Complete genome sequence of *Propionibacterium freudenreichii* DSM 20271T

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Abstract

*Propionibacterium freudenreichii* subsp. *freudenreichii* DSM 20271T is the type strain of species *Propionibacterium freudenreichii* that has a long history of safe use in the production dairy products and B12 vitamin. *P. freudenreichii* is the type species of the genus *Propionibacterium* which contains Gram-positive, non-motile and non-sporeforming bacteria with a high G + C content. We describe the genome of *P. freudenreichii* subsp. *freudenreichii* DSM 20271T consisting of a 2,649,166 bp chromosome containing 2320 protein-coding genes and 50 RNA-only encoding genes.

Keywords: +*Propionibacterium*, Type strain, Dairy starter, B12 vitamin

Introduction

Strain DSM 20271T (= van Niel 1928T = ATCC 6207) is the type strain of species *Propionibacterium freudenreichii*, which is the type species of its genus *Propionibacterium* [1]. There are traditionally two groups described in *Propionibacterium* genus; the “classical” or “dairy” and the “cutaneous” propionibacteria. *P. freudenreichii* belongs to the dairy group and is divided into two subspecies on the basis of lactose fermentation and nitrate reductase activity. The DSM 20271T strain represents the *P. freudenreichii* subsp. *freudenreichii* distinguished from subsp. *shermanii* by nitrate reduction and by a lack of lactose fermentation. [1]. Dairy propionibacteria do not belong to human microbiota but can be isolated from various habitats including raw milk, dairy products, soil and fermenting food and plant materials such as silage and fermenting olives [1]. Strains of *P. freudenreichii* have a long history of safe use in human diet and for instance in the production of Swiss-type cheeses, in which they play central role as ripening starters [1, 2]. Industrial applications of *P. freudenreichii* include production of vitamin B12 (cobalamin), as well as several other biomolecules like propionic acid, trehalose and conjugated linoleic acid [3]. Recently, there has been growing interest to study *P. freudenreichii* for its probiotic properties. Complete genome sequence of the type strain *P. freudenreichii* subsp. *shermanii* CIRM-BIA1 has been reported [4], but lack of other complete genome sequences has prevented the genomic level comparisons between the two subspecies. Thus, the genomic analysis of DSM 20271T strain should help us in *P. freudenreichii* subspecies definition that has been under debate [4, 5].

Here we present a summary classification and a set of features for *P. freudenreichii* DSM 20271T, together with the description of the complete genome sequence and annotation.

Organism information

Classification and features

*P. freudenreichii* subsp. *freudenreichii* DSM 20271 T is a Gram-positive, non-motile, non-sporulating, anaerobic to aerotolerant, mesophilic *Actinobacteria* belonging to the order *Propionibacterium*. The strain was originally isolated as one of the three propionic acid-producing strains from Emmental cheese by von Freudenreich and Orla-Jensen as *Bacterium acidi propionici a* [6] during their work in Bern, Switzerland [7]. The strain was further studied by van Niel and renamed to *Propionibacterium freudenreichii* [6]. Figure 1 shows the phylogenetic neighborhood of DSM 20271T in a 16S rRNA sequence
based tree. Cells of DSM 20271<sup>T</sup> are short rods with length of approximately 1.5 μm (Fig. 2). According to API 50 CH (Biomerieux, France) carbohydrate fermentation test the growth of DSM 20271<sup>T</sup> is supported by carbon sources including glucose, fructose, mannose, glycerol, adonitol, inositol, erythritol and galactose (Table 1).

**Genome sequencing information**

**Genome project**

This organism was selected for sequencing on the basis of its importance in food fermentations and in metabolite production.

**Growth conditions and genomic DNA preparation**

The strain was grown to early stationary growth phase in propionic medium (PPA), composed of 5.0 g. tryptone (Sigma-Aldrich), 10.0 g. yeast extract (Becton, Dickinson), 14.0 ml 60 % w/w DL-sodium lactate (Sigma-Aldrich) per liter and pH adjusted to 6.7. The cells were harvested by centrifugation for 5 min at 21,000 g at 4 °C and washed once with 0.1 M Tris–HCl pH 8.0. The DNA extraction was performed with ILLUSTRA™ bacteria genomicPrep Mini Spin Kit (GE Healthcare) according to the manufacturer’s instruction for Gram-positive bacteria using

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**Table 1** Classification and general features of *Propionibacterium freudenreichii* subspecies *freudenreichii* DSM20271<sup>T</sup> according to the MIGS recommendations [24]

| MIGS ID | Property                        | Term                                         | Evidence code<sup>a</sup> |
|---------|---------------------------------|----------------------------------------------|---------------------------|
|         | Classification                  | Domain Bacteria                              | TAS [25]                  |
|         | Phylum                          | Actinobacteria                               | TAS [26, 27]              |
|         | Class                           | Actinobacteria                               | TAS [26, 27]              |
|         | Order                            | Propionibacteriales                          | TAS [28]                  |
|         | Family                          | Propionibacteriaceae                         | TAS [1]                   |
|         | Genus                            | Propionibacterium                            | TAS [1, 29]               |
|         | Species                         | *Propionibacterium freudenreichii* subspecies *freudenreichii* | TAS [1, 29, 30] |
|         | (Type) strain: van Niel 1928<sup>T</sup>, (DSM 20271<sup>T</sup> = ATCC 6207) |                               |                           |
|         | Gram stain                      | Positive                                      | TAS [1]                   |
|         | Cell shape                      | Rod                                           | TAS [1]                   |
|         | Motility                        | Non-motile                                    | TAS [1]                   |
|         | Sporulation                     | Not reported                                  | NAS                       |
|         | Temperature range                | Mesophile                                     | TAS [1]                   |
|         | Optimum temperature             | 30 °C                                         | TAS [1]                   |
|         | pH range; Optimum               | ~4.5–8; ~7                                    | NAS                       |
|         | Carbon source                   | Glucose, fructose, mannose, glycerol, adonitol, inositol, erythritol, galactose | IDA                       |

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<sup>a</sup>Evidence codes - IDA inferred from direct assay, 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 the Gene Ontology project [31]
100 mg/ml of lysozyme (Sigma-Aldrich) and 30 min incubation time in the lysis step.

Genome sequencing and assembly
The complete finished genome sequence of *P. freudenreichii* strain DSM 20271<sup>T</sup> was generated at the Institute of Biotechnology, University of Helsinki, using Pacific Biosciences RS II sequencing platform [8](Table 2). One standard PacBio 10 kb library was constructed and sequenced using two SMRTCells with 180 min runtime on the RS II instrument, which generated 145,463 reads totaling up to 608.98 Mbp. For the assembly, the data was filtered using default HGAP parameters. The resulting 130,046 reads totaling up to 557.87 Mbp were used to generate the initial genome sequence. 498.74 Mbp of the filtered data mapped to the assembled genome afterwards. The assembled genome sequence was generated using SMRTAnalysis (2.1.0), HGAP2. For the annotation, 100 mg/ml of lysozyme (Sigma-Aldrich) and 30 min incubation time in the lysis step.

### Genome annotation
Genes were identified using Prodigal v2.50 tool [11] with manual curation in ARGO Genome Browser [12]. The predicted genes were translated and functionally annotated with description lines, Gene Ontology (GO) classes and Enzyme Commission (EC) numbers with PANNZER program [13] using UniProtKB, Enzyme and GOA databases. PfamA domains were identified using InterProScan 48.0 [14], transmembrane helices and signal peptides were found with TMHMM [15] and SignalP [16], respectively. Clusters of Orthologous Groups (COG) assignments were done by using CD-Search [17]. The tRNAscanSE tool [18] was used to identify tRNA genes and ribosomal RNA were predicted with RNAmmer v1.2 [19].

### Genome properties
The circular genome of *Propionibacterium freudenreichii* subsp. *freudenreichii* DSM 20271<sup>T</sup> is 2,649,166 nucleotides with 67.34 % GC content (Table 3) and contains one finalized chromosome with no plasmids. From total number of 2370 genes 2320 (97.9 %) are protein coding and 50 (2.1 %) are RNA genes (Fig. 3). 91.80 % of all proteins were functionally annotated whilst the remaining genes were annotated as “functionally unknown putative proteins”. The distribution of genes into COGs functional categories is presented in Table 4. Three sequence motifs containing methylated bases were also detected in the genome by PacBio sequencing and SMRTAnalysis Modification and Motif detection protocol. Two of these motifs, 5′-GG<sub>A</sub>NNNNNNNNCTT-3′ and 5′-A<sub>A</sub>GNNNNNNNTCC-3′, are partner motifs and correspond to same modifications on different strands with m<sub>6</sub>A as a modified base on 3rd and 2nd position respectively. The modified base is

| Table 2 | Genome sequencing project information for *Propionibacterium freudenreichii* DSM 20271<sup>T</sup> |
|---------|--------------------------------------------------|
| MIGS ID | Property                                          |
| MIGS 31 | Finishing quality                                | Finished |
| MIGS-28 | Libraries used                                   | One PacBio 10 kb standard library |
| MIGS 29 | Sequencing platforms                            | PacBio RS II |
| MIGS 31.2 | Fold coverage                                    | 198x |
| MIGS 30 | Assemblers                                       | SMRTAnalysis (2.1.0), HGAP2 |
| MIGS 32 | Gene calling method                              | Prodigal v2.50 |
| Locus Tag | RM25                                             |
| Genbank ID | CP010341                                          |
| GenBank Date of Release | February 1<sup>st</sup> 2015 |
| GOLD ID | Gs0113908                                         |
| BIOPROJECT | PRJNA269789                                       |
| MIGS 13 | Source Material Identifier                       | DSM 20271<sup>T</sup> |
| Project relevance | Type strain, dairy starter, B12 vitamin |

| Table 3 | Genome statistics |
|---------|-------------------|
| Attribute | Value | % of Total |
| Genome size (bp) | 2,649,166 | 100.00 |
| DNA coding (bp) | 2,321,778 | 87.64 |
| DNA G + C (bp) | 1,783,838 | 67.34 |
| DNA scaffolds | 1 | 100.00 |
| Total genes | 2353 | 100.00 |
| Protein coding genes | 2320 | 97.90 |
| RNA genes | 50 | 2.10 |
| Pseudo genes | NA | NA |
| Genes in internal clusters | NA | NA |
| Genes with function prediction | 2160 | 91.80 |
| Genes assigned to COGs | 1751 | 74.42 |
| Genes with Pfam domains | 1958 | 83.21 |
| Genes with signal peptides | 113 | 4.80 |
| Genes with transmembrane helices | 577 | 24.52 |
| CRISPR repeats | 0 | 0 |
shown in bold. Each motif is found 664 times in the genome and the marked bases are methylated in all of the 1328 motifs. The structure of the motifs and similarity to existing methyltransferases in REBASE [20] suggests that this is a Type I restriction-modification (RM) system. The third modified motif detected in the genome is 5′-TCGWGA-3′ which partners with itself and is found 4258 times in the genome. In 3676 of these motifs (86.3 %) the 5th nucleotide (C) is found to be modified. The type of this modification could not be reliably identified. However, 319 of the detected modifications are identified as $\text{m}_4\text{C}$ although with low confidence. This finding is supported by the similarity of the recognition site to existing methyltransferases found in REBASE [20] and suggests that there is a Type II RM system acting on this motif in the genome. Therefore also the unidentified modifications are probably $\text{m}_4\text{C}$ bases. This data together suggest that there are two active RM systems present in P. freudenreichii. Comprehensive analysis of these RM systems and corresponding methylations requires further study.

**Conclusions**

Prior to this report only a single genome sequence was available for *Propionibacterium freudenreichii*, from the
type strain of *P. freudenreichii* subsp. *shermanii* CIRM-BIA1 [4]. In the present study the first genome sequence of a *P. freudenreichii* subsp. *freudenreichii* strain was described. *P. freudenreichii* is an industrially important species and a rare producer of biologically active form of vitamin B12. Probably the characteristics of *P. freudenreichii* DNA such as high G + C content and regions of repeated sequences have hampered the unraveling the complete genomes of this species. The results presented here indicate that PacBio RS II sequencing platform is well-suited to overcome these potential obstacles. In this study three DNA sequence motifs containing methylated bases were detected. Our future investigations include using this platform for sequencing of several additional strains for establishing core and pan-genomes as well as methylomes to gain understanding of genome structure and evolution of *P. freudenreichii*.

### Abbreviations

PPA: Propionic medium; HGAP: Hierarchical genome-assembly process; RM: Restriction-modification.

### Competing interests

The authors declare that they have no competing interests.

### Authors’ contributions

PV and VP supplied the strain and background information for this project. PA, PV, LP, KS and PD conceived and designed the experiments. PD performed microbiological experiments and DNA isolation. PK, OPS, FT, JK, LP and PA performed the sequencing, assembly experiments and annotation. PK, OPS, PD, KS and PV wrote the manuscript. All authors read and approved the final manuscript.

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