



## Short Genome Communications

Complete genome sequence of the nematocidal *Bacillus thuringiensis* MYBT18247

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## ARTICLE INFO

## Keywords:

*Bacillus thuringiensis*

Genome sequence

Nematicidal

Crystal toxins

Evolution

## ABSTRACT

The Gram-positive spore forming bacterium *Bacillus thuringiensis* MYBT18247 encodes three *cry* toxin genes, (*cry6Ba2*, *cry6Ba3* and *cry21*-like) which are active against nematodes. For a better understanding of the evolution of virulence and *cry* toxins, we present here the complete genome sequence of *Bacillus thuringiensis* MYBT18247. Various additional virulence factors such as bacteriocins, proteases and hemolysins were identified. In addition, the methylome and the metabolic potential of the strain were analyzed and the strain phylogenetically classified.

*Bacillus thuringiensis* is a ubiquitous, Gram-positive, spore-forming, bacterium (Schnepf et al., 1998). Strains of the species are used as a biopesticide because of their ability to produce parasporal protein crystals (Bechtel and Bulla, 1976; Ibrahim et al., 2010). These protein crystals consist of  $\delta$ -endotoxins which are active against a broad spectrum of invertebrates including species of the orders Lepidoptera, Diptera, Coleoptera, Hymenoptera, Homoptera, Orthoptera, Mallophaga as well as mites, protozoa and nematodes (Feitelson, 1993; Schnepf et al., 1998). Here we present the annotated genome sequence of *B. thuringiensis* MYBT18247 that was isolated and used for single as well as multiple infection co-evolution experiments within the nematode *Caenorhabditis elegans* (Masri et al., 2015; Schulte et al., 2010).

Genomic DNA was isolated using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) and the Genomic-Tip 100/G Kit (Qiagen, Hilden, Germany). For 454 pyrosequencing the genomic DNA was sheared (~700 bp), end repaired and universal barcoded sequencing adaptors were ligated (Rapid-GSFLX Titanium, Roche 454, Branford CT). The library preparation was done with the GS Titanium Sequencing Kit XLR70t (Roche 454, Branford CT). Illumina sequencing DNA libraries were generated with the Nextera XT DNA Library Prep Kit (Illumina, San Diego, USA). For SMRT-sequencing (Pacific Biosciences, Menlo Park, USA) the C2/P4 chemistry was applied for three SMRT-cells and C4/P6 chemistry were used for two additional SMRT-cells, respectively. Whole-genome

sequencing was performed using the 454 GS-FLX instrument, the Genome analyzer Ilx (Illumina, San Diego, USA) and the PacBio RSII system (Pacific Biosciences, Menlo Park, USA). The 454 shotgun sequencing produced 335,141 single-end reads with an average read length of 420 bp. The Newbler 2.8 *de novo* assembler (Roche Diagnostics) assembled the reads into 411 contigs with a coverage of 18 x. A hybrid assembly was performed with Mira 4.0.3 (<http://mira-assembler.sourceforge.net/docs/DefinitiveGuideToMIRA.html>) by using 4,000,000 (112 bp) paired-end Illumina reads and 30,952 PacBio reads (C2-chemistry) with an average read length of 5053 bp. An HGAP 2.3.0 assembly using 67,045 PacBio-reads (P6-chemistry) with a mean length of 13,802 bp resulted in an average coverage of 124.46 x. The assemblies were manually combined and contradictions were resolved by Sanger sequencing using BigDye 3.0 chemistry and an ABI3730XL capillary sequencer (Applied Biosystems, Life Technology GmbH, Darmstadt, Germany). All sequence positions were manually checked using Gap4 (v4.11) of the Staden package (Staden et al., 1999) to ensure the sequence quality.

Annotation was conducted with Prokka v.1.9 (Seemann, 2014). The initial annotation was performed using *Bacillus thuringiensis* Bt407 as a high quality species reference (Sheppard et al., 2013) and a comprehensive toxin protein database (including all Cry, Cyt, Vip, Sip toxins) as a feature reference set. The annotations of detected *cry* toxin genes were manually corrected and confirmed by Crickmore and deposited at

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**Table 1**  
Genome statistics of *Bacillus thuringiensis* MYBT18247.

Genome Feature	Value
Genome size (bp)	6,138,199
DNA coding (bp)	5,143,010
DNA G + C (bp)	2,159,739
DNA scaffolds	7
Total genes	6366
Protein coding genes	6210
RNA genes	156
rRNA genes	42
Genes in internal clusters	536
Genes with function prediction	4767
Genes assigned to COG	1443
Genes assigned to Mobilome	268
Genes with Pfam domains	5054
Genes with signal peptides	424
Genes with transmembrane helices	1692
CRISPR repeats	–

[http://www.bionomenclature.info/\(Crickmore et al., 1998\)](http://www.bionomenclature.info/(Crickmore et al., 1998)).

The genome size of *B. thuringiensis* MYBT18247 is 6,138,199 bp with an average GC-content of 35% (Table 1). The data set consists of a chromosome of 5.6 Mbp and six plasmids which vary in size from 12.5 kb to 175 kb. One plasmid contig of 130 kb could not be circularized due to large repetitive regions at both contig ends. The chromosome encodes 6210 protein-coding and 156 RNA genes, including 14 rRNA clusters (5S, 16S, 23S rRNA). The plasmids encode 556 protein coding genes. There were 3727 genes assigned to COG database (Table 2) and 1114 genes were identified in MetaCyc metabolic pathways (Caspi et al., 2016). Three nematocidal cry toxin genes have been identified on plasmid p174778 (*cry6Ba2*; BTI247\_60340), p113275 (*cry6Ba3*, BTI247\_62380) and p15092 (*cry21*-like, BTI247\_64000) (Table 3).

*B. thuringiensis* MYBT18247 comprises a plethora of virulence factors which might all contribute to pathogenicity. The chromosome encodes two chitinases, which enable the degradation of chitin in the midgut peritrophic membrane of many insects and have been identified as insect virulence factors (Sampson and Gooday, 1998). Additionally,

**Table 2**  
COG categories of *B. thuringiensis* MYBT18247.

Letter	Name	Count	Percent [%]
E	Amino acid transport and metabolism	365	8.83
G	Carbohydrate transport and metabolism	227	5.49
D	Cell cycle control, cell division, chromosome partitioning	57	1.38
N	Cell motility	52	1.26
M	Cell wall/membrane/envelope biogenesis	216	5.22
B	Chromatin structure and dynamics	1	0.02
H	Coenzyme transport and metabolism	213	5.15
Z	Cytoskeleton	2	0.05
V	Defense mechanisms	111	2.69
C	Energy production and conversion	194	4.69
W	Extracellular structures	4	0.1
S	Function unknown	295	7.14
R	General function prediction only	368	8.9
P	Inorganic ion transport and metabolism	226	5.47
U	Intracellular trafficking, secretion, and vesicular transport	32	0.77
F	Lipid transport and metabolism	138	3.34
F	Nucleotide transport and metabolism	126	3.05
O	Posttranslational modification, protein turnover, chaperones	156	3.77
L	Replication, recombination and repair	190	4.6
Q	Secondary metabolites biosynthesis, transport and catabolism	89	2.15
T	Signal transduction mechanisms	185	4.48
K	Transcription	364	8.81
J	Translation, ribosomal structure and biogenesis	255	6.17
	Not in COG	2907	42.92

**Table 3**  
Predicted virulence factors of *B. thuringiensis* MYBT18247.

Virulence factor	No. of proteins	Locus_Taq	Location
Cry toxins	3		
pesticidal crystal protein <i>Cry6Ba2<sup>a</sup></i>		BTI247_60340	p174778
pesticidal crystal protein <i>Cry6Ba3<sup>a</sup></i>		BTI247_62380	p113275
putative pesticidal crystal protein <i>Cry21</i> -like <sup>a</sup>		BTI247_64000	p15092
Bacteriocins	4		
Bacteriocin > 10 kD (bacteriocinIII) <sup>b</sup>			chromosome
LAP (linear azol(ine) containing peptides) <sup>b</sup>			chromosome
Head to tail cyclized bacteriocin <i>Ild<sup>b</sup></i>			p174778
Head to tail cyclized bacteriocin <i>Ild<sup>b</sup></i>			p81952
Proteases			
Bacillolysin	4		
<i>npr1<sup>c</sup></i>		BTI247_07440	chromosome
<i>npr2<sup>c</sup></i>		BTI247_24310	chromosome
<i>nprM<sup>c</sup></i>		BTI247_29880	chromosome
<i>npr3<sup>c</sup></i>		BTI247_36120	chromosome
Collagenases	6		
<i>colA1<sup>c</sup></i>		BTI247_06950	chromosome
<i>colA2_1<sup>c</sup></i>		BTI247_32780	chromosome
<i>colA4<sup>c</sup></i>		BTI247_36420	chromosome
<i>colA3<sup>c</sup></i>		BTI247_37900	chromosome
<i>colA5<sup>c</sup></i>		BTI247_46580	chromosome
<i>colA2_2<sup>c</sup></i>		BTI247_59250	chromosome
Immune inhibitor A	5		
<i>ina1_1<sup>c</sup></i>		BTI247_08150	chromosome
<i>ina1_2<sup>c</sup></i>		BTI247_08170	chromosome
<i>ina2_1<sup>c</sup></i>		BTI247_15830	chromosome
<i>ina3<sup>c</sup></i>		BTI247_32580	chromosome
<i>ina2_2<sup>c</sup></i>		BTI247_33630	chromosome
Phospholipases	4		
<i>cerA<sup>c</sup></i>		BTI247_08230	chromosome
phospholipase, patatin family		BTI247_22620	chromosome
<i>plcA<sup>c</sup></i>		BTI247_37570	chromosome
<i>ypA<sup>c</sup></i>		BTI247_50630	chromosome
Chitinases	2		
<i>chiA<sup>c</sup></i>		BTI247_05680	chromosome
<i>chiD<sup>c</sup></i>		BTI247_39810	chromosome
Camelysins	4		
<i>calY1_1<sup>c</sup></i>		BTI247_07820	chromosome
<i>calY1_2<sup>c</sup></i>		BTI247_15800	chromosome
<i>calY1_3<sup>c</sup></i>		BTI247_59500	p174778
<i>calY1_4<sup>c</sup></i>		BTI247_63500	p81952
N-acyl homoserine lactonase	1		
<i>AiiA<sup>c</sup></i>		BTI247_36860	chromosome
Enterotoxins			
Hemolysins			
Hemolysin BL			
<i>hblA1_1<sup>c</sup></i>		BTI247_21380	chromosome
<i>hblA2<sup>c</sup></i>		BTI247_21390	chromosome
<i>hblD<sup>c</sup></i>		BTI247_26830	chromosome
<i>hblA3<sup>c</sup></i>		BTI247_26840	chromosome
<i>hblA4_1<sup>c</sup></i>		BTI247_26850	chromosome
<i>hblA4_2<sup>c</sup></i>		BTI247_26880	chromosome
<i>hblA1_2<sup>c</sup></i>		BTI247_60570	p130548
<i>hblA1_3<sup>c</sup></i>		BTI247_61620	p130548
Hemolysin II	3		
<i>hly_1<sup>c</sup></i>		BTI247_37800	chromosome
<i>hly_2<sup>c</sup></i>		BTI247_59850	p174778
<i>hly_3<sup>c</sup></i>		BTI247_63990	p15092
Hemolysin III	2		
<i>hlyIII_1<sup>c</sup></i>		BTI247_24750	chromosome

(continued on next page)

Table 3 (continued)

Virulence factor	No. of proteins	Locus_Taq	Location
<i>hlyIII</i> 2 <sup>c</sup>		BTI247_58090	chromosome
Hemolysin A <i>thyA</i> 2 <sup>c</sup>	1	BTI247_44490	chromosome
Gamma hemolysin <i>hlgB</i> 2 <sup>c</sup>	1	BTI247_13860	Chromosome
Non-hemolysins	4		
<i>nhe_1</i> 2 <sup>c</sup>		BTI247_21370	chromosome
<i>nhe_2</i> 2 <sup>c</sup>		BTI247_60560p	p130548
<i>nhe_3</i> 2 <sup>c</sup>		BTI247_61630	p130548
Alveolysin 2 <sup>c</sup>		BTI247_54170	chromosome

<sup>a</sup> all protein sequences of the genome were scanned with generic HMM models constructed from representative sequences of the known Cry/Cyt/VIP-toxins extracted from SwissProt and the Bt toxin database (<http://www.btmomenclature.info/>). Identified sequences were characterized by a procedure described by the international committee for cry-Toxins (Crickmore et al., 1998), verified by Crickmore and deposited at the BT toxin database.

<sup>b</sup> AntiSMASH3.0.5 (Weber et al., 2015) and BAGEL3 (van Heel et al., 2013) were used for identification.

<sup>c</sup> Predicted by Prodigal (Hyatt et al., 2010) and annotated by BLAST+ (Camacho et al., 2009) comparisons to reference-proteins from the Prokka genome annotation pipeline (Seemann, 2014). Annotation was primarily derived from *Bacillus thuringiensis* Bt407 as a high quality species reference (Sheppard et al., 2013).

proteases including four camelysins, six collagenases, and four phospholipases were identified and may play an important role in activating protoxins (Nisnevitch et al., 2010), destruction of the intestine of *C. elegans* (Peng et al., 2016) and in hydrolyzing phospholipids of host cell membranes (Hergenrother and Martin, 1997). Furthermore, five immune inhibitor A metalloproteases, four bacillolysins and an N-acyl homoserine lactonase were detected, suspicious for boosting the nematocidal or insecticidal activity, as well as overcoming the host immune system by cleaving host antibacterial peptides (Fedhila et al., 2002; Park et al., 2008; Raymond et al., 2010). The host gut microbiome is part of the immune response and bacterial secondary metabolites such as bacteriocins and microcins are able to suppress other pathogenic microbes. In the *B. thuringiensis* MYBT18247 genome eleven biosynthetic clusters including bacteriocins, siderophores, NRPS (non-ribosomal peptide synthetases) and terpene clusters were detected with AntiSMASH3.0.5 (Weber et al., 2015) (Table 4). Four potential bacteriocins were further validated with BAGEL3 (van Heel et al., 2013) (Table 3). In the chromosome a putative bacteriocin class III and a LAP (linear azol(ine) containing peptide) were classified. In the plasmids p174778 and p81952 one head to tail cyclized bacteriocin class IId (PF09221) were identified. The cyclic bacteriocins are active against a broad spectrum of Gram-positive and Gram-negative bacteria (Finn et al., 2016). *B. thuringiensis* MYBT18247 encodes as well genes for hemolysis and non-haemolytic enterotoxins (Table 3), which have an important effect during the infection of hosts (Argolo-Filho and

Loguercio, 2014; Kim et al., 2015). However, a test on Columbia agar revealed a hemolytic negative phenotype. This might correlate to the observation that the corresponding genome locus misses the hemolysin BL lytic component L2, one essential part of the tripartite toxin. The plasmids encode a number of genes dedicated to genome fluidity such as transposases, insertion sequences, transcriptional regulators and recombinases.

The metabolic versatility of *B. thuringiensis* MYBT18247 has been evaluated using BIOLOG Phenotypic Microarrays (PM1-PM2). The strain utilized substrates assigned feeding into glycolysis, citrate cycle and pentose phosphate pathway. It metabolizes various carbon sources including sugars, sugar alcohols and sugar acids which have been found in fruits, fungi, insect compartments or plant compartments. Apparently, the strain is a generalist which is able to adapt to various ecological niches.

The methylome analysis of *B. thuringiensis* MYBT18247 was performed using the SMRT Portal v2.3.0 analysis platform. Four methylated N<sup>6</sup>-methyladenine (m6A) motifs were observed. The non-palindromic motif (CRTANNNNNNNRTTNC/GNAAYNNNNNNNTAYG) was found to be methylated in more than 65% of all instances. Additionally, three N4-methylcytosin (m4C) motifs were detected with a methylation grade of 6–21% and in four putative motifs the identification of the methylation was not possible due to insufficient coverage. The SMRT data DNA methyltransferase recognition motifs are deposited in REBASE at [http://rebase.neb.com/rebase/private/pacbio\\_Liesegang23.html](http://rebase.neb.com/rebase/private/pacbio_Liesegang23.html) (Roberts et al., 2015).

Phylogenetic classification of *B. thuringiensis* MYBT18247 within the *Bacillus cereus sensu lato* group, was performed by multi-locus-sequence typing (MLST) according to Priest et al. (Priest et al., 2004) (Fig. 1). The strain clusters within a subgroup, comprising *B. thuringiensis* MYBT18246, *B. thuringiensis* YBT-1518, *B. thuringiensis* Bt407, and *B. thuringiensis* serovar *chinensis* CT-43. Strikingly, all members of the cluster except *B. thuringiensis* 407, which is an artificially cured laboratory strain, encode at least one nematocidal or insecticidal cry toxin gene.

Summarized, *B. thuringiensis* MYBT18247 comprises a variety of promising genes, including the rare cry6Ba which show high potential for the development of new biotechnological relevant nematocidal and insecticidal control agents.

## Nucleotide sequence accession numbers

The whole genome sequence has been deposited at the DDBJ/EMBL/GenBank with the accession numbers CP015250.1-CP015256.1. The strain is available from DSMZ (Braunschweig, Germany) under accession 104068.

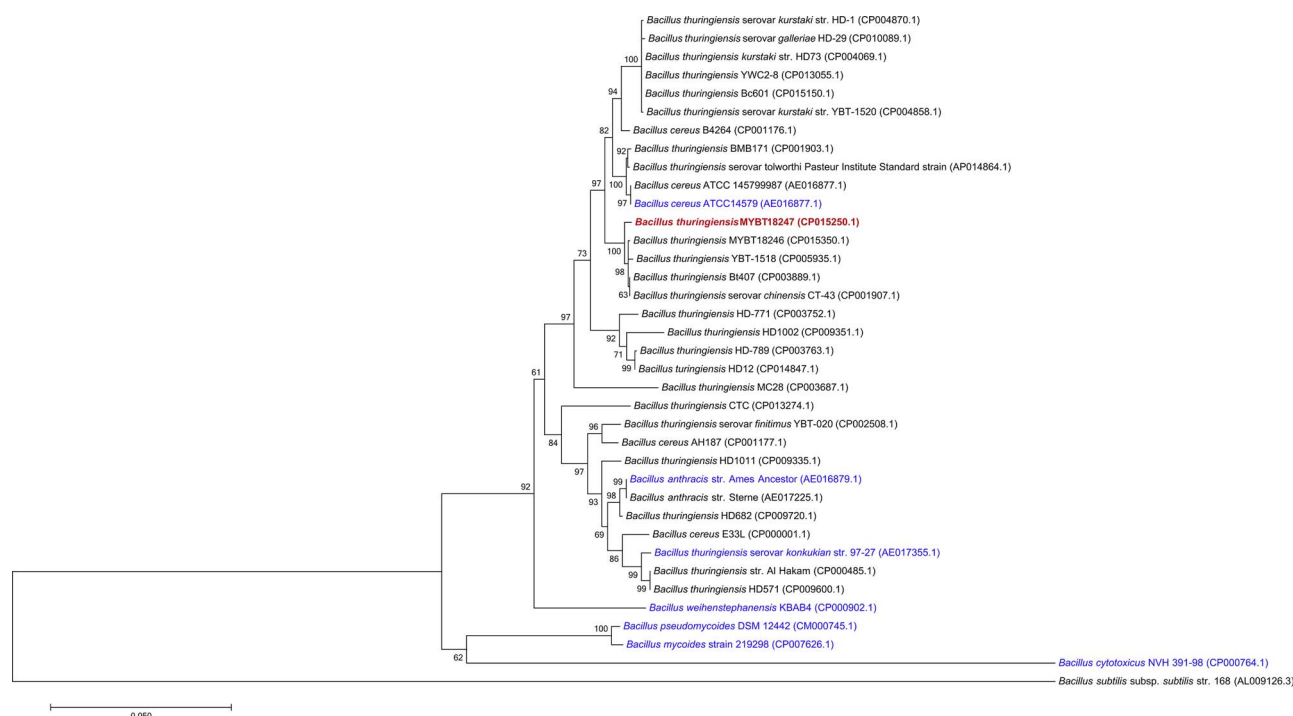
## Acknowledgements

We thank Dr. Andrea Thürmer, Nicole Heyer and Simone Severitt for sequencing support. This project was funded by the German

Table 4

Secondary metabolite clusters identified in *B. thuringiensis* MYBT18247 with antiSMASH3.0.

Cluster	Type	Location	Start	Stop	Similarity to known cluster	MIBiG BGC-ID
1	Other	chromosome	496,393	539,974	–	–
2	Bacteriocin	chromosome	1,444,119	1,457,984	–	–
3	Siderophore	chromosome	2,120,707	2,134,414	Petrobactin biosynthetic gene cluster (83% of genes show similarity)	BGC0000942_c1
4	NRPS	chromosome	2,433,678	2,483,403	Bacillibactin biosynthetic gene cluster (46% of genes show similarity)	BGC0000309_c1
5	Bacteriocin	chromosome	2,666,725	2,677,045	–	–
6	NRPS	chromosome	2,736,144	2,783,151	–	–
7	Bacteriocin	chromosome	2,800,158	2,810,424	–	–
8	NRPS	chromosome	3,472,892	3,532,849	–	–
9	Terpene	chromosome	3,659,931	3,681,784	Molybdenum cofactor biosynthetic gene cluster (11% of genes show similarity)	BGC0000916_c1
10	Bacteriocin	p174778	132,176	142,397	–	–
11	Bacteriocin	p81952	56,165	66,488	–	–



**Fig. 1.** Phylogenetic tree highlighting the taxonomic relation of *B. thuringiensis* MYBT18247 (red) based on Multi-Locus-Sequence-Typing within the *Bacillus cereus* sensu lato species group. GenBank accession numbers are given in parentheses. Comparison includes representative strains of Bcsl group members (blue). *Bacillus subtilis* subsp. *subtilis* str. 168 has been used as outlier to root the tree. Sequences were aligned using ClustalW 1.6 (Thompson et al., 1994). The phylogenetic tree was constructed by using the Neighbor-Joining method (Saitou and Nei, 1987) and evolutionary distances were computed by the Maximum Composite Likelihood method (Tamura et al., 2004) within MEGA7.0 (Kumar et al., 2016). Numbers at the nodes are bootstrap values calculated from 1000 replicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Research Foundation (DFG-SPP1399, Grant LI 1690/2-1 to HL; SCHU 1415/9 to HS, and RO 2994/3 to PR). We acknowledge support by the German Research Foundation and the Open Access Publication Funds of the University of Goettingen.

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