Complete genome sequence of *Sanguibacter keddieii* type strain (ST-74<sup>T</sup>)

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**Abstract**

*Sanguibacter keddieii* is the type species of the genus *Sanguibacter*, the only described genus within the family of *Sanguibacteraceae*. Phylogenetically, this family is located in the neighbourhood of the genus *Oerskovia* and the family *Cellulomonadaceae* within the actinobacterial suborder *Micrococcineae*. The strain described in this report was isolated from blood of apparently healthy cows. 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 family *Sanguibacteraceae*, and the 4,253,413 bp long single replicon genome with its 3735 protein-coding and 70 RNA genes is part of the Genomic Encyclopedia of Bacteria and Archaea project.

**Introduction**

Strain ST-74<sup>T</sup> (DSM 10542 = ATCC 51767 = JCM 11429 = NCIMB 703025 and other collections) is the type strain of the *Sanguibacter keddieii* species, and the type species of the genus *Sanguibacter* [1, Figure 1]. *S. keddieii* strain ST-74<sup>T</sup> was isolated in 1995 by Fernandez-Garayzabal et al. from the blood of apparently healthy dairy cows in Spain [1] as the first member of the genus *Sanguibacter* and the family of *Sanguibacteraceae* [2]. On the basis of 16S rRNA sequence phylogeny, the small (six type strains) family *Sanguibacteraceae* is located in the neighbourhood to the genus *Oerskovia* [3] and the much larger micrococcineal families *Promicromonosporaceae* [2] and *Cellulomonadaceae* [2].

Like strain ST-74<sup>T</sup>, two more type strains from the genus *Sanguibacter* (*S. suarezii* ST-26<sup>T</sup> [1], and *S. inulinus* [4]) have been isolated from blood of cows. The type strains of the other
Sanguibacter species have been isolated from coastal sediment in the Eastern China Sea [5], from surface soil of a ginseng field in South Korea [6], from alpine subnival plants (DQ339590), and from a sea sand sample collected on the Weaver Peninsula on King George Island, Antarctica [7], which may suggest a global ecological versatility of this genus. Only two related but yet uncultivated phylotypes with more than 98.5% 16S rRNA sequence identity were reported from the gastrointestinal tract of pigs (AF371710), and from glacial meltwater at 6350 m on Mount Everest (EU584523), and no significant matches with any 16S rRNA sequences from environmental genomic samples and surveys are reported at the NCBI BLAST server (March 2009).

S. keddieii ST-74T cells are facultatively anaerobic, Gram-positive, short, irregular shaped motile rods [1, Figure 2]. The colonies on tryptose soy agar (TSA, Difco) are circular, convex, with entire edges and yellow in color. Strain ST-74T is Voges-Proskauer negative and does not reduce nitrate. Casein and gelatin are hydrolysed. Cellulose and Tween 80 are not hydrolysed. Acid is produced from a broad range of substrates: α-methyl-D-mannoside, α-methyl-D-glucoside, N-acetylgulcosamine, amygdalin, rhamnose, D-rafinose, glycerol, L-arabinose, ribose, D-xylose, β-methyl-xyloside, galactose, glucose, fructose, D-mannose, rhamnose, arbutin, sorbitol, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, raffinose, glycogen, β-gentibiose, turanose and lyxose [1]. The optimum growth temperature of strain ST-74T is 25-30°C [1]; it grows at 35°C on agar [7] but not at 42°C [1].

Here we present a summary classification and a set of features for S. keddieii ST-74T, (Table 1), together with the description of the complete genomic sequencing and annotation.

Classification and features

Figure 1 shows the phylogenetic neighborhood of S. keddieii strain ST-74T in a 16S rRNA based tree. Analysis of the four 16S rRNA gene sequences in the genome of strain ST-74T indicated that the genes differ by up to two nucleotides from each other, with two of the copies being identical with the previously published 16S rRNA sequence (X87755) generated from DSM 10542.
bootstrap replicates if larger than 60%. Strains with a genome sequencing project registered in GOLD [11] are printed in blue; published genomes in bold.

**Figure 2.** Scanning electron micrograph of *S. keddieii* ST-74\(^T\) (M. Rohde, Helmholtz Centre for Infection Biology, Braunschweig)

Only little is known about the chemotaxonomy of strain ST-74\(^T\). Saturated straight chain and branched chain are the major cellular fatty acids. In strain ST-74\(^T\) the straight chain fatty acids 16:0 (53.3%), 18:0 (10.1%), 14:0 (5.8%) predominate lower amounts of branched chain anteiso-15:0 (11.4%) and iso-16:0 (5.4%) fatty acids. This is in contrast to other species in the genus *Sanguibacter* and in the neighbouring taxa *Oerskonia* and *Cellulomonadaceae*, where branched chain fatty acids dominate [12]. Only traces of unsaturated acids, anteiso-15:1 (1.6%), and no mycolic acids were detected [1], as in the neighbouring taxa *Oerskonia* and *Cellulomonadaceae*. The murein of *S. keddieii* contains L-Lys-Ser-D-Glu, variation A4\(\alpha\) [1], strikingly different from members of the genus *Oerskonia* and in members of the family *Cellulomonadaceae* [1]. Menaquinones are the sole respiratory lipoquinones present, with a partially saturated menaquinone containing nine-isoprene subunits MK-9(H\(_4\)) dominating [1]. The location of the points of unsaturation are in the 2\(^{nd}\) and 3\(^{rd}\) isoprene units, adjacent to the naphthoquinone nucleus (MK-9 (II, III-H\(_4\)), in *O. turbata*. The phospholipid composition has not been reported, but phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, together with phosphoglycolipids have been reported in members of the neighbouring taxa *Oerskonia* and *Cellulomonadaceae* [12].

**Table 1.** Classification and general features of *S. keddieii* ST-74\(^T\) in accordance to the MIGS recommendations [13]
| MIGS ID | Property          | Term                                | Evidence code |
|---------|-------------------|-------------------------------------|---------------|
|         | Domain            | Bacteria                            |               |
|         | Phylum            | Actinobacteria                      |               |
| Current classification | Order            | Actinomycetales                     | TAS [2]       |
|         | Family            | Sanguibacteraceae                   | TAS [2]       |
|         | Genus             | Sanguibacter                        | TAS [14]      |
|         | Species           | Sanguibacter keddieii               | TAS [1]       |
|         | Type strain       | ST-74                               |               |
| Gram stain | positive         |                                     | TAS [1]       |
| Cell shape | short, irregular rods |                                 | TAS [1]       |
| Motility | motile            |                                     | TAS [1]       |
| Sporulation | not reported     |                                     |               |
| Temperature range | mesophilic      |                                     | TAS [1]       |
| Optimum temperature | 25-30°C         |                                     | TAS [1]       |
| Salinity | not reported      |                                     |               |
| MIGS-22 | Oxygen requirement | primarily aerobe; facultatively anaerobic; no nitrate reduction | TAS [1] |
| Carbon source | broad variety of sugars |                             | TAS [1]       |
| Energy source | carbohydrates    |                                     | NAS          |
| MIGS-6  | Habitat           | animal blood                        | TAS [1]       |
| MIGS-15 | Biotic relationship | free living                       | NAS          |
| MIGS-14 | Pathogenicity     | none                                | NAS          |
|         | Biosafety level   | 2                                   | TAS [15]      |
| Isolation | blood of apparently healthy cow |                 | TAS [1]       |
| MIGS-4  | Geographic location |                                 |               |
| MIGS-5  | Sample collection time | before 1995                      | TAS [1]       |
| MIGS-4.1 | Latitude – Longitude | not reported                   |               |
| MIGS-4.2 | 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](http://www.geneontology.org/GO.evidence.shtml) of the Gene Ontology project [16]. 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

#### Genome project history
This organism was selected for sequencing on the basis of each phylogenetic position, and is part of the *Genomic Encyclopedia of Bacteria and Archaea* project. The genome project is deposited in the Genome OnLine Database [11] 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 | 10.4 x Sanger; 20 x pyrosequence                                      |
| MIGS-30  | Assemblers        | Newbler version 1.1.02.15, phrap Genemak 4.6b, tRNAScan-SE-1.23, infernal 0.81 |
| MIGS-32  | Gene calling method | INSDC / Genbank ID N/A                                               |
|          |                   | Genbank Date of Release N/A                                          |
|          |                   | GOLD ID Gi02151                                                      |
|          |                   | Database: IMG-GEBA 2500901759                                        |
|          | Project relevance | Tree of Life, GEBA                                                   |

**Growth conditions and DNA isolation**

*S. keddieii* ST-74, DSM10542, was grown in DSMZ medium 92 (3% trypticase soy broth, 0.3% yeast extract; see http://www.dsmz.de/microorganisms/media_list.php) at 30°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 protocol, but with with an extended (one hour) incubation at 37°C for cell lysis with lysozyme and proteinase K.

**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 4,746 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 [17] or transposon bombing of bridging clones (Epicentre Biotechnologies, Madison, WI). Gaps between contigs were closed by editing in Consed, custom primer walk or PCR amplification. 2,397 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 30.4 x coverage of the genome.

**Genome annotation**

Genes were identified using GeneMark [18] as part of the genome annotation pipeline in the Integrated Microbial Genomes Expert Review (IMG-ER) system (http://img.jgi.doe.gov/er)
followed by a round of manual curation using JGI’s GenePRIMP pipeline (http://geneprimp.jgi-psf.org) [20]. 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. The tRNAscanSE tool [21] was used to find tRNA genes, whereas ribosomal RNAs were found by using the tool RNAmmer [22]. Other non coding RNAs were identified by searching the genome for the Rfam profiles using INFERNAL (v0.81) [23]. Additional gene prediction analysis and manual functional annotation was performed within the Integrated Microbial Genomes (IMG) platform (http://img.jgi.doe.gov/) [24].

**Metabolic network analysis**
The metabolic Pathway/Genome Database (PGDB) was computationally generated using Pathway Tools software version 12.5 [25] and MetaCyc version 12.5 [26], based on annotated EC numbers and a customized enzyme name mapping file. It has undergone no subsequent manual curation and may contain errors, similar to a Tier 3 BioCyc PGDB [27].

**Genome properties**
The genome is 4,253,413 bp long and comprises one main circular chromosome with a 71.9% GC content (Table 3 and Figure 3). Of the 3805 genes predicted, 3735 were protein coding genes, and 70 RNAs. 25 pseudogenes were also identified. 74.4% of the genes were assigned with a putative function while the remaining are annotated as hypothetical proteins. The properties and the statistics of the genome are summarized in Table 3. The distribution of genes into COGs functional categories is presented in Table 4, and a cellular overview diagram is presented in Figure 4, followed by a summary of metabolic network statistics shown in Table 5.

| Attribute                  | Value     | % of Total |
|----------------------------|-----------|------------|
| Genome size (bp)           | 4,253,413 | 100.00%    |
| DNA Coding region (bp)     | 3,872,139 | 91.04%     |
| DNA G+C content (bp)       | 3,057,630 | 71.89%     |
| Number of replicons        | 1         |            |
| Extrachromosomal elements  | 0         |            |
| Total genes                | 3805      | 100.00%    |
| RNA genes                  | 70        | 2.37%      |
| rRNA operons               | 4         |            |
| Protein-coding genes       | 3735      | 98.16%     |
| Pseudo genes               | 25        | 0.66%      |
| Genes with function prediction | 2832 | 74.43%     |
| Genes in paralog clusters  | 501       | 13.17%     |
| Genes assigned to COGs     | 2706      | 71.12%     |
| Genes assigned Pfam domains | 2785 | 73.19%     |
| Genes with signal peptides | 1126      | 29.59%     |
| Genes with transmembrane helices | 937  | 24.63%     |
| CRISPR repeats             | 0         |            |
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 |
|------|--------------------------------------------------|-------------|
| J    | 166 5.0                                         | Translation |
| A    | 1 0.0                                           | RNA processing and modification |
| K    | 317 10.0                                        | Transcription |
| L    | 120 4.0                                         | Replication, recombination and repair |
| B    | 1 0.0                                           | Chromatin structure and dynamics |
| D    | 25 1.0                                          | Cell cycle control, mitosis and meiosis |
| Y    | 0 0.0                                           | Nuclear structure |
| V    | 69 2.0                                          | Defense mechanisms |
| T    | 173 6.0                                         | Signal transduction mechanisms |
| M    | 134 4.0                                         | Cell wall/membrane biogenesis |
| N    | 55 2.0                                          | Cell motility |
| Z    | 3 0.0                                           | Cytoskeleton |
| W    | 0 0.0                                           | Extracellular structures |
| U    | 41 1.0                                          | Intracellular trafficking and secretion |
| O    | 84 3.0                                          | Posttranslational modification, protein turnover, chaperones |
| C    | 174 6.0                                         | Energy production and conversion |
Table 5. Metabolic Network Statistics

| Attribute               | Value |
|-------------------------|-------|
| Total genes             | 3805  |
| Enzymes                 | 714   |
| Enzymatic reactions     | 935   |
| Metabolic pathways      | 205   |
| Metabolites             | 676   |

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