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BACTERIAL, FUNGAL AND VIRUS MOLECULAR BIOLOGY - SHORT COMMUNICATION

Genome sequencing of *Burkholderia contaminans* LTEB11 reveals a lipolytic arsenal of biotechnological interest

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Abstract

Burkholderia contaminans LTEB11 is a Gram-negative betaproteobacterium isolated as a contaminant of a culture in mineral medium supplemented with vegetable oil. Here, we report the genome sequence of *B. contaminans* LTEB11, identifying and analyzing the genes involved in its lipolytic machinery and in the production of other biotechnological products.

Keywords Lipases · Polyhydroxyalkanoates · Rhamnoplipids · Non-ribosomal peptide synthetases

The genus *Burkholderia* is widely distributed in the environment [1]. It is divided into two well-established clusters: the non-pathogenic cluster, which comprises beneficial plant symbionts, and the pathogenic cluster, which comprises opportunistic human, animal, and plant pathogens [1]. The species *Burkholderia contaminans* was described in 2009 and received this name because it was isolated as a contaminant of a Sargasso Sea DNA sample [2]. Currently, *B. contaminans* is classified in the *Burkholderia cepacia* complex (BCC), a group of at least 18 species that infect immunocompromised individuals, especially sufferers of cystic fibrosis [3, 4].

Despite their role as disease agents, *B. contaminans* and other BCC species have biotechnological applications. For example, they have been applied as biocontrol and bioremediation agents [5], for the production of biosurfactants [6] and for the production of extracellular lipases [7].

B. contaminans LTEB11 is a Gram-negative betaproteobacterium isolated in our laboratory as a contaminant of a fungal culture in mineral medium supplemented with vegetable oil. It was previously classified, erroneously, as both *B. cepacia* and *B. lata*. This strain produces an extracellular lipase (LipBC) that is active and highly stable in media containing organic solvents [8, 9]. Indeed, we have produced LipBC by submerged fermentation and by solid-state fermentation and applied it in esterification and transesterification reactions for biodiesel synthesis [10–12] and resolution of racemates [13]. Recently, genes encoding lipase LipBC (*lipA*) and foldase LifBC (*lipB*) were identified and co-expressed in *Escherichia coli*, with a recombinant LipLifBC complex being purified and characterized [14]. However, little is known about the genome of this bacterium and whether it might have other biotechnological applications. Here, we report the genome sequence of *B. contaminans* LTEB11, identifying and analyzing the genes involved in its lipolytic machinery.

Genomic DNA was isolated using phenol-chloroform extraction [15]. The whole-genome sequencing was performed on two different platforms: MiSeq Illumina (2,698,078 paired-end reads, 250 bp) and Ion Proton System (5,661,193 fragments, 125 bp long). The sequence data were de novo assembled using CLC Genomics Workbench 6.5.1 [16], Velvet 1.2.07 [17] and Masurca 2.3.2 [18] and the final assembly was optimized and finished using GFinisher [19]. The average nucleotide identity (ANI) [20] was calculated by a script developed by Kostas's lab (http://enve-omics.gatech.edu/). BLASTn comparison of genomes was visualized by BRIG

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Table 1. Features of the genome of *Burkholderia contaminans* LTEB11

	Size (bp)	Contigs	Protein-coding genes[a]	tRNAs	G + C content (%)
Chromosome 1	3,548,326	4	3366	56	66.7
Chromosome 2	3,254,142	2	3074	6	66.6
Chromosome 3	1,196,160	1	1113	3	65.8
Total	7,998,628	7	7553	65	66.3

[a] Note that the number of genes presented in Table 1 is different from the number of genes annotated in the genome in GenBank (accession number GCA_001865715.1). For the present paper, we annotated the genome using the RAST annotator, which considers pseudogenes in the final gene count. The PGAP annotator applied by GenBank disregards pseudogenes

[21]. Coding sequences (CDS) and open reading frames (ORFs) were predicted using the RAST server [22]. Phylogenetic analysis was carried out with the neighbor-joining method and bootstrapping (1000 replicates) was used to estimate the confidence levels of phylogenetic reconstructions [23].

The *B. contaminans* LTEB11 genome was assembled in 7 contigs organized in three replicons of 3,548,326 bp (chromosome 1), 3,254,142 bp (chromosome 2), and 1,196,160 bp (chromosome 3) (Table 1). The estimated genome size is 7.9 Mb and the GC content is 66.5%. This genome size falls within the range of 7.4 to 9.7 Mb described for the genus *Burkholderia* [24]. *Burkholderia contaminans* LTEB11 showed ANI values greater than 97% when compared with *B. contaminans* strains LMG 23361, FFI-28, FFH2055, and MS14 (Table S1). Also, the highest nucleotide sequence identity of *B. contaminans* LTEB11 was for *B. lata* FL7530S1D0 (95%) and *B. lata* 383 (94%), values that indicate high genome relatedness (Fig.1).

In total, 7553 protein-coding genes were predicted (Table 1). Sixty genes were annotated as coding for α/β hydrolases, with 17 of these being classified as esterases or lipases, organized in different superfamilies (Table 2). Among these sequences, the *lipAB* operon, which codes for the lipase LipA (LipBC) and the lipase-specific foldase LipB (LifBC), was annotated in chromosome 2. In addition, we identified

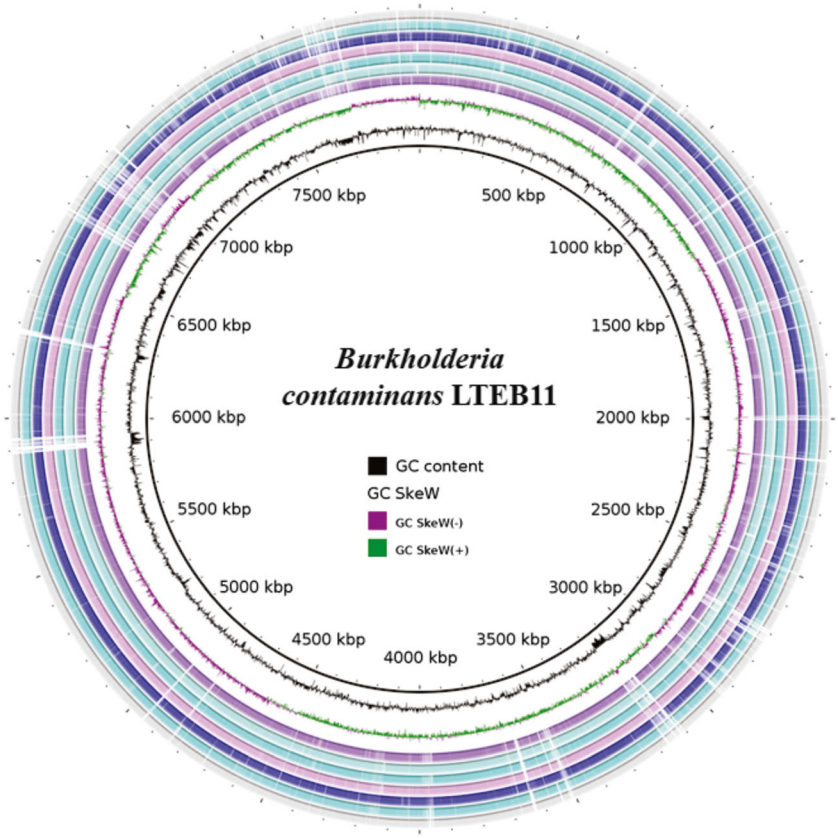

Fig. 1 Circular representation of the genome of *B. contaminans* LTEB11 and comparison with the whole-genome sequences of seven *Burkholderia* strains. Rings from the inside to outside: [1] GC content (black), [2] GC skew (purple and green), [3] BLASTn comparison with *B. contaminans* MS14, [4] BLASTn comparison with *B. contaminans* LGM23361, [5] BLASTn comparison with *B. contaminans* FFH2055, [6] BLASTn comparison with *B. contaminans* FFI-28, [7] BLASTn comparison with *B. lata* FL7530S1D0, [8] BLASTn comparison with *B. lata* 383, and [9] BLASTn comparison with *B. cepacia* ATCC 25416

Table 2. Lipase and esterase genes identified in the genome of *Burkholderia contaminans* LTEB11 and compared with those of other strains of *B. contaminans* and other species of *Burkholderia*

B. contaminans LTEB11				*B. contaminans* MS14		*B. contaminans* LMG 23361		*B. cepacia* ATCC 25416		*B. lata* 383		*B. lata* FL7530S1D0	
Gene	COG	Pfam	Accession number	%	Accession number	%	Accession number	%	Accession number	%	Accession number	%	Accession number
lip01(LipA)	1075	00561	WP_071332898.1	99	WP_047850286.1	99	WP_039358205.1	96	WP_027791104.1	98	WP_011355767.1	96	WP_069251115.1
lip02(LipE)	1075	00561	WP_083417736.1	–	NI	–	NI	69	WP_034196290.1	–	NI	88	WP_083265862.1
lip03(LipC)	–	03583	WP_071333792.1	98	WP_047851367.1	99	WP_039340439.1	96	WP_027790279.1	98	WP_011353700.1	–	NI
lip04(LipD)	0657	07859	WP_071335730.1	99	WP_047853081.1	99	WP_039355306.1	95	WP_027792157.1	97	WP_011350355.1	94	WP_069249857.1
lip05 -	1647	00561	WP_071333461.1	25	WP_047850525.1	98	WP_039344411.1	95	WP_027790064.1	98	WP_011356666.1	97	WP_069250458.1
lip06	0657	07859	WP_039367881.1	99	WP_047851233.1	100	WP_039367881.1	99	WP_027790468.1	99	WP_011353908.1	99	WP_069252570.1
lip07	0657	07859	WP_071332108.1	98	WP_047849544.1	99	WP_039366558.1	95	WP_027789698.1	94	WP_011354223.1	96	WP_069252334.1
lip08	0657	07859	WP_071332532.1	97	WP_047849908.1	98	WP_039367521.1	93	WP_027791693.1	95	WP_041493289.1	40	WP_069248845.1
lip09	0657	07859	WP_071332595.1	98	WP_047849999.1	99	WP_039367460.1	94	WP_027791591.1	95	WP_011355223.1	96	WP_069251569.1
lip10	0657	07859	WP_071334485.1	97	WP_047850871.1	99	WP_039354955.1	92	WP_027787386.1	95	WP_041492832.1	95	WP_069247987.1
lip11	0657	07859	WP_071334944.1	99	WP_047851135.1	98	WP_039367198.1	97	WP_021162652.1	96	WP_011352337.1	97	WP_069248161.1
lip12	0657	07859	WP_071335074.1	99	WP_047852215.1	99	WP_039360143.1	97	WP_027789139.1	98	WP_011353179.1	98	WP_069248845.1
lip13	0657	07859	WP_071335986.1	98	WP_047853319.1	98	WP_039369974.1	–	NI	–	NI	–	NI
lip14	0657	07859	WP_071336069.1	38	WP_047851135.1	38	WP_039367460.1	34	WP_027790074.1	38	WP_011356710.1	39	WP_069251569.1
lip15	3545	06821	WP_071334327.1	98	WP_046546320.1	98	WP_039347442.1	95	WP_027787757.1	95	WP_011351717.1	97	WP_069247743.1
lip16	3545	06821	WP_039346467.1	99	WP_047852299.1	100	WP_039346467.1	97	WP_027788972.1	99	WP_011353407.1	98	WP_069249030.1
lip17	3150	05728	WP_071333903.1	98	WP_047851640.1	99	WP_039353030.1	97	WP_021161885.1	99	WP_011350884.1	99	WP_069247086.1
lipB	5380	03280	WP_071332897.1	97	WP_047850285.1	99	WP_039358208.1	93	WP_027791105.1	94	WP_011355766.1	97	WP_069251116.1
lipF	5380	03280	WP_071334273.1	–	NI	–	NI	–	NI	–	NI	72	WP_069247680.1

[%] Represents identities of amino acid sequences. *COG*, clusters of orthologous group; *Pfam*, protein families database; *Accession number*, National Center for Biotechnology Information. *NI*, not identified

Table 3. Lipolytic activity of the crude extracts obtained from *B. contaminans* LTEB11 and recombinant LipA (LipBC) against triacylglycerols

	Lipolytic activity (U mg^{-1})[a]	
Substrate	Crude extract	LipA
Tributyrin	90 ± 14	1330 ± 86
Olive oil	44 ± 8	790 ± 29

[a] The activity was determined by the titrimetric method using a pHStat, at pH 8.0 and 37 °C. Results are expressed as the average of triplicate assays ± the standard error of the mean

another operon, *lipEF*, in chromosome 1, with this operon coding for a lipase (LipE) and a foldase (LipF) that have 65% and 53% of identity, respectively, with LipA and LipB. Sequence analysis showed that LipA, LipE, and two more lipases (LipC and LipD) have the typical *N*-terminal signal sequence, suggesting that these lipases may be secreted by *B. contaminans* LTEB11. The genes encoding a Sec-translocase complex as well as a type II secretion system (T2SS) were annotated in chromosome 1. These systems are required for the secretion of lipases by Gram-negative bacteria [25].

Comparative analysis showed that LipA, LipC, and LipD are also present in genomes of other isolates of *B. contaminans* (FFH2055, LMG 23361, MS14, FFI-28, and FFH 2055) and *B. lata* (383 and FL7530S1D0). However, in these genomes, the operon *lipEF* described here was only identified in *B. lata* FL (Table 2). Phylogenetic analysis classified LipA and LipE in the family I.2 of bacterial lipases, next to lipases of *B. glumae*, *B. lata*, and *C. viscosum*, whereas LipC and LipD were classified close to I.3 family members (Fig. S1).

In order to evaluate the lipolytic activity of *B. contaminans* LTEB11, a crude extract was obtained by submerged fermentation using olive oil 1% (*v*/*v*) as an inducer for lipase expression [9]. In addition, recombinant LipA (LipBC) was overexpressed in *Escherichia coli* and purified according to Alnoch et al. [14]. The activities of both crude extract and recombinant LipA were determined by the titrimetric method using a pHStat (as described in the Supplementary material). The crude extract of *B. contaminans* LTEB11 showed higher activity (90 U mg^{-1}) against tributyrin than against olive oil (44 U mg^{-1}) (Table 3). The same profile was observed for the recombinant LipA, with activities of 1330 U mg^{-1} against tributyrin and 790 U mg^{-1} against olive oil (Table 3). These results suggest that *B. contaminans* LTEB11 secretes LipA into the medium; however, other lipases or esterases might be produced and secreted during the cultivation. The high activity presented in the crude extract of *B. contaminans* LTEB11 and shown by recombinant LipA suggests that it would be interesting to characterize further the lipases and esterases produced by *B. contaminans* LTEB11.

The genome of *B. contaminans* LTEB11 also contains the *pha* genes, *phaC*, *phaA*, *phaB*, coding for enzymes involved in the biosynthesis of polyhydroxyalkanoates (PHA); *phaR*, coding for the transcriptional regulator (PhaR) of the phasin gene *phaP*; and *phaZ*, the gene encoding the PHA

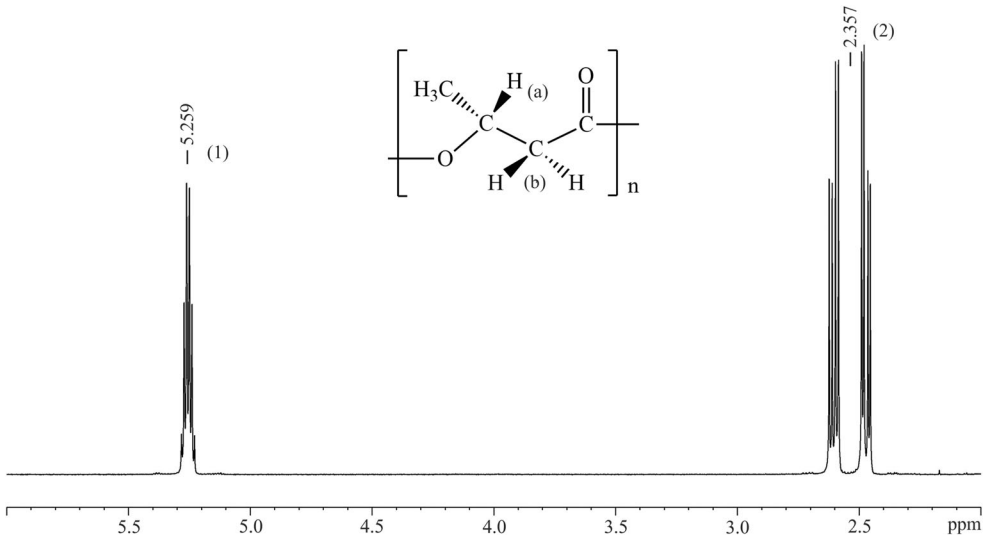

Fig. 2 [1]H-NMR spectrum of 3-hydroxybutyrate (P3HB) produced by *Burkholderia contaminans* LTEB11. (1) The multiplet at 5.25 ppm corresponds to 1H (a) in the asymmetric carbon; (2) The doublet of the quadruplet at 2.35 ppm corresponds to 2H (b) in the methylene group adjacent to an asymmetric carbon atom. PHB samples (10 mg) were dissolved in $CDCl_3$ and subjected to analysis. [1]H-NMR spectra were acquired for each sample at 600 MHz using an AscendTM 600 spectrometer (Bruker) equipped with a 5-mm QXI inverse probe and a sample case autosampler. PHB accumulation assays were performed according to Matias et al. [28]. Flask cultures containing 500 mL of liquid ISP9 medium (2% (*w*/*v*) of glucose) were incubated in a shaker at 30 °C for 72 h, 120 rpm. Culture samples were harvested by centrifugation, lyophilized, and pretreated with two acetone baths and treated with chloroform at 60 °C for 48 h under agitation. After the treatment, the contents of flasks were filtered through Whatman no. 1 filter paper and dried at room temperature until PHB film formation

depolymerase involved in PHA mobilization (Table S2) [26]. PHAs are classified, according to the carbon chain length of the monomers, as either short-chain or medium-chain, the best known PHAs being polyhydroxybutyrate (PHB) and the co-polymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [27]. PHB accumulation assays were performed according to Matias et al. [28], involving 72 h of cultivation in liquid ISP9 medium containing 2% (*w*/*v*) of glucose. After chloroform extraction, only poly-3-hydroxybutyrate (P3HB) was identified by [1]H-nuclear magnetic resonance (NMR) (Fig. 2).

The genome of *B. contaminans* LTEB11 also contains genes that code for enzymes required for the synthesis of rhamnolipids (*rhlA*, *rhlB*, and *rhlC*), biosurfactants of particular interest for cosmetic, pharmaceutical, and detergent manufacturers [29]. The genome also contains the *ocf* gene cluster that has been previously described in *B. contaminans* MS14, with an identity greater than 90% (Table 4). This cluster includes the ATP-binding cassette (*ocfA*) and the genes encoding non-ribosomal peptide synthetases (*ocfD*, *ocfE*, *ocfF*, *ocfH*, and *ocfJ*). These genes are required for the production of the antifungal compound occidiofungin, which is active against a broad range of plant and animal fungal pathogens [3].

The *B. contaminans* LTEB11 genome sequence reported here can underpin further studies into the production of new lipases and esterases and mechanisms involved in the regulation of lipase expression, as well as the potential of this bacterium to produce polyhydroxyalkanoates, rhamnolipids, and antifungal compounds with biotechnological relevance.

Table 4. Comparison of genes encoding for occidiofungin biosynthesis in *Burkholderia contaminans* LTEB11 and *B. contaminans* MS14

Burkholderia contaminans LTEB11		*Burkholderia contaminans* MS14	
Gene	Accession number	%[a]	Accession number
Orf1 -	WP_071335792.1	98	ACN32485.1
ambR1	WP_071335793.1	98	ACN32486.1
ambR2	WP_071335794.1	95	ACI01437.2
ocfA	WP_071335795.1	100	ACJ24909.2
ofcB	WP_071336295.1	97	ACL81525.1
ofcC	WP_039355063.1	99	ACL81526.1
ofcD	WP_071335796.1	98	ACL81527.1
ofcE	WP_083417853.1	98	ACL81528.1
ofcF	WP_071335798.1	99	ACN32487.1
ofcG	WP_071335799.1	95	ACN32488.1
ofcH	WP_071335800.1	99	ACN32489.1
ofcI	WP_071335801.1	98	ADT64845.1
ofcJ	WP_071335802.1	97	ADT64846.1
ofcK	WP_071335803.1	97	ADT64847.1
ofcL	WP_071335804.1	99	ADT64848.1
ofcM	WP_039362393.1	98	ADT64849.1
ocfN	WP_071335805.1	96	ADT64850.1

[a] Represents identities of amino acid sequences. *Accession number*, National Center for Biotechnology Information

Nucleotide sequence accession numbers This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession MLFG00000000 and BioSample: SAMN04287748.

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