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Bioinformatics Analysis of NprR-NprX Quorum-Sensing System of *Bacillus thuringiensis* Isolates from the Papaloapan Region, Oaxaca-Mexico

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Abstract

Quorum sensing is a chemical communication process that bacteria use to regulate collective behaviors. In Gram-positive bacteria, oligopeptides (called autoinducers) are the signaling molecules to elicit quorum sensing. In *Bacillus thuringiensis*, NprR is a transcriptional regulator whose activity depends on the NprX signalling peptide. *Bacillus thuringiensis* is closely related to *Bacillus cereus* and *Bacillus anthracis*. The principal difference between them is that *Bacillus thuringiensis* is the only one that produced Cry protein. The aim of this study is to explore the relation of *nprR* and 16S rRNA genes in *Bacillus thuringiensis.* Phylogenetic trees of nucleotide sequences of *nprR* and 16S rRNA genes were built. Sequences of fourteen new isolates from Papaloapan region were included in those phylogenetic trees. In order to identify the isolates, a simple and fast methodology considering the Cry protein formation was used. The 16S rRNA phylogenetic tree allows identify eight isolates as *Bacillus thuringiensis* and the others as *Bacillus spp*. The *nprR* phylogenetic tree does not match with the 16S rRNA phylogenetic tree. This confirms that *nprR* is not a molecular marker for evolution. Most of the new isolates have the same NprR sequence (WTSDIVG). However, the SKPDIVG is the most common NprR sequence in *thuringiensis* species.

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Keywords

Quorum-Sensing, *Bacillus thuringiensis*, NprR-NprX, Phylogenetic Tree

1. Introduction

Several bacterial species use the Quorum Sensing (QS, cell-cell communication) to coordinate their behavior as a whole community [1]. In Gram-positive bacteria, the signaling molecules are mostly small secreted peptides that are actively released into the extracellular environment [2]. The QS regulators of soil bacteria have been grouped in a new protein family called RNPP (Rap, NprR, PlcR and PrgX). Genome analysis indicated that genes encoding a putative regulator (NprR) and a putative signaling peptide (NprX or NprRB) were found upstream from *nprA* in the bacteria of the *Bacillus cereus* (*B. cereus*) group [3] [4]. These genes encode for a receptor and for a small protein. The small protein has a putative signal sequence used in the export pathway and a secreted domain [5]. The signaling peptide exported is processed by extracellular proteases and it is internalized by an oligopeptide permease [6]. The secreted QS factor allowing the activation of *nprA* expression corresponds to the central part of NprX and it is at least seven amino acids long [3]. The NprR-NprX system was found in all the species of *B. cereus* group, where 31 different NprR polypeptide sequences were identified [3].

The *B. cereus* group comprises a number of closely related pathogenic species (*Bacillus thuringiensis*, *B. thuringiensis*; *Bacillus anthracis*, *B. anthracis*; and *B. cereus*) [7]. *B. thuringiensis* is a Gram-positive endospore-forming bacterium which is the only one in *B. cereus* group that synthesizes a crystalline delta-endotoxin protein (named Cry) [8]. Cry protein is formed during sporulation process and it is an insoluble and crystalline protein. The main application of the Cry protein is in the biological control and it has been also reported that some Cry proteins are highly cytotoxic to a wide range of human cancer cells [9].

The aim of the present study is to determine if the genes encoding the signaling peptide (*nprX*) and the 16S rRNA genes are related in *B. thuringiensis*. New isolates from the Papaloapan region were used in the study. Also, a simple and rapid isolation method for *B. thuringiensis* is proposed.

2. Material and Methods

2.1. Soil Samples

The new *B. thuringiensis* isolates were obtain from uncultivated and cultivated soil samples from the Papaloapan region. The cultivated soil samples were from sugarcane, coffee and banana crops. The geographic location and coordinates of the soil sampling is show in the Electronic Supplementary Material, **Table S1**. Papaloapan region is a humid tropical region with annual average conditions of 70% of humidity, 33˚C and 3000 mm of precipitation. Five samples of 500 g at 15 cm deep of soil per crop (four corners and the center) were collected. The samples were stored at 4˚C for 24 h.

2.2. Isolation and Identification Method

The isolation method was design considering the principal characteristics of *B. thuringiensis* which is forming an insoluble and crystalline protein (Cry protein). The method consist them in two steps: 1) colony and microscopically morphology and 2) production of an insoluble protein at the end of the submerged culture. In order to compare the isolates *B. thuringiensis* var. *kurstaki* HD-73 (ATCC-35866), which produces a 133.3 kDa Cry1A(c) insecticidal crystal protein, was used as a reference. The isolates were phylogenetically identified comparing the molecular chronometer 16S rRNA from the isolates with 16S rRNA from *B. thuringiensis* strains reported in databases.

Steps 1: *Colony and Microscopically Morphology*. From the collected sample serial dilutions were done with sterile distilled water. The 1×10^{-3} dilutions were inoculated by triplicate in nutrient agar and were incubated at 30˚C for 24 hours. Colonies were selected by their morphology looking for white to off-white color, opaque, slightly raised elevation, and regular outlined colonies [10]. The selected colonies were inoculated in serial subcultures of nutrient agar until pure cultures were obtained. After that, the selected isolates were inoculated in 250 ml of nutrient broth by duplicate at 30˚C and 180 rpm during 48 h. At eight hours of culture (during the expo-

nential growth) samples were collected for Gram staining, looking for gram positive bacteria. At 24 hours of culture (during the sporulation phase) samples were collected for spore formation staining. The stains were analyzed with a microscope under dark field illumination, with a 40× objective and 10× ocular lenses. It is important for a proper Gram staining of *B. thuringiensis* to take the sample during the exponential growth; otherwise, according to our experiments, the bacteria could be stained as a gram negative. At the end of the cultures the broth were collected for Cry protein detection.

Step 2: *Detection of Insoluble Protein.* The Cry proteins are insoluble at neutral pH, they are soluble just under alkaline conditions [9] [11]. The detection of Cry protein was done by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Bovine serum albumin (BSA) was used as protein standard. Considering the insolubility of Cry protein, the culture broth were centrifuged in 50 ml corning tubes at 5580 g during 30 min. The pellets were resuspended in 2 ml of distilled water, concentrated in a 15 ml corning tube and centrifuged at 5580 g during 30 min. The samples preparation and the solubilization process were done using the methodology described by Navarro *et al.* [12]. Four replicates were done for each sample.

2.3. Bioinformatic Analysis

The selected isolates were inoculated in Gerry Rowe culture medium, and incubated in orbital shaker (Thermo Scientific) at 30˚C and 200 rpm during 48 h. At eight hours of culture, 5 ml samples were centrifuged at 11,180 g during 15 min at 4˚C. Genomic DNA was extracted using the Ultra Clean microbial DNA isolation kit (MOBIO) following the manufacturer's recommendations. DNA was quantified using a nano-spectrophotometer (Nanodrop 2000, Thermo Scientific).

The PCR amplification of 16S DNA gene was done using 100 ng of genomic DNA, 10 pM fD1 primer (CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG) and rD1 primer (CCCGGGATCCAAGCTTAA GGAGGTGATCCAGCC) [13]. 16S DNA PCR was performed in a MaxyGene (Axygen Scientific®) thermal cycler under the following conditions: 95˚C 5 min; 35 cycles of 95˚C for 1 min, 55˚C for 40 s and 72˚C for 30 s; finally, 72˚C for 10 min. The PCR amplification of *nprX* gene was done using the primers NprR-1 (GGGCAT TTGTTCTGTCTC) and NprR-2 (GCTAACACTAACGCTAAAC) [4] for NprR-NprX in 50 µL reaction (DreamTaq kit ™ 2X Green PCR Master Mix, Fermentas). *nprX* gene PCR was performed in a MaxyGene (Axygen Scientific®) thermal cycler under the following conditions: 95˚C 5 min; 35 cycles of 95˚C for 1 min, Ta 1 min and 72˚C for 1 min; finally, 72˚C for 10 min. The amplifications were observed with an imaging and analyzing system INGENIUS SYNGENNE®. The PCR products were purified using Thermo Scientific GeneJET PCR Purification Kit. The sequencing service was performed in Macrogen Inc. Korea. The nucleotide sequences obtained in this study were compared with sequences retrieved from databases in a pairwise mode, with the BLAST2 sequences tool (http://www.ncbi.nlm.nih.gov/BLAST/bl2seq/wblast2.cgi). The identification criteria proposed by Pothathil and Lazzazzera [6], and Perchat *et al.* [3] were used to identify the isolates putative signal pentapeptide sequence.

Likelihood trees of 16S rRNA gene and *nprX* gene nucleotide sequences were built for isolates bioinformatic analysis. For phylogenetic tree based on 16S rRNA, the sequences from 29 isolates and 42 sequences from *B. cereus* group strains were included. For phylogenetic tree based on *nprX* gene, 24 isolates and 33 sequences from *B. cereus* group strains were included (Electronic Supplementary Material **Table S2** and **Table S3**). The *B. cereus* group strains sequences were obtained from the GenBank database of the NCBI (http://www.ncbi.nlm.nih.gov/genbank) and IMG database (Integrated Microbial genomes, http://img.jgi.doe.gov). The phylogenetic tree based on 16S rRNA gene was built under the Statistical Method Maximum Likelihood. The Substitution Model used was Tamura Nei [14]. The phylogenetic tree based on 16S rRNA gene topology was optimized with the nearest neighbor interchanges (NNI). The phylogenetic tree based on *nprX* gene was built under the general time reversible (GTR) substitution model using Phyml [15], while the topology was optimized with the nearest neighbor interchanges and sub-tree pruning and regrafting approaches. Statistical support was determined by 10,000 bootstrap replicates. The phylogenetic trees based on 16S rRNA and *nprX* gene were built using MEGA 6.0 software and CLUSTALW analysis.

3. Results

3.1. Isolation and Identification of Strains

The results of the isolation method are shown in **Table 1**. From colony and microscopically morphology 45 iso-

lates were selected as *B. thuringiensis*; from those, 41 isolates produce an insoluble protein between 60 and 130 kDa (91%). Considering just colony morphology, approximately 63% of the isolates produce an insoluble protein. Therefore, the combination of colony and microscopically morphology increase the possibility to isolate correctly *B. thuringiensis* strains.

3.2. Molecular Characterization and Phylogenetic Analysis

For isolates identification three consecutive phylogenetic trees were built. The first one was built using 25 reported sequences of 16S rRNA gene: 19 of *B. cereus* group strains (10 for *B. thuringiensis*, 6 for *B. cereus*, 3 for *B. anthracis*), 4 of *Bacillus subtilis* (*B. subtilis*), 1 of *Bacillus licheniformis* (*B. licheniformis*) and 1 of *Escherichia coli* (*E. coli*), **Figure 1**. This phylogenetic tree shows three groups, one with all the strains of *B. cereus*

Table 1. Results from the isolation method.

	Isolates of soil crop			
	Uncultivated	Banana	Coffee	Sugarcane
Colony morphology	2	6	5	52
Microscopically morphology	2	4	3	36
Insoluble protein detection	2	4	3	32
16S rRNA gene amplification	2	4	3	32
Forward and reverse positive sequencing	2	4	2	21
Phylogenetic identification	2	2	-	20

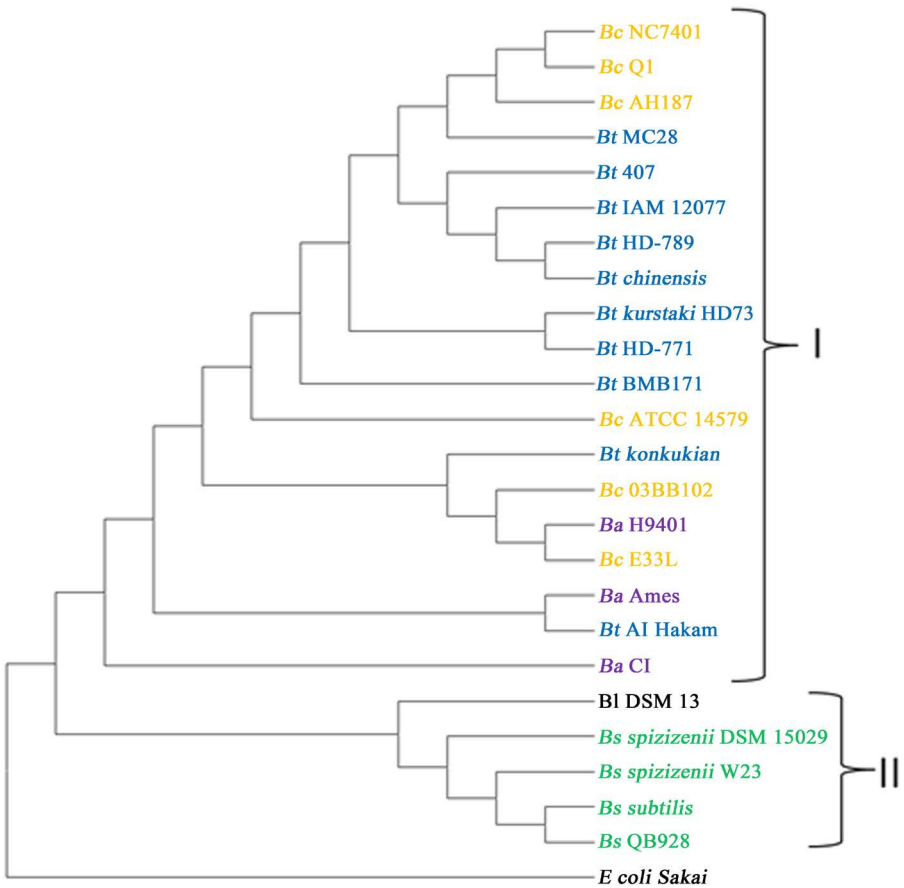

Figure 1. Rectangular phylogenetic tree built with the 16S rRNA genes sequences (first tree) for several strains of *B. cereus* group (obtained from the GenBank and IMG). Abbreviations: Bt: *Bacillus thuringiensis* (blue), Bc: *B. cereus* (orange), Ba: *B. anthracis* (purple), Bs: *B. subtilis* (green), BI DSM 13: *B. licheniformis* DMS 13 (black). Group I corresponds to *B. cereus* group strains and group II corresponds to *B. subtilis* strains.

group, the second one with *B. subtilis* and *B. licheniformis* and the last one with *E. coli*.

A ClustalW analysis [16] using the isolates 16S rRNA gene sequences and the sequences used for the first tree with the MEGA 6.0 was done. With this alignment the second phylogenetic tree was built using the Tamura Nei model [14] (**Figure 2**). The second phylogenetic tree shows two groups, one with the *B. cereus* strains and

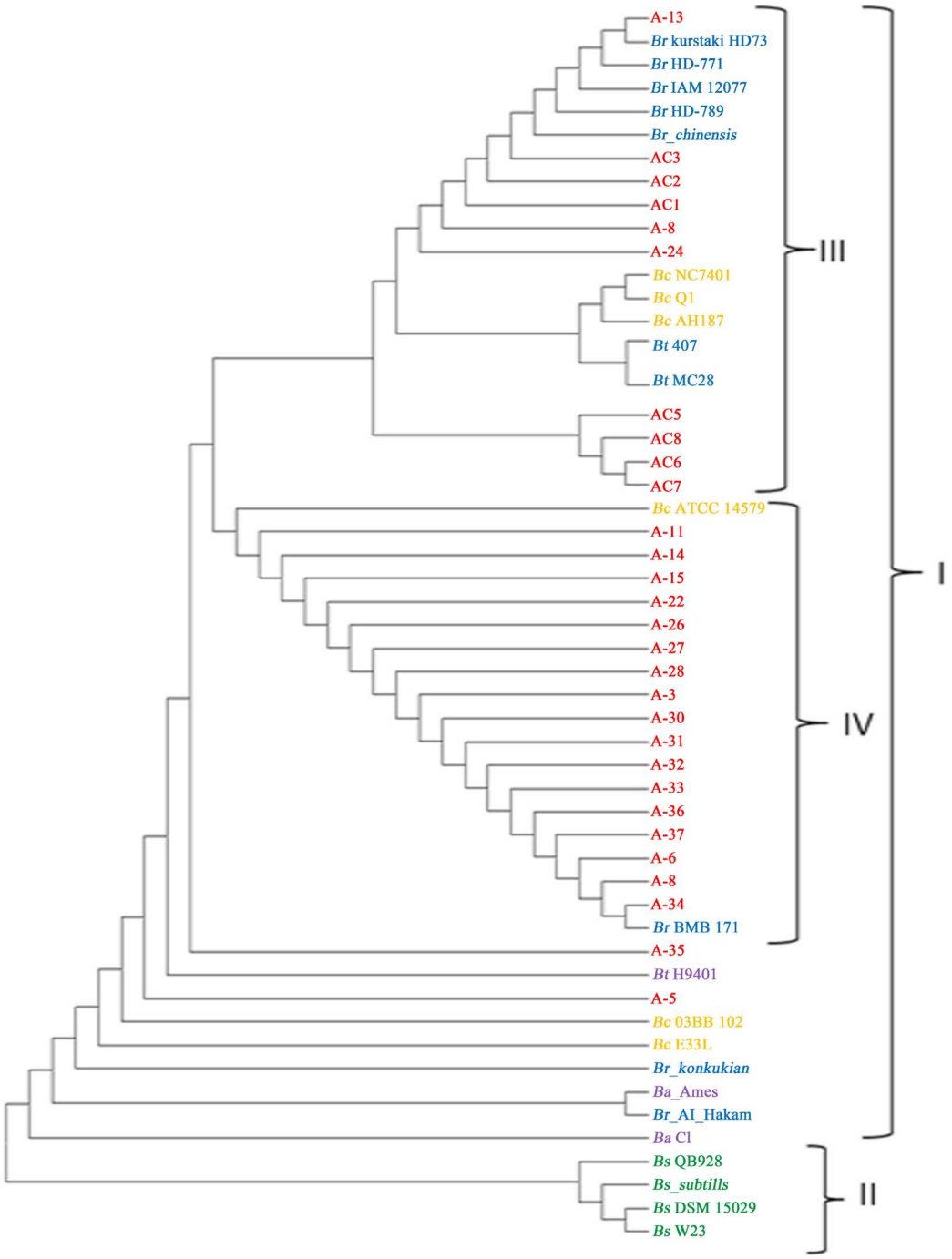

Figure 2. Rectangular phylogenetic tree built with the 16S rRNA gene sequences (second tree) for isolates strains of Papaloapan region (red) and for several strains of *B. cereus* group (obtained from the GenBank and IMG). Abbreviations: Bt: *Bacillus thuringiensis* (blue), Bc: *B. cereus* (orange), Ba: *B. anthracis* (purple), Bs: *B. subtilis* (green). Group I corresponds to *B. cereus* group strains, group II corresponds to *B. subtilis* strains, group III corresponds to *B. thuringiensis* strains and group IV corresponds to A-X isolates group.

the other one with *B. subtilis*. All the isolates sequences are closer to the *B. cereus* group (**Figure 2**). The 16S rRNA gene sequences of 16 isolates (A-8, A-6, A-37, A-36, A-33, A-32, A-31, A-30, A-3, A-28, A-27, A-26, A-22, A-15, A-14 and A-11) have a comb-like configuration. Through the alignment of these sequences, it was observed that all the sequences are identical. Therefore, they were considered as one group (A-X group).

Considering the results of the first and the second phylogenetic tree, the third one was built (**Figure 3**). For the third phylogenetic tree 22 sequences of *B. thuringiensis* strains were added (Electronic Supplementary Material **Table S2**). In this third phylogenetic tree (**Figure 3**) it can be observed that isolated AC1 presents similarity with *B. thuringiensis alesti*; as well as AC2 with *B. thuringiensis* HD-771, AC3 with *B. thuringiensis higo* and A-13 with *B. thuringiensis* IAM 12077. Besides, A-9 and A-24 show similarity not only with *B. thuringiensis* IAM 12077 but also with different varieties of *B. thuringiensis* such as *chinensis*, *oswaldocruzi*, *kenyae* and *thompsoni*. Isolates AC5, AC6, AC7 and AC8 show similarity with *B. thuringiensis* and *B. cereus*. The A-X group share information with *B. thuringiensis* (*ostriniae*, *seoulensis* and BMB) and with *B. cereus* (ATCC 14579). The isolated A-34 shows similarity with *B. thuringiensis tolworthi*. The isolated A-35 and A-5 show similarity with the group of *B. thuringiensis*, *B. cereus* and *B. anthracis* species. **Table 2** shows the results from

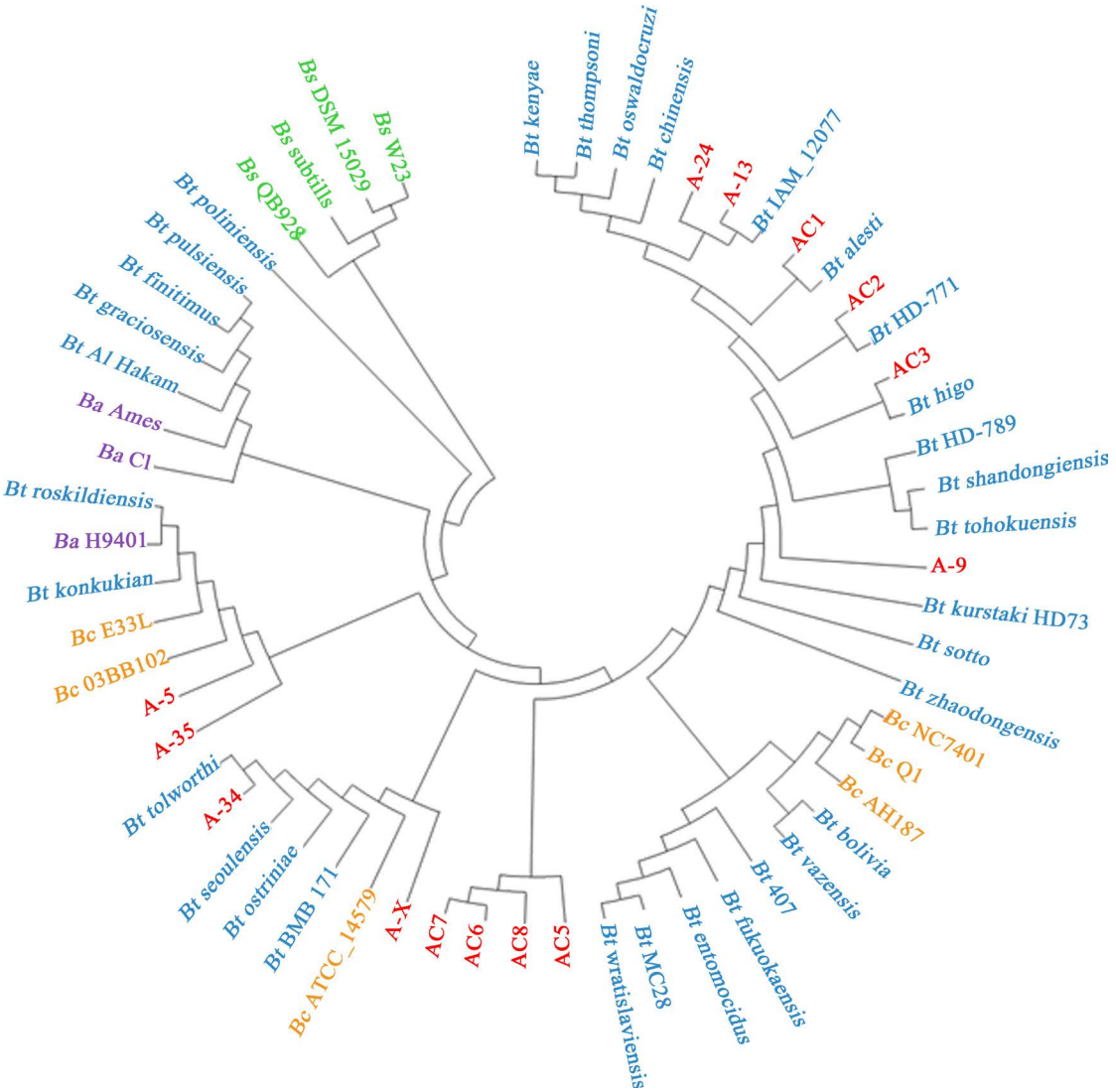

Figure 3. Circular phylogenetic tree built with the 16S rRNA gene sequences (third tree) for isolates strains of Papaloapan region (red) and several strains of *B. cereus* group (obtained from the GenBank and IMG). Abbreviations: Bt: *Bacillus thuringiensis* (blue), Bc: *B. cereus* (orange), Ba: *B. anthracis* (purple), Bs: *B. subtilis* (green). A-X group represent A-8, A-6, A-37, A-36, A-33, A-32, A-31, A-30, A-3, A-28, A-27, A-26, A-22, A-15, A-14 and A-11 isolates.

Table 2. Phylogenetic analysis results of 16S rRNA gene sequences of isolates strains of Papaloapan region.

Isolated	Soil type	Species	Heptapeptide type
AC-1	Uncultivated	*thuringiensis* var *alesti*	WTSDIVG
AC-2	Uncultivated	*thuringiensis* HD-771	WTSDIVG
AC-3	Banana	*thuringiensis* var *higo*	Unidentified
AC-5	Coffe	*spp*	WTSDIVG
AC-6	Banana	*spp*	WTSDIVG
AC-7	Coffe	*spp*	WTSDIVG
AC-8	Banana	*spp*	WTSDIVG
A-X	Sugarcane	*thuringiensis*	WTSDIVG
A-5	Sugarcane	*spp*	SRPDVLT
A-9	Banana	*thuringiensis*	SKPDIVG
A-13	Sugarcane	*thuringiensis* IAM 12077	SKPDIVG
A-24	Sugarcane	*thuringiensis*	SKPDIVG
A-34	Sugarcane	*thuringiensis* var *tolworthi*	Unidentified
A-35	Sugarcane	*spp*	SRPDVLT

the phylogenetic identification of the isolates from the Papaloapan region. For AC-5, AC-6, AC-7, AC-8, A-5 and A-35 isolates the phylogenetic analysis is not conclusive, therefore, they were identified as *Bacillus spp.* However, all of these isolates produce an insoluble protein at the end of the culture.

3.3. NprX Bioinformatic Analysis

The phylogenetic tree based on *nprR* gene nucleotide sequence built on 29 strains isolated and 32 strains of *B. cereus* group from the literature (**Figure 4**, Electronic Supplementary Material **Table S3**) show a perfect correlation of seven putative mature NprX heptapeptide sequences [3]. Analysis of the NprX peptide was performed using the software SignalP 3 [15], which incorporates a prediction of cleavage sites and a signal peptide/non-signal peptide prediction based on a combination of several artificial neural networks and hidden Markov models. Alignment of the secreted region was done using ClustalX to identify putative mature signaling peptides. The putative mature NprX for isolated strains are show in **Table 2**.

In phylogenetic tree based on *nprR* gene (**Figure 4**) different putative mature NprX can be observed: SKPDIVG for 15 *B. thuringiensis* strains, 3 *B. anthracis* strains and one *B. cereus* strain; WTSDIVG for 5 *B. thuringiensis* strains and 4 *Bacillus spp.* strains; SRPDVLT** for 2 strains of *B. thuringiensis*, one of *B. cereus* and two of *Bacillus spp.*; WKPDVLG* for 2 *B. thuringiensis* strains; WKPDTLG* for 2 *B. thuringiensis* strains and one *B. cereus* strain; SNPDIY** for one *B. thuringiensis* strain and one *B. cereus* strain; SKPDTYG for 2 strains of *B. cereus*.

The phylogenetic tree based on *nprR* gene also shows the type of Cry protein produced for the reported strains (**Figure 4**, Electronic Supplementary Material **Table S3**). No relationship was found between the Cry protein type produced and the putative mature peptides of all strain studied.

4. Discussion

The isolation method results show that the phase contrast microscopy strategy is not enough for *B. thuringiensis* isolation. Just 63% of the isolates produce an insoluble protein (Cry protein) at the end of the culture. In general, phase contrast microscopy is the backbone of the isolation strategy [17]. Another disadvantage of the phase contrast microscopy strategy is that a large proportion of isolates contain *cry*1 gene (bipyramidal shaped crystal) and relatively few novel isolates [17].

According to the isolation method results (**Table 1**), the critical step in the method is to obtain forward and reverse sequencing. With both sequences, around 83% of the strains were identified by phylogenetic analysis. In this study, the phylogenic analysis of the 16S rRNA gene from 30 isolates from the Papalopan region 24 isolates was identified as *B. thuringiensis* while 6 of them were unidentified (**Table 2**). However, *B. thuringiensis* is the

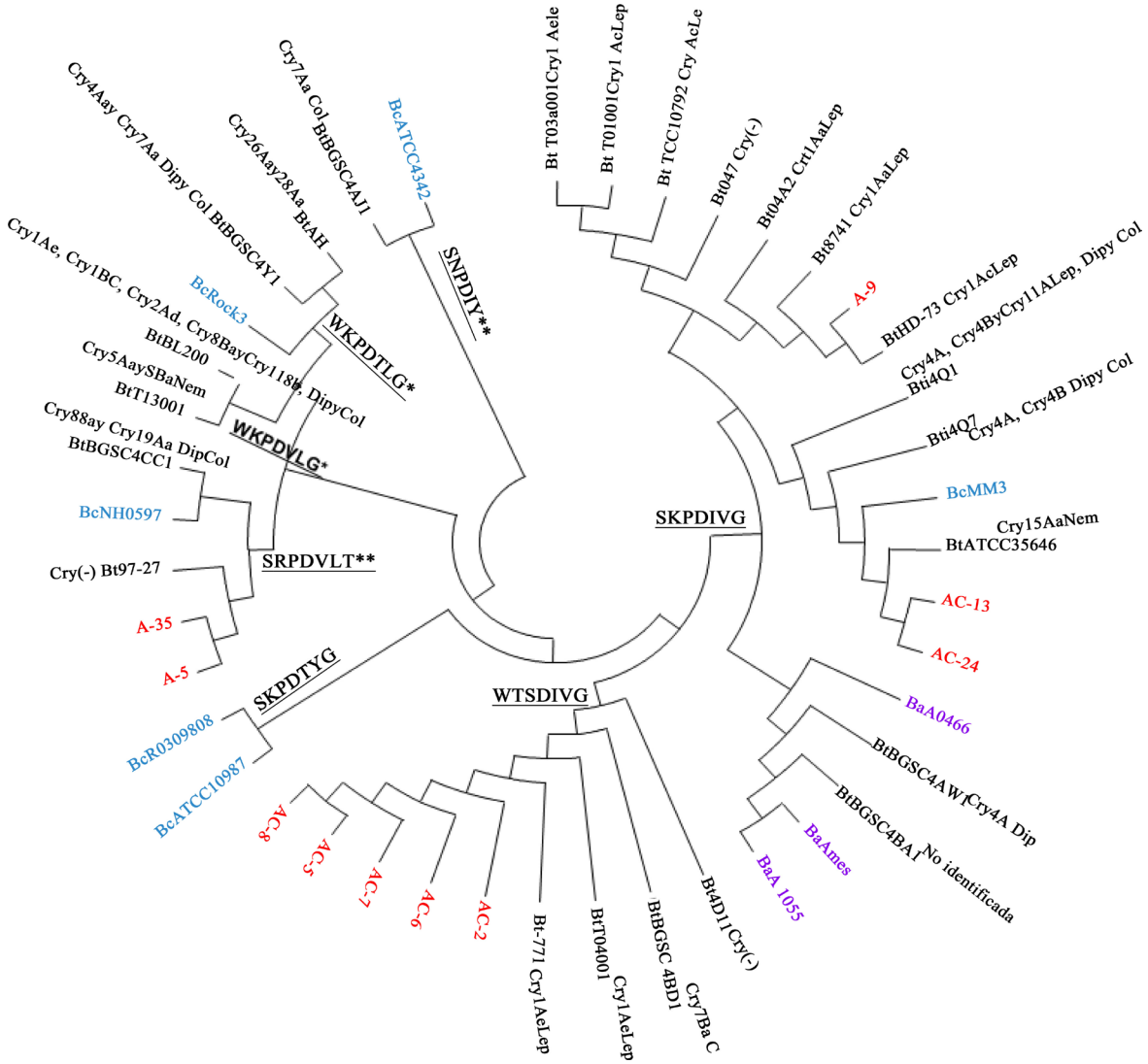

Figure 4. Phylogenetic tree based on *nprX* gene sequence. 34 sequences of the *B. cereus* group were used under the model of amino acid substitution. One asterisk represents sequences of NprX that are lowly predicted to be exported from the cell. Two asterisks represent NprX sequences that are not predicted to be secreted by the cell.

only strain in *B. cereus* group that produces an insoluble protein at the end of the culture, named Cry protein. The 6 unidentified isolates produce this insoluble protein; therefore, they cannot be discarded as *B. thuringiensis*.

Several methods have been used in order to classify the *B. cereus* group. However, the classification results depend on the method, the strains, and the molecular marker used for each study. For example, multi-locus enzyme electrophoresis and the analysis of nine chromosomal genes showed that *B. cereus*, *B. anthracis*, and *B. thuringiensis* are the same species [18]. On the other hand, analysis of 16S rRNA, 23S, *rpoB* and *gyrB* genes from *B. cereus* group strains indicated that *B. anthracis* can be distinguished from *B. cereus* and *B. thuringiensis* strains [17] [19].

The phylogenetic tree based on *nprR* gene does not match with the phylogenetic tree based on 16S rRNA gene indicating that the *nprR* is not specific for the species but it is specific for the strain. Several species of *Bacillus* could have the same heptapeptide sequence. For *B. thuringiensis* five different heptapeptide sequences were found. Although the SKPDIVG is the most common heptapeptide in *thuringiensis* species. For the isolates strain, the most common peptide sequence is WTSDIVG and there is no relationship with the soil type. Considering A-X as the same strain, for *thuringiensis* species SKPDIVG is the most common peptide. It has been re-

ported that the SKPDIVG and the SKPDI are the most common peptide in *thuringiensis* species [3] [4]. The NprR-NprX quorum-sensing system was found to be strain-specific with a possible cross-talk between some pherotypes. The phylogenic relationship between NprR and NprX suggests a coevolution of the regulatory protein and its signalling peptide [3]. The incongruence between the phylogenetic trees based on *nprR* and 16S rRNA genes indicates that NprR is not a molecular marker for evolution. Therefore, the *nprR* gene is not useful for strain identification. Although phylogenetic tree based on *nprR* gene provides significant biological information, as it constitutes a system that controls functions like extracellular enzyme productions and plays a role in sporulation and *cry* transcription [3].

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Supplementary Tables

Table S1. Soil type and location of sampling in the Papaloapan region in the state of Oaxaca.

Soil type	Geographic location	Geographic coordinates	Meters above sea level
Uncultivated	San Bartolo Tuxtepec, Oaxaca	N18˚00'744"; WO96˚10'146"	55
Cane	Higway Tuxtepec-Palomares	N31˚37'514"; W131˚21'293"	89
		N45˚03'5044"; W115˚52'022"	92
		N17˚59'597"; W96˚06'895"	45
		N17˚59'597"; W96˚06'882"	53
		N17˚59'597"; W96˚06'884"	49
		N18˚06'342"; W96˚15'249"	50
		N18˚06'349"; W96˚15'264"	59
		N18˚06'357"; W96˚15'291"	61
		N18˚06'366"; W96˚15'241"	66
		N18˚06'402"; W96˚15'234"	35
Coffe	Armadillo Chico, Valle Nacional, Oaxaca	N17˚50'764"; W96˚18'992"	605
Banana	San Bartolo Tuxtepec, Oaxaca	N18˚05'924"; W96˚06'767"	53

Table S2. Strains used for phylogenetic bioinformatics study of the 16S rRNA gene.

Species	Access Number	Species	Access Number
Bacillus anthracis CI	NC_014335	*Bacillus thuringiensis aralesti*	EF210300
Bacillus cereus E33L	NC_006274	*Bacillus thuringiensis fukuokaensis*	EF210301
Bacillus cereus NC7401	NC_016771	*Bacillus thuringiensis sotto*	EF210309
Bacillus subtilis QB928	CP003783	*Bacillus thuringiensis kenyae*	EF210315
Bacillus subtilis spizizenii DSM 15029	NC_016047	*Bacillus thuringiensis entomocidus*	EF210312
Bacillus subtilis subsp. *spizizenii* str. W23	NC_014479	*Bacillus thuringiensis ostriniae*	EF210314
Bacillus thuringiensis BMB171	NC_014171	*Bacillus thuringiensis tolworthi*	EF210288
Bacillus thuringiensis Bt407	CP003889	*Bacillus thuringiensis thompsoni*	EF210310
Bacillus thuringiensis HD-771	CP003752	*Bacillus thuringiensis tohokuensis*	EF210294
Bacillus thuringiensis HD-789	CP003763	*Bacillus thuringiensis shandongiensis*	EF210287
Bacillus thuringiensis MC28	CP003687	*Bacillus thuringiensis seoulensis*	EF210286
Bacillus thuringiensis var. *chinensis* CT-43	CP001907	*Bacillus thuringiensis oswaldocruzi*	EF210284
Bacillus thuringiensis var *konkukian* str. 97-27	NC_005957	*Bacillus thuringiensis higo*	EF210308
*Bacillus thuringiensis*str. *Al Hakam*	NC_008600	*Bacillus thuringiensis roskildiensis*	EF210304
Bacillus anthracis str. *Ames*	NR_074453	*Bacillus thuringiensis wratislaviensis*	EF210311
Bacillus cereus ATCC 14579	NC_004722	*Bacillus thuringiensis poloniensis*	EF210305
Bacillus licheniformis DSM 13 ATCC 14580	NC_006322	*Bacillus thuringiensis zhaodongensis*	EF210289
Bacillus subtilis subsp. *subtilis* 6051-HGW	NC_020507	*Bacillus thuringiensis Bolivia*	EF210299
Bacillus thuringiensis var. *kurstaki* str. HD73	NC_020238	*Bacillus thuringiensis pulsiensis*	EF210285
Bacillus thuringiensis strain IAM 12077	NR_043403	*Bacillus thuringiensis graciosensis*	EF210302
Bacillus thuringiensis finitimus	EF210290	*Bacillus thuringiensis vazensis*	EF210293

Table S3. Strains used for phylogenetic study of the NprRB gene.

Strain	Abbreviation	GenBank Access Number	Cry protein	Target groups	Isolation location	Heptapeptide type
Bacillus thuringiensis strain 407	Bt407	ACMZ01000019.1	Cry1Ab	Lepidoptera	-	SKPDIVG
Bacillus thuringiensis var. *andalousiensis* BGSC 4AW1	BtBGSC4AW1	ACNG01000020.1	Cry 4Aa	Diptera	Spain	SKPDIVG
Bacillus thuringiensis var. *berliner* ATCC 10792	BtATCC10792	NZ_ACNF01000019.1	Cry1Ac	Lepidoptera	-	SKPDIVG
Bacillus thuringiensis var. *kurstaki* str. T03a001	BtT03a001	NZ_ACND01000019.1	Cry1Ae	Lepidoptera	Japan	SKPDIVG
Bacillus thuringiensis var. *pondicheriensis* BGSC 4BA1	BtBGSC4BA1	NZ_ACNH01000016.1	-	-	India	SKPDIVG
Bacillus thuringiensis var. *thuringiensis* str. T01001	BtT01001	NZ_ACNA01000020.1	Cry1Ac	Lepidoptera	-	SKPDIVG
Bacillus thuringiensis var. *israelensis* 4Q1	Bti4Q1	JN637469	Cry4A, Cry4B and Cry11A	Diptera, Lepidoptera and Coleoptera	Israel	SKPDIVG
Bacillus thuringiensis var. *israelensis* 4Q7	Bti4Q7	JN637470	Cry4A and Cry4B	Diptera	-	SKPDIVG
Bacillus thuringiensis var. *thuringiensis* 4A2	Bt4A2	JN637471	Cry1Aa	Lepidoptera	-	SKPDIVG
Bacillus thuringiensis var. *thuringiensis* 8741	Bt8741	JN637473	Cry1Aa	Lepidoptera	-	SKPDIVG
Bacillus thuringiensis var. *kurstaki* 4D11	Bt4D11	JN637468	Cry(-)	-	-	SKPDIVG
Bacillus thuringiensis var. *huazhongensis* BGSC 4BD1	BtBGSC 4BD1	NZ_ACNI01000018.1	Cry7Ba	Coleoptera	China	WTSDIYG
Bacillus thuringiensis var. *monterrey* BGSC 4AJ1	BtBGSC4AJ1	NZ_ACNE01000016.1	Cry7Aa	Coleoptera	México	SNPDIYG
Bacillus thuringiensis var. *pakistani* str. T13001	BtT13001	NZ_ACNC01000023.1	Cry5Ba and Cry5Aa	Nematode	-	WKPDVLG
Bacillus thuringiensis var. *pulsiensis* BGSC 4CC1	BtBGSC4CC1	NZ_ACNJ01000017.1	Cry8Ba and Cry19Aa	Coleoptera and Diptera	Pakistan	SRPDVLT
Bacillus thuringiensis var. *sotto* str. T40001	BtT04001	NZ_ACNB01000029.1	Cry1Ae	Lepidoptera	China	WTSDIYG
Bacillus thuringiensis var. *tochigiensis* BGSC 4Y1	BtBGSC4Y1	NZ_ACMY01000020.1	Cy4Aa and Cry7Aa	Diptera and Coleoptera	Japan	WKPDTLG
Bacillus thuringiensis var. *israelensis* ATCC 35646	BtATCC35646	AAJM01000002	Cry15Aa	Nematode	-	SKPDIVG
Bacillus thuringiensis str. *Al Hakam*	BtAH	CP000485	Cry26Aa and 28Aa	Nonpathogenic	-	WKPDTLG
Bacillus thuringiensis IBL 200	BtIBL200	CM000758	Cry1Ae, Cry1Bc, Cry2Ad, Cry8Ba and Cry11Bb	Lepidoptera, Coleoptera and Diptera	-	WKPDVLG
Bacillus thuringiensis var. *konkukian* str. 97-27	Bt97-27	NC_005957	Cry(-)	-	-	SRPDVLT
Bacillus thuringiensis BMB171	BtBMB171	ADH05341.1	Cry1Ac	Lepidoptera	-	SKPDIVG
Bacillus thuringiensis HD-73	BtHD-73		Cry1Ac	Lepidoptera	-	SKPDIVG
Bacillus thuringiensis HD-771	Bt-771	CP003752	Cry1Aa	Lepidoptera	-	WTSDIYG