Draft genome sequence of *Bacillus thuringiensis* strain s908, an isolate toxic for coleopteran

Projeto de sequência genómica de *Bacillus thuringiensis* strain s908, um tóxico isolado para coleopteran

DOI:10.34115/basrv6n3-019

Recebimento dos originais: 18/02/2022
Aceitação para publicação: 14/03/2022

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ABSTRACT

Bacillus thuringiensis (Bt) produces pore forming toxins that have been used for Diptera, Lepidoptera and Coleoptera pest control in agriculture for many years. This work describes the draft genome sequence of B. thuringiensis S908, which contains the genes cry1B, Vpa, Vpb and Sip. Genes coding for bacteriocin, enhancin, plant growth promotion pathway and tyrosinase were identified

Keywords: cry toxins, vip toxins, ngs, bioinformatics.

RESUMO
Bacillus thuringiensis (Bt) produz toxinas formadoras de poros que têm sido utilizadas para o controlo de pragas de Diptera, Lepidoptera e Coleoptera na agricultura durante muitos anos. Este trabalho descreve o projecto da sequência genómica de B. thuringiensis S908, que contém os genes cry1B, Vpa, Vpb e Sip. Foram identificados os genes que codificam a bacteriocina, a melhoria, a via de promoção do crescimento das plantas e a tirosinase.

Palavras-chave: toxinas de grito, vip toxinas, ngs, bioinformática.

1 INTRODUCTION

Bacillus thuringiensis (Bt) is a Gram-positive bacterium that produces proteins like *cry1B, Vpa*, *Vpb* and *Sip* with a wide variety of insecticidal properties against insects. These microbial insecticides have been used for decades as pest control agents and they represent an alternative to chemical pesticides in a modern agriculture (1). Bt crystal (Cry) proteins damage the midgut post ingestion and have been used successfully for management of other insect pests. While individual Cry toxins are generally toxic to a particular order of insects, collectively they exhibit activity across orders, particularly the Lepidoptera, Diptera and Coleoptera (2).

Purified genomic and plasmid DNA`s from strain S908 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 239 contigs totaling 6,736,357 bp (Q20 = 99.23%), with a maximum scaffold size of 402,268 bp, an N_{50} length of 99,59 bp, and 34.57% 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 7,080 coding sequences (4), 96 tRNA (5), 7 rRNA operons (6), and 5 ncRNA were predicted. It was identified 10 plasmid ranging from 585,993 bp to 2,061 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*, *vpa/vpb*, *sip*, *cyt*, growth promotion, parasporin 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/). The strain S908 draft genome sequence carries three insecticidal toxin genes showing identities to the *cry1Ba1*, *Vpb1Bc1*, *Vpa2Bb4* and *Sip1Aa1*. The genes which encode the metabolic pathway of plant growth promotion were identified. The genomic analyses could identify the operon related to the antimicrobial peptide bacteriocin (nisin and thuricin 4A), and one gene which encodes the tyrosinase. No parasporin gene was identified. Although this strain possesses the tyrosinase gene in its genome, the strain did not show any black colony during its growing in culture. This strategy was useful to identify the *Vpa* and *Vpb* genes isolated in the genome once *Vpa/Vpb* genes can be identified in the *B. thuringiensis* genomes isolated or in operon. Using the new nomenclature of proteins toxic to insects proposed by Crickmore et al. (11) we found *Mpp5A* and *Spp1A* genes. This strain showed potential to control *A. grandis* showed a LC_{50} 233 ug.mL^{-1} and CI with P≤0.05 (140-348 ug.mL^{-1}) using methodology described by Martins et al (12).

Nucleotide sequence accession numbers. The sequence of the *B. thuringiensis* strain S908 has been deposited in GenBank with the accession number SAMN12206555.

ACKNOWLEDGMENT

We would like to thank Dr. Lilian Praça for the technical assistance.

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