

Draft genome sequence of *Bacillus thuringiensis* strain S1287, an isolate showing insecticidal activity against coleoptera

Projeto de sequência do genoma de *Bacillus thuringiensis* strain S1287, um isolado mostrando actividade insecticida contra coleópteros

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ABSTRACT

Bacillus thuringiensis is an important bacterium showing insecticide proteins effective to control several important medical pests, including several Diptera species, like Diptera, Coleoptera and Lepidoptera. This work describes the draft genome sequence of *B. thuringiensis* S1287, which contains genes encoding the parasporal crystal cry, like Cry1Ab, Cry1Bb, Cry1C, Cry1D, Cry1Fb, Cry1Hb, Cry1Id, Cry1Ja, Cry1Ka and

Cry1Nn. The genes belonging to the Vip Family were identified, Vpb1Aa, Vpa2Aa and Vip3Ag. Genes coding for plant growth promotion pathway were found.

Keywords: insecticidal proteins, cry genes, boll weevil.

RESUMO

Bacillus thuringiensis é uma bactéria importante mostrando proteínas insecticidas eficazes para controlar várias pragas médicas importantes, incluindo várias espécies de Diptera, como a Diptera, Coleoptera e Lepidoptera. Este trabalho descreve o projecto da sequência genómica de *B. thuringiensis* S1287, que contém genes que codificam o grito de cristal parasporal, como Cry1Ab, Cry1Bb, Cry1C, Cry1D, Cry1Fb, Cry1Hb, Cry1Id, Cry1Ja, Cry1Ka e Cry1Nn. Foram identificados os genes pertencentes à Família Vip, Vpb1Aa, Vpa2Aa e Vip3Ag. Foram encontrados genes que codificam a via de promoção do crescimento das plantas.

Palavras-chave: proteínas inseticidas, genes de choro, gorgulho de boll.

1 INTRODUCTION

Bacillus thuringiensis is a soil bacterium of considerable economic importance used as a biocontrol agent (1), including the cotton Boll weevil (*Anthonomus grandis*).

The toxic action of some *B. thuringiensis* strains is due the parasporal inclusions that are composed of several toxic polypeptides named Cry toxins. And Vip toxins which can act together to affect the coleopteran larvicidal activity (2).

Purified genomic and plasmid DNA's from strain S1287 were extracted by Masterpure Gram-positive DNA purification kit (Epicentre) and QIAGEN Plasmid Maxi Kit (QiAGEN). Afterwards, these DNA's were sequenced at Macrogen, Inc. (Seoul, Korea) using high-throughput HiSeq2000 and GS-FLX Plus platforms getting one lane of 100 bp and 1/8 region plate, respectively. The reads were assembled using SOAP de novo (version 1.05) and produced 218 contigs totaling 6,636,210 bp (Q20 = 99.34%), with a maximum scaffold size of 359,449 bp, an N₅₀ length of 115,122 bp, and 34.22% G+C content and genome coverage depth was approximately 100x. De novo assembly was carried out using the Geneious version (8.0.4) (3). A total of 6,554 coding sequences (4), 71 tRNA (5), 5 rRNA operons (6) and 5 ncRNA were predicted. We identified 5 plasmids ranging from 342,958 to 8,235 bp size. The functions of encoding genes were annotated by using the NCBI nr, Swiss-Prot (7), Clusters of Orthologous Groups (COG) (8), KEGG (9), and InterProScan (10) databases.

The custom insecticidal toxin database was constructed with nucleotide and amino acid sequences of *cry*, *vip*, *cyt*, growth promotion, parasporyin and bacteriocin genes using

the complete sequences of other *B. thuringiensis* strains deposited in public databases (<http://www.ncbi.nlm.nih.gov/genome/> or [http://www.https://www.bpprc.org/](https://www.bpprc.org/)). The strain S1785 draft genome sequence carries several insecticidal toxin genes showing identities to the *Cry1Ab*, *Cry1Bb*, *Cry1C*, *Cry1D*, *Cry1Fb*, *Cry1Hb*, *Cry1Id*, *Cry1Ja*, *Cry1Ka* and *Cry1Nn*. and the *Vip* family genes *Vpb1Aa*, *Vpa2Aa* and *Vip3Ag*. The genes which encode the metabolic pathway of plant growth promotion 1-aminocyclopropane-1-carboxylate deaminase, indolepyruvate decarboxylase, putative acid phosphatase and siderophore biosynthesis protein were identified. Using the new nomenclature of proteins toxic to insects proposed by Crickmore et al. (11) we found the *Spp1Aa* gene. The genomic analyses could not identify any operon related to the antimicrobial peptide bacteriocin (thuricin or nisin).

Nucleotide sequence accession numbers. The sequence of the *B. thuringiensis* strain S1287 has been deposited in GenBank with the accession number SAMN12285942.

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