Complete genome sequence of *Beutenbergia cavernae* type strain (HKI 0122\textsuperscript{T})

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*Beutenbergia cavernae* (Groth et al. 1999) is the type species of the genus and is of phylogenetic interest because of its isolated location in the actinobacterial suborder *Micrococcineae*. *B. cavernae* HKI 0122\textsuperscript{T} is a Gram-positive, non-motile, non-spore-forming bacterium isolated from a cave in Guangxi (China). *B. cavernae* grows best under aerobic conditions and shows a rod-coccus growth cycle. Its cell wall peptidoglycan contains the diagnostic L-lysine $\xrightarrow{\text{L-glutamate}}$ interpeptide bridge. Here we describe the features of this organism, together with the complete genome sequence, and annotation. This is the first completed genome sequence from the poorly populated micrococcal family *Beutenbergiaceae*, and this 4,669,183 bp long single replicon genome with its 4225 protein-coding and 53 RNA genes is part of the *Genomic Encyclopedia of Bacteria and Archaea* project.

Introduction

*Beutenbergia cavernae* strain HKI 0122\textsuperscript{T} (DSM 12333 = ATCC BAA-8 = JCM 11478) is the type strain of the species, which represents the type species of the genus *Beutenbergia*, the type genus of the family *Beutenbergiaceae* [1]. *B. cavernae* was described by Groth et al. 1999 as Gram-positive, non-motile and non-spore-forming [1]. The organism is of significant interest for its position in the tree of life within the small (2 type strains) family *Beutenbergiaceae* Zhi, et al., 2009 emend. Schumann et al. 2009 in the actinobacterial suborder *Micrococcineae* [2], which in addition to the genus *Beutenbergia* contains only the genus *Salana* [3,4] (Figure 1), also otherwise stated in a
Beutenbergia cavernae type strain (HKI 0122T)

Recent overview on the class Actinobacteria [2]. Here we present a summary classification and a set of features for B. cavernae strain HKI 0122T (Table 1), together with the description of the complete genome sequencing and annotation.

In addition to strain HKI 0122T, only one other strain (HKI 0132) was isolated from the soil sample collected in the Reed Flute Cave near Guilin, Guangxi, China. HKI 0132 was also classified in the species B. cavernae [1]. No closely related isolates and uncultivated clones with more than 97% 16S rRNA gene sequence identity are recorded in the microbiological literature, nor can any phylotype from environmental samples or genomic surveys be directly linked to B. cavernae.

B. cavernae cells vary in shape and colonies grown on rich medium vary in color from cream to bright yellow. In young cultures, cells are irregular rods arranged in palisades, clusters or in pairs at an angle to give V-formations [1]. Cells in stationary cultures are predominantly coccoid, occurring singly, in pairs, irregular clusters and short chains. During growth in complex media a rod-coccus growth cycle was observed [1]. B. cavernae grows well under aerobic and microaerophilic conditions, but not under anaerobic conditions [1]. The optimal growth temperature is 28°C [1].

B. cavernae is able to degrade casein, esculin, gelatin and potato starch. Acids are produced from L-arabinose, D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, inulin, maltose, D-mannose, D-raffinose, L-rhamnose, D-ribose, salicin, sucrose, starch, trehalose and D-xylose. There is no acid production from D-glucitol, lactose and D-mannitol. Nitrate is reduced to nitrite, H2S is produced [1].

Classification and features

Figure 1. shows the phylogenetic neighborhood of B. cavernae strain HKI 0122T in a 16S rRNA based tree. Analysis of the two identical 16S rRNA gene sequences in the genome of strain HKI differed by four nucleotides from the previously published 16S rRNA sequence generated from DSM 12333 (Y18378). The slight differences between the genome data and the reported 16S rRNA gene sequence is most likely due to sequencing errors in the previously reported sequence data.

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**Figure 1.** Phylogenetic tree of B. cavernae HKI 0122T and all type strains of the genus Beutenbergia, inferred from 1,411 aligned characters [5, 6] of the 16S rRNA sequence under the maximum likelihood criterion [7]. The tree was rooted with species from the genera Isoptericola and Oerskovia, both also members of the actinobacterial suborder Micrococcineae. The branches are scaled in terms of the expected number of substitutions per site. Numbers above branches are support values from 1,000 bootstrap replicates if larger than 60%. Strains with a genome-sequencing project registered in GOLD [8] are printed in blue; published genomes in bold.
Chemotaxonomy

The peptidoglycan of *B. cavernae* HKI 0122<sup>T</sup> contains D- and L-alanine, D- and L-glutamic acid and L-lysine, with the latter widely distributed among actinobacteria [1]. The strain possesses a type A4<interpeptide bridge, type A11.54 according to DSMZ. Glucose, mannose and galactose are the cell wall sugars [1]. The fatty acid profile of strain *B. cavernae* HKI 0122<sup>T</sup> is dominated by 13-methyl tetradecanoic (iso-C<sub>15:0</sub>; 43.7%) and 12-methyl tetradecanoic (anteiso-C<sub>15:0</sub>; 34.6%) saturated, branched chain acids. Other predominantly saturated fatty acids play a minor role in the cellular fatty acid composition of the strain: iso-C<sub>14:0</sub> (0.9%), C<sub>14:0</sub> (1.9%); C<sub>15:0</sub> (0.9%) iso-C<sub>16:0</sub> (2.3%), C<sub>16:0</sub> (6.8%), iso-C<sub>17:0</sub> (3.1%), anteiso-C<sub>17:0</sub> (4.9%), und C<sub>18:1</sub> (0.9%) [1]. Mycolic acids are not present [1]. MK-8(H<sub>4</sub>) is the major menaquinone, complemented by minor amounts of MK-8(H<sub>2</sub>), MK-8 and MK-9(H<sub>4</sub>) [1]. The combination of the L-Lys<interpeptide bridge and MK-8(H<sub>4</sub>) as the dominating menaquinone is shared with the organisms from the neighboring genera *Bogoriella* and *Georgenia*. The polar lipids of strain HKI 0122<sup>T</sup> consist of phosphatidylinositol and diphosphatidylglycerol together with three yet unidentified phospholipids [1].

**Figure 2.** Scanning electron micrograph of *B. cavernae* HKI 0122<sup>T</sup>
Beutenbergia cavernae type strain (HKI 0122T)

Table 1. Classification and general features of B. cavernae HKI 0122\(^{T}\) based on the MIGS recommendations [9]

| MIGS ID | Property               | Term                                                      | Evidence code |
|---------|------------------------|-----------------------------------------------------------|---------------|
|         | Current classification |                                                           |               |
|         | Domain                 | Bacteria                                                 |               |
|         | Phylum                 | Actinobacteria                                            |               |
|         | Class                  | Actinobacteria                                            | TAS [10]      |
|         | Order                  | Actinomycetales                                           | TAS [10]      |
|         | Suborder               | Micrococcineae                                            | TAS [2]       |
|         | Family                 | Beutenbergiaceae                                          | TAS [2]       |
|         | Genus                  | Beutenbergia                                              | TAS [1]       |
|         | Species                | Beutenbergia cavernae                                     | TAS [1]       |
|         | Type strain            | HKI 0122                                                 |               |
|         | Gram stain             | positive                                                  | TAS [1]       |
|         | Cell shape             | varies; rod-coccus growth cycle                           | TAS [1]       |
|         | Motility               | nonmotile                                                 | TAS [1]       |
|         | Sporulation            | non-sporulating                                            | TAS [1]       |
|         | Temperature range       | mesophile                                                 | TAS [1]       |
|         | Optimum temperature    | 28°C                                                      | TAS [1]       |
|         | Salinity               | tolerance of 2-4% (w/v) NaCl                              | TAS [1]       |
| MIGS-22 | Oxygen requirement     | aerobic and microaerobic, no growth under anaerobic       | TAS [1]       |
|         |                        | conditions                                                |               |
| MIGS-6  | Carbon source          | glucose, maltose, mannose, cellobiose                     | TAS [1]       |
| MIGS-15 | Energy source          | unknown                                                   |               |
| MIGS-14 | Pathogenicity          | none                                                      | NAS           |
|         | Biosafety level        | 1                                                         | TAS [11]      |
|         | Isolation              | cave, soil between rocks                                  | TAS [1]       |
| MIGS-4  | Geographic location    | Guangxi, China                                            | TAS [1]       |
| MIGS-5  | Sample collection time | about 1999                                                | TAS [1]       |
| MIGS-4.1| Longitude - Latitude   | 110.263306 - 25.307878                                    | TAS [1]       |
| MIGS-4.2| Depth                  | not reported                                              |               |
| MIGS-4.3| Altitude               | not reported                                              |               |

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 the Gene Ontology project [12]. If the evidence code is IDA the property was directly observed for a live isolate by one of the authors or an expert mentioned in the acknowledgements.

Genome sequencing and annotation

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 [8] and the complete genome sequence in GenBank (CP001618). 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                                                             |
|         |                           | Three genomic libraries: two Sanger libraries - 8 kb pMCL200 and fosmid pcc1Fos - and one 454 pyrosequence standard library |
| 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      | 8.56x Sanger; 10.86x pyrosequence                                    |
| MIGS-30 | Assemblers                | Newbler version 1.1.02.15, phrap                                       |
| MIGS-32 | Gene calling method       | Prodigal                                                             |
|         | INSDC / Genbank ID        | CP001618                                                             |
|         | Gold Date of Release      | 07-MAY-2009                                                          |
|         | GOLD ID                   | Gc01025                                                              |
|         | NCBI project ID           | 20827                                                                |
|         | Database: IMG-GEBA        | 2501416922                                                            |
| MIGS-13 | Source material identifier| DSM 12333                                                            |
|         | Project relevance         | Tree of Life, GEBA                                                   |

Growth conditions and DNA isolation

*B. cavernae* HKI 0122T, DSM 12333, was grown in DSMZ medium 736 (Rich Medium) [13] at 28°C. DNA was isolated from 0.5-1 g of cell paste using Qiagen Genomic 500 DNA Kit (Qiagen, Hilden, Germany) with a modification of the standard protocol for cell lysis in first freezing for 20 min. (-70°C), then heating 5 min. (98°C), and cooling 15 min to 37°C; adding 1.5 ml lysozyme (standard: 0.3 ml, only), 1.0 ml a chromatopeptidase, 0.12 ml lysostaphine, 0.12 ml mutanolysine, 1.5 ml proteinase K (standard: 0.5 ml, only), followed by overnight incubation at 35°C.

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 the [JGI website](http://standardsingenomics.org). 454 Pyrosequencing reads were assembled using the Newbler assembler version 1.1.02.15 (Roche). Large Newbler contigs were broken into 5,256 overlapping fragments of 1,000 bp and entered into the 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 [14]. Gaps between contigs were closed by editing in Consed, custom primer walking or PCR amplification. A total of 1,627 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 19.42x coverage of the genome.

Genome annotation

Genes were identified using Prodigal [15] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the [JGI GenePRIMP pipeline](http://standardsingenomics.org) [16]. 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 [17].

Genome properties

The genome is 4,669,183 bp long and comprises one main circular chromosome with a 73.1% GC content. (Table 3 and Figure 3). Of the 4,278 genes predicted, 4,225 were protein coding genes, and 53 RNAs. Twenty eight pseudogenes were also identified. The majority of the genes...
Beutenbergia cavernae type strain (HKI 0122T) (74.3%) were assigned a putative function while the remaining ones were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 4.

Table 3. Genome Statistics

| Attribute                               | Value  | % of Total |
|-----------------------------------------|--------|------------|
| Genome size (bp)                        | 4,669,183 |            |
| DNA Coding region (bp)                  | 4,347,731 | 93.12%     |
| DNA G+C content (bp)                    | 3,413,947 | 73.12%     |
| Number of replicons                     | 1      |            |
| Extrachromosomal elements               | 0      |            |
| Total genes                             | 4278   | 100.00%    |
| RNA genes                               | 53     | 1.24%      |
| rRNA operons                            | 2      |            |
| Protein-coding genes                    | 4225   | 98.76%     |
| Pseudo genes                            | 28     | 0.65%      |
| Genes with function prediction          | 3183   | 74.40%     |
| Genes in paralog clusters               | 689    | 16.11%     |
| Genes assigned to COGs                  | 3109   | 72.67%     |
| Genes assigned Pfam domains             | 3246   | 75.88%     |
| Genes with signal peptides              | 1034   | 24.17%     |
| Genes with transmembrane helices        | 1135   | 26.53%     |
| CRISPR repeats                          | 1      |            |

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

| Code | Value | %   | Description                                         |
|------|-------|-----|-----------------------------------------------------|
| J    | 169   | 4   | Translation, ribosomal structure and biogenesis     |
| A    | 4     | 0.1 | RNA processing and modification                      |
| K    | 384   | 9.1 | Transcription                                        |
| L    | 122   | 2.9 | Replication, recombination and repair                |
| B    | 1     | 0   | Chromatin structure and dynamics                     |
| D    | 25    | 0.6 | Cell cycle control, mitosis and meiosis             |
| Y    | 0     | 0   | Nuclear structure                                    |
| V    | 95    | 2.3 | Defense mechanisms                                  |
| T    | 138   | 3.3 | Signal transduction mechanisms                       |
| M    | 166   | 3.9 | Cell wall/membrane biogenesis                        |
| N    | 1     | 0   | Cell motility                                        |
| Z    | 0     | 0   | Cytoskeleton                                         |
| W    | 0     | 0   | Extracellular structures                             |
| U    | 27    | 0.6 | Intracellular trafficking and secretion              |
| O    | 89    | 2.1 | Posttranslational modification, protein turnover, chaperones |
Table 4. Number of genes associated with the 21 general COG functional categories

| Code | Value | %  | Description                                           |
|------|-------|----|-------------------------------------------------------|
| J    | 169   | 4  | Translation, ribosomal structure and biogenesis      |
| G    | 546   | 12.9 | Carbohydrate transport and metabolism                |
| E    | 264   | 6.3 | Amino acid transport and metabolism                  |
| F    | 92    | 2.2 | Nucleotide transport and metabolism                  |
| H    | 129   | 3.1 | Coenzyme transport and metabolism                    |
| I    | 101   | 2.4 | Lipid transport and metabolism                       |
| P    | 183   | 4.3 | Inorganic ion transport and metabolism               |
| Q    | 62    | 1.5 | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 433   | 10.3| General function prediction only                     |
| S    | 249   | 5.9 | Function unknown                                     |
| -    | 1116  | 26.4| Not in COGs                                          |

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, rRNAs red, other RNAs black), GC content, GC skew
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