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Complete genome sequence of *Leptotrichia buccalis* type strain (C-1013-b^T)

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Keywords

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Abstract

Leptotrichia buccalis (Robin 1853) Trevisan 1879 is the type species of the genus, and is of phylogenetic interest because of its isolated location in the sparsely populated and neither taxonomically nor genomically adequately accessed family '*Leptotrichiaceae*' within the phylum '*Fusobacteria*'. Species of *Leptotrichia* are large fusiform non-motile, non-sporulating rods, which often populate the human oral flora. *L. buccalis* is anaerobic to aerotolerant, and saccharolytic. Here we describe the features of this organism, together with the complete genome sequence and annotation. This is the first complete genome sequence of the order '*Fusobacteriales*' and no more than the second sequence from the phylum '*Fusobacteria*'. The 2,465,610 bp long single replicon genome with its 2306 protein-coding and 61 RNA genes is a part of the *Genomic Encyclopedia of Bacteria and Archaea* project.

Introduction

Leptotrichia buccalis strain C-1013-b^T (DSM 1135 = ATCC 14201 = JCM 12969, and other strain collections) is the type strain of the species [1], which also represents the type species of the genus first adequately and validly described in 1879 by Trevisan to accommodate the oral filamentous bacteria and to separate them from the algae [2, 3]. For a while two entirely different organisms have been termed *L. buccalis* in the literature [3]. One of these was '*Leptothrix buccalis*', a name originally employed by Robin in 1853 for filamentous forms which he had seen in wet mounts of tooth scrapings [4]. Over a century of the history of classification and misclassification of *L. buccalis* was documented by Gilmore *et al.* 1961 [3]. *L. buccalis* was among the first bacteria to be described and drawn in the letters of Antoni van

Leeuwenhoek [5]. Next to *Fusobacterium nucleatum* [6], *L. buccalis* is only the second species from the phylum *Fusobacteria* for which a complete genome sequence is described.

Here we present a summary classification and a set of features for *L. buccalis* strain C-1013-b^T (Tab. 1), together with the description of the complete genomic sequencing and annotation.

Classification and features of organism

The primary habitat of *L. buccalis* and most other *Leptotrichia* species is the human oral cavity, especially dental plaque, or the female genitourinary tract and the intestinal tract [7, 8]. Although *L. buccalis* and *L. buccalis*-like bacteria have also occasionally been recovered from blood mostly in immunocompromised patients, they are not known as causative agents of systemic infections [7, 9] even if an endotoxin was documented for the *L. buccalis* [5, 9]. Almost all of the cultivated *Leptotrichia* isolates cluster in 16S rRNA sequence comparisons with one of the five other type strains of the genus *Leptotrichia* [7, Fig. 1]. Except for the uncultured clone GI5-008-C04 (FJ192568), which has been recovered from screening of a spacecraft assembly clean room during the Phoenix mission, all significantly related phylotypes were from the usual habitats as described above. No phylotypes from environmental screening or genomic surveys could be linked with more than 85% 16S rRNA sequence similarity to *L. buccalis* (status May 2009).

Fig. 1 shows the phylogenetic neighborhood of *L. buccalis* strain C-1013-b^T in a 16S rRNA based tree. The sequences of the five 16S rRNA gene copies in the genome of strain C-1013-b^T differ from each other by 5 to 20 nucleotides (up to 1.3%), and by 4 to 16 nucleotides plus 38 ambiguities (total up to 3.6%) from the previously published 16S rRNA sequence (X90831) generated from NCTC 10429.

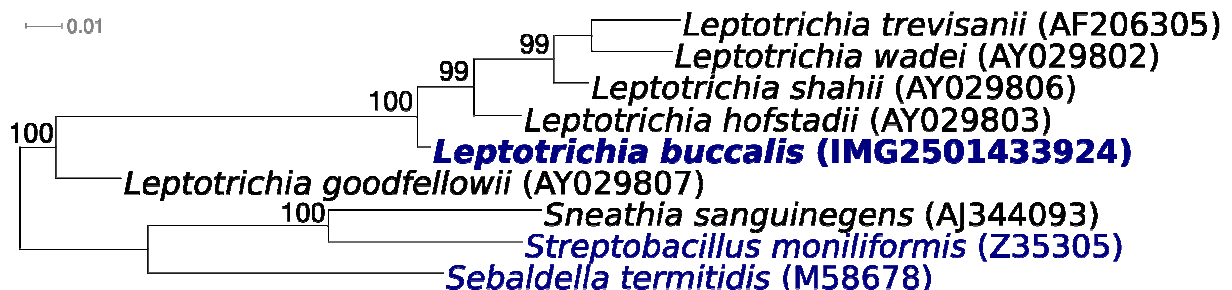
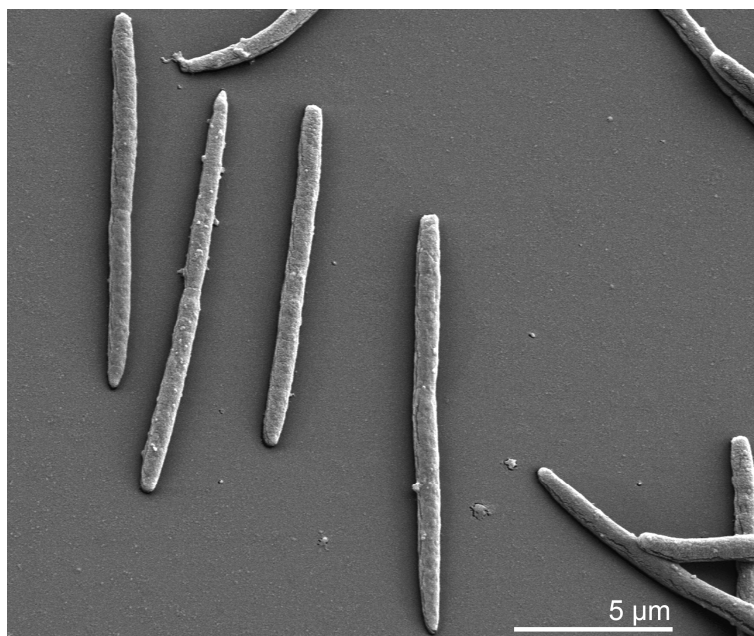


Figure 1. Phylogenetic tree highlighting the position of *L. buccalis* C-1013-b^T relative to all type strains of the genus *Leptotrichia* inferred from 1421 aligned characters [10, 11] of the 16S rRNA sequence under the maximum likelihood criterion [12], and rooted with all type strains of the family *Leptotrichiaceae*. The branches are scaled in terms of the expected number of substitutions per site. Numbers above branches are support values from 1000 bootstrap replicates if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [13] are shown in blue, published genomes in bold.

Older cells of *L. buccalis* strain C-1013-b^T are gram negative, but younger cells that have been in culture for less than six hours show Gram-positiveness [5]. The organism forms long rods, commonly occurring in pairs, and is non-motile [5, Fig. 2]. Young colonies are colourless, smooth, shiny, raised and described as “medusa-head” colonies because of filamentous edges [5]. On first isolation, *L. buccalis* is anaerobic but becomes aerotolerant upon transfer and grows in the presence of air and CO₂ [5, 8]. *L. buccalis* is susceptible to many antibiotics but resistant to aminoglycosides [5]. The organism is highly saccharolytic and ferments a range of

different sugars [5, 8]. The main metabolic end product is lactic acid [8]. The G+C content was already in 1982 described as ‘unusually low’ (25 %) [5].

Figure 2. Scanning electron micrograph of *L. buccalis* stain C-1013-b^T (M. Rohde, HZI Braunschweig)



Chemotaxonomy. The murein of strain C-1013-b^T contains *meso*-2,6-diaminopimelic acid (A₂pm), D- and L-alanine, and D-glutamic acid [14]. The strain possesses muramic acid and glucosamine as principal components of its peptidoglycan [14]; typeA1γ according to the classification of Schleifer and Kandler [15]. As in other *Leptotrichia* strains, the fatty acid pattern of *L. buccalis* is an almost equal mixture of saturated and unsaturated straight chain acids: C_{16:0} (39%), C_{14:0} (10%), C_{18:1} (42%), with about 7% hydroxy acids (C_{14:0}) [7]. The type of menaquinones and polar lipids used by *L. buccalis* has not been described yet.

Table 1. Classification and general features of *L. buccalis* strain C-1013-b^T in accordance to the MIGS recommendations [16]

| MIGS ID | Property | Term | Evidence code ^{a,b} |
|---------|------------------------|--------------------------------------|------------------------------|
| | | Domain <i>Bacteria</i> | |
| | | Phylum ' <i>Fusobacteria</i> ' | |
| | | Class ' <i>Fusobacteria</i> ' | |
| | Current classification | Order ' <i>Fusobacteriales</i> ' | |
| | | Suborder | |
| | | Family ' <i>Leptotrichiaceae</i> ' | |
| | | Genus <i>Leptotrichia</i> | TAS [2] |
| | | Species <i>Leptotrichia buccalis</i> | TAS [2] |
| | | Type strain C-1013-b | TAS [1] |
| | Gram stain | negative | TAS [5] |
| | Cell shape | long rods | TAS [5] |
| | Motility | nonmotile | TAS [5] |
| | Sporulation | nonsporulating | TAS [5] |

| | | | |
|----------|------------------------|--|----------|
| | Temperature range | mesophile | NAS |
| | Optimum temperature | 37°C | NAS |
| | Salinity | normal | NAS |
| MIGS-22 | Oxygen requirement | anaerobic on isolation, becomes aerotolerant on further transfer | TAS [5] |
| | Carbon source | mono- and disaccharides | TAS [5] |
| | Energy source | carbohydrates | NAS |
| MIGS-6 | Habitat | oral cavities | TAS [5] |
| MIGS-15 | Biotic relationship | Free living | |
| MIGS-14 | Pathogenicity | opportunistic pathogen | TAS [5] |
| | Biosafety level | 1 | TAS [17] |
| | Isolation | human oral flora | TAS [7] |
| MIGS-4 | Geographic location | global | NAS |
| MIGS-5 | Sample collection time | Mid of 19 th century | TAS [3] |
| MIGS-4.1 | Latitude – Longitude | not reported | |
| MIGS-4.2 | | | |
| MIGS-4.3 | Depth | not reported | |
| MIGS-4.4 | Altitude | not reported | |

- a) Evidence codes - IDA: Inferred from Direct Assay (first time in publication); TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from <http://www.geneontology.org/GO.evidence.shtml> of the Gene Ontology project [18]. If the evidence code is IDA, then the property should have been directly observed, for the purpose of this specific publication, for a live isolate by one of the authors, or an expert or reputable institution mentioned in the acknowledgements.

Genome sequencing information

Genome project history

This organism was selected for sequencing on the basis of its phylogenetic position, and is part of the *Genomic Encyclopedia of Bacteria and Archaea* project. The genome project is deposited in the Genomes OnLine Database [13] and the complete genome sequence in GenBank NOT YET. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI). A summary of the project information is shown in Table 2.

Table 2. Genome sequencing project information

| MIGS ID | Property | Term |
|-----------|----------------------|---|
| MIGS-31 | Finishing quality | Finished |
| MIGS-28 | Libraries used | Three genomic libraries: two Sanger libraries - 8 kb pMCL200 and fosmid pcc1Fos and one 454 pyrosequence standard library |
| MIGS-29 | Sequencing platforms | ABI3730, 454 GS FLX |
| MIGS-31.2 | Sequencing coverage | 9.7x Sanger; 42x pyrosequence |
| MIGS-30 | Assemblers | Newbler version 1.1.02.15, phrap |

| | | |
|---------|-------------------------|--------------------|
| MIGS-32 | Gene calling method | Prodigal |
| | INSDC / Genbank ID | not yet available |
| | Genbank Date of Release | not yet available |
| | GOLD ID | Gi02240 |
| | NCBI project ID | 29445 |
| | Database: IMG-GEBA | 2501416906 |
| | Project relevance | Tree of Life, GEBA |

Growth conditions and DNA isolation

L. buccalis strain C-1013-b^T, DSM 1135, was grown anaerobically in DSMZ medium 104 (<http://www.dsmz.de>) at 37°C. DNA was isolated from 1-1.5 g of cell paste using Qiagen Genomic 500 DNA Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions, but with a modified protocol for cell lysis, using more lysozyme (1.6x) and an extended incubation time (1 hour).

Genome sequencing and assembly

The genome was sequenced using a combination of Sanger and 454 sequencing platforms. All general aspects of library construction and sequencing performed at the JGI can be found at <http://www.jgi.doe.gov/>. 454 pyrosequencing reads were assembled using the Newbler assembler version 1.1.02.15 (Roche). Large Newbler contigs were broken into 2,747 overlapping fragments of 1000bp and entered into assembly as pseudo-reads. The sequences were assigned quality scores based on Newbler consensus q-scores with modifications to account for overlap redundancy and to adjust inflated q-scores. A hybrid 454/Sanger assembly was made using the parallel phrap assembler (High Performance Software, LLC). Possible mis-assemblies were corrected with Dupfinisher or transposon bombing of bridging clones [19]. Gaps between contigs were closed by editing in Consed, custom primer walk or PCR amplification. 908 Sanger finishing reads were produced to close gaps, to resolve repetitive regions, and to raise the quality of the finished sequence. The error rate of the completed genome sequence is less than 1 in 100,000. Together all sequence types provided 51.7 x coverage of the genome. The final assembly contains 28,754 Sanger reads in addition to the 454 based pseudo reads.

Genome annotation

Genes were identified using Prodigal [20] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using JGI's GenePRIMP pipeline (<http://geneprimp.jgi-psf.org>) [21]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and functional annotation was performed within the Integrated Microbial Genomes (IMG-ER) platform (<http://img.jgi.doe.gov/er>) [22].

Genome properties

The genome is 2,345,610 bp long and comprises one circular chromosome with a 29.7% GC content (Tab. 3). Of the 2367 genes predicted, 2306 were protein coding genes, and 61 RNAs; 86 pseudogenes were also identified. 65.4% of the genes were assigned with a putative function while the remaining are annotated as hypothetical proteins. The distribution of genes into GOGs functional categories is presented in Table 4.

Table 5. Genome Statistics

| Attribute | Value | % of Total |
|----------------------------------|--------------|-------------------|
| Genome size (bp) | 2,465,610 | |
| DNA Coding region (bp) | 2,139,206 | 86.76% |
| DNA G+C content (bp) | 730,947 | 29.65% |
| Number of replicons | 1 | |
| Extrachromosomal elements | 0 | |
| Total genes | 2367 | 100.00% |
| RNA genes | 61 | 2.58% |
| rRNA operons | 5 | |
| Protein-coding genes | 2306 | 97.42% |
| Pseudo genes | 86 | 3.63% |
| Genes with function prediction | 1547 | 65.36% |
| Genes in paralog clusters | 402 | 16.98% |
| Genes assigned to COGs | 1533 | 64.77% |
| Genes assigned Pfam domains | 1577 | 66.62% |
| Genes with signal peptides | 432 | 18.25% |
| Genes with transmembrane helices | 530 | 22.39% |
| CRISPR repeats | 4 | |

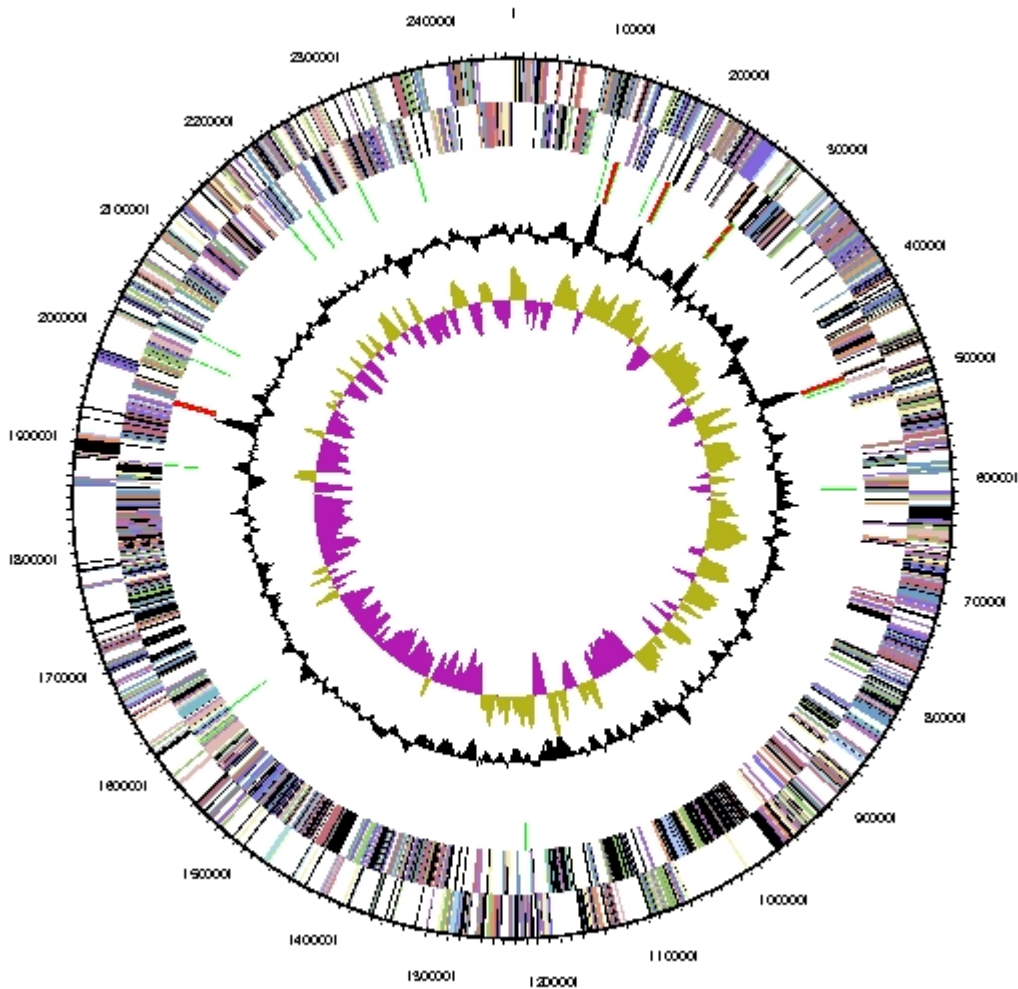


Figure 3. Graphical circular map of the genome. From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, sRNAs red, other RNAs black), GC content, GC skew.

Table 4. Number of genes associated with the 21 general COG functional categories

| Code | COG counts and percentage of protein-coding genes | | Description |
|------|---|------------|---|
| | Genome value | % of total | |
| J | 144 | 6.2 | Translation, ribosomal structure and biogenesis |
| A | 0 | 0.0 | RNA processing and modification |
| K | 86 | 3.7 | Transcription |
| L | 124 | 5.4 | Replication, recombination and repair |
| B | 0 | 0.0 | Chromatin structure and dynamics |
| D | 24 | 1.0 | Cell cycle control, mitosis and meiosis |

| | | | |
|---|-----|------|--|
| Y | 0 | 0.0 | Nuclear structure |
| V | 28 | 1.2 | Defense mechanisms |
| T | 47 | 2.0 | Signal transduction mechanisms |
| M | 112 | 4.9 | Cell wall/membrane biogenesis |
| N | 6 | 0.3 | Cell motility |
| Z | 0 | 0.0 | Cytoskeleton |
| W | 0 | 0.0 | Extracellular structures |
| U | 34 | 1.5 | Intracellular trafficking and secretion |
| O | 68 | 3.0 | Posttranslational modification, protein turnover, chaperones |
| C | 79 | 3.4 | Energy production and conversion |
| G | 110 | 4.8 | Carbohydrate transport and metabolism |
| E | 176 | 7.6 | Amino acid transport and metabolism |
| F | 54 | 2.3 | Nucleotide transport and metabolism |
| H | 80 | 3.5 | Coenzyme transport and metabolism |
| I | 44 | 1.9 | Lipid transport and metabolism |
| P | 84 | 3.6 | Inorganic ion transport and metabolism |
| Q | 11 | 0.5 | Secondary metabolites biosynthesis, transport and catabolism |
| R | 206 | 8.9 | General function prediction only |
| S | 141 | 6.1 | Function unknown |
| - | 773 | 28.1 | Not in COGs |

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