Draft Genome Sequence of *Bacillus thuringiensis* Strain 907, an Isolate Toxic for Coleopteran

Projecto de Sequência Genómica de *Bacillus thuringiensis* Strain 907, um Tóxico Isolado para Coleopteran

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Paulo Queiroz
Doutorado em Biologia Animal pela Universidade de Brasília (UNB)
Institution: Centro Universitário de Brasília (CEUB)
Address: 707/907, Campus Universitário, Asa Norte, Brasília, DF, CEP: 70790-075
E-mail: prqzqueiroz@gmail.com

Roberto Togawa
Doutorado pela University of Bedfordshire
Institution: Embrapa Recursos Genéticos e Biotecnologia
Address: Parque Estação Biológica, PqEB, Av. W5 Norte (final) Caixa Postal 02372, Brasília, DF, CEP: 70770-917
E-mail: roberto.togawa@embrapa.br

Priscila Grynberg
Doutorado em Bioinformática pela Universidade Federal de Minas Gerais (UFMG)
Institution: Embrapa Recursos Genéticos e Biotecnologia
Address: Parque Estação Biológica, PqEB, Av. W5 Norte (final) Caixa Postal 02372, Brasília, DF, CEP: 70770-917
E-mail: priscila.grynberg@embrapa.br

Érica Martins
Doutorado em Ciências Biológicas (Biologia Molecular) pela Universidade de Brasília
Institution: Universidade de Brasília
Address: SBN Quadra 02 Bloco C, Edifício Phenícia, CEP: 70040-020
E-mail: es.martins2@gmail.com

Rose Monnerat
Doutorado em Agronomia na Ecole Nacionale Agronomique de Montpellier
Institution: Embrapa Recursos Genéticos e Biotecnologia
Address: Parque Estação Biológica, PqEB, Av. W5 Norte (final) Caixa Postal 02372, Brasília, DF, CEP: 70770-917
E-mail: rose.monnerat@embrapa.br

ABSTRACT

Bacillus thuringiensis is an important bactéria which shows insecticide action effective to control several Lepidoptera like Anticarsia gemmatalis, Helicoverpa armigera, Plutella xylostella and S. frugiperda, and Coleoptera like A. grandis. This work describes the draft genome sequence of B. thuringiensis S907, which contains the genes cry1Ba1, Vpb1Bc1,

Vpa2Bb4 and Sip1Aa1. Genes coding for bacteriocin, plant growth promotion pathway and tyrosinase were identified.

Keywords: cry toxin, boll weevil, ngs.

RESUMO
Bacillus thuringiensis é uma bactéria importante que mostra uma acção insecticida eficaz para controlar vários Lepidoptera como Anticarsia gemmatalis, Helicoverpa armigera, Plutella xylostella e S. frugiperda, e Coleoptera como A. grandis. Este trabalho descreve o projecto da sequência genómica de B. thuringiensis S907, que contém os genes cry1Ba1, Vpb1Bc1, Vpa2Bb4 e Sip1Aa1. Foram identificados os genes que codificam a bacteriocina, a via de promoção do crescimento das plantas e a tirosinase.

Palavras-chave: cry toxin, boll weevil, ngs.

1 INTRODUÇÃO

Bacillus thuringiensis (Bt) is a bacteria that produce insecticidal proteins showing entomopathogenic properties useful to be extensively in agriculture to protect plants against (1). Among the different insecticidal proteins produced by Bt, the Cry toxins are the most widely used (2).

Purified genomic and plasmid DNA`s from strain S907 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 233 contigs totaling 6,779,822 bp (Q20 = 99.35%), with a maximum scaffold size of 528,564 bp, and N_{50} length of 99,735 bp, and 33.72% 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 7,088 coding sequences (4), 72 tRNA (5), 3 rRNA operons (6) and 5 ncRNA were described. It was identified 13 plasmids wich sizes ranged from 585,452 bp to 5,380 bp. The chromosome was sized in approx. 5,295,017 bp long. 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 aminoacid sequences of *cry1Ba1*, *Vpb1Bc1*, *Vpa2Bb4* and *Sip1Aa1*, 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 S907 draft genome sequence carries insecticidal toxin genes showing identities to the *cry1Ba1*, *Vpb1Bc1*, *Vpa2Bb4* and *Sip1Aa1*. The genes which encode the metabolic pathway of plant growth promotion 1-aminocyclopropane-1-carboxylate (ACC) deaminase, acid phosphatase, indole pyruvate decarboxylase (ipdC) and siderophore biosynthesis protein 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/Vpb* genes isolated in the genome once *vip* 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 *Mpp5Aa1* and *Spp1Aa1*. This strain showed potential to control *A. grandis* showed a LC_{50} 195 ug.mL^{-1} and CI with P≤0.05 (43-369 ug.mL^{-1}) using methodology described by Martins et al. (12).

Nucleotide sequence accession numbers. The sequence of the *B. thuringiensis* strain S907 has been deposited in GenBank with the accession number SAMN12200924.

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