Complete genome sequence of *Tsukamurella paurometabola* type strain (no. 33T)

A. Christine Munk1,2, Alla Lapidus1, Susan Lucas1, Matt Nolan1, Hope Tice1, Jan-Fang Cheng1, Tijana Glavina Del Rio1, Lynne Goodwin1,2, Sam Pitluck1, Konstantinos Liolios1, Marcel Huntemann1, Natalia Ivanova1, Konstantinos Mavromatis1, Natalia Mikhailova1, Amrita Pati1, Amy Chen1, Krishna Palaniappan1, Roxanne Tapia1,2, Cliff Han1,2, Miriam Land1,2, Loren Hauser1,4, Yun-Juan Chang1,4, Cynthia D. Jeffries1,4, Thomas Brettin1,4, Montri Yasawong1, Evelyne-Marie Brambilla6, Manfred Rohde5, Johannes Sikorski5, Markus Göker6, John C. Detter1,2, Tanja Woyke1, James Bristow1, Jonathan A. Eisen1,7, Victor Markowitz3, Philip Hugenholtz1,8, Nikos C. Kyrpides1, and Hans-Peter Klenk6*

1 DOE Joint Genome Institute, Walnut Creek, California, USA
2 Los Alamos National Laboratory, Bioscience Division, Los Alamos, New Mexico, USA
3 Biological Data Management and Technology Center, Lawrence Berkeley National Laboratory, Berkeley, California, USA
4 Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA
5 HZI – Helmholtz Centre for Infection Research, Braunschweig, Germany
6 DSMZ - German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany
7 University of California Davis Genome Center, Davis, California, USA
8 Australian Centre for Ecogenomics, School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia

*Corresponding author: Hans-Peter Klenk*

**Keywords**: obligately aerobic, non-motile, mesophilic, chemoorganotrophic, Gram-positive, metachromatic granules, opportunistic pathogen, *Tsukamurellaceae*, GEBA

*Tsukamurella paurometabola* corrig. (Steinhaus 1941) Collins *et al.* 1988 is the type species of the genus *Tsukamurella*, which is the type genus to the family *Tsukamurellaceae*. The species is not only of interest because of its isolated phylogenetic location, but also because it is a human opportunistic pathogen with some strains of the species reported to cause lung infection, lethal meningitis, and necrotizing tenosynovitis. This is the first completed genome sequence of a member of the genus *Tsukamurella* and the first genome sequence of a member of the family *Tsukamurellaceae*. The 4,479,724 bp long genome contains a 99,806 bp long plasmid and a total of 4,335 protein-coding and 56 RNA genes, and is a part of the Ge-nomic Encyclopedia of Bacteria and Archaea project.

**Introduction**

Strain no. 33T (DSM 20162 = ATCC 8368 = JCM 10117) is the type strain of the species *Tsukamurella paurometabola*, which in turn is the type species of the genus *Tsukamurella* [1,2]. Currently, there are eleven species within the genus *Tsukamurella* [1,3], which is named in honor of Michio Tsukamura, a Japanese microbiologist [1]. The species epithet derives from the Greek words *pauros* meaning little and *metabolus* meaning changeable, referring to a metabolism that is little changeable [1]. Strain no. 33T was first isolated from the mycetome and ovaries of *Cimex lectularis* (bedbug) in a study on the bacterial flora of *Hexapoda* by Edward A. Steinhaus in 1941 [2]. *T. paurometabola* was formerly also known as *Corynebacterium paurometabolum* (basonym) [1,4] as well as under its heterotypic synonym *Rhodococcus aurantiacus* [5,6], until Collins *et al.* revised the controversial taxonomic position of the species in 1988 [1] and J. P. Euzéby corrected the species epithet according to the rules of the International Code of Nomenclature of Bacteria (1990 Revision) [7]. *T. paurometabola* is known, albeit rarely, to be an opportunistic pathogen for humans, especially in patients with predisposing conditions, such as immunosuppression (leukemia, solid tumors, and HIV infection) [8,9],
chronic lung disease (tuberculosis) [9], and most often indwelling foreign bodies (long-term use of indwelling catheters) [10-13]. Here we present a summary classification and a set of features for *T. paurometabola* no. 33', together with the description of the complete genomic sequencing and annotation.

**Classification and features**

The phylogenetic neighborhood of *T. paurometabola* no. 33' in a 16S rRNA based tree is shown in Figure 1. The sequences of the two identical 16S rRNA gene copies in the genome differ by one nucleotide from the previously published 16S rRNA sequence (AF283280).

![Phylogenetic tree highlighting the position of *T. paurometabola* relative to the other type strains within the genus *Tsukamurella*. The tree was inferred from 1,447 aligned characters [14,15] of the 16S rRNA gene sequence under the maximum likelihood criterion [16] and rooted with the members of the closely related genus *Dietzia*. 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 [17] if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [18] are labeled with one asterisk, those registered as 'Complete and Publish.'

A representative genomic 16S rRNA sequence of strain no. 33 was compared using NCBI BLAST under default settings (e.g., considering only the high-scoring segment pairs (HSPs) from the best 250 hits) with the most recent release of the Greengenes database [19] and the relative frequencies, of taxa and keywords (reduced to their stem [20]) were determined, weighted by BLAST scores. The most frequently occurring genera were *Tsukamurella* (34.7%), *Mycobacterium* (32.5%), *Dietzia* (20.6%) and *Rhodococcus* (12.1%) (220 hits in total). Regarding the seven hits to sequences from members of the species, the average identity within HSPs was 99.3%, whereas the average coverage by HSPs was 96.7%. Regarding the 45 hits to sequences from other members of the genus, the average identity within HSPs was 99.2%, whereas the average coverage by HSPs was 96.2%. Among all other species, the one yielding the highest score was *Tsukamurella strandjordii*, (NR_025113), which corresponded to an identity of 99.5% and a HSP coverage of 100.0%. (Note that the Greengenes

http://standardsingenomics.org
Tsukamurella paurometabola type strain (no. 33T)
database uses the INSDC (= EMBL/NCBI/DDBJ)
annotation, which is not an authoritative source for
nomenclature or classification.) The highest-scoring
environmental sequence was DQ366095
('on Oil Degrading Consortium oil polluted soil
clone MH1 Pitesti'), which showed an identity of
99.2% and an HSP coverage of 99.0%. The most
frequently occurring keywords within the labels of
environmental samples which yielded hits were
'skin' (9.6%), 'human' (4.8%), 'microbiom, tempor,
topograph' (4.2%), 'sea' (3.8%) and 'sediment'
(1.8%) (30 hits in total). Environmental samples
which yielded hits of a higher score than the high-
est scoring species were not found. These envir-
onmental labels are in line with the locations re-
ported for the isolation of Tsukamurella strains,
such as soil, human sputum, and bed bug [2,21].
The cells of T. paurometabola are straight to
slightly curved rods with a size of 0.5-0.8 × 1.0-5
µm and occur singly, in pairs, or in masses [2,21]
(Figure 2). The organism is Gram-positive, weakly
acid-fast (some strains are strongly acid-fast),
non-sporeforming and non-motile [2,21] (Table
1). The organism contains metachromatic gra-
nules [2]. Colonies of T. paurometabola are small
(diameter, 0.5-2.0 mm) with convex elevation,
have entire edges (sometimes rhizoidal), are
dryish but easily emulsified and are white to
creamy to orange in color [3.15]. T. paurometabola
is strictly aerobic and chemoorganotrophic bacte-
rium [1]. Reaction is positive for catalase and py-
razinamidase [1]. Acid is produced from some su-
gars [1]. The organism does not produce nitriles
from nitrates [2]. Indole is not produced by T.
paurometabola [2]. The organism is non-
pathogenic for guinea pigs [2]. In general T. pau-
rometabola strains grow in the range 10°C to 35°C.
Strain no. 33T does not grow at 45°C [1]. The
strain did not survive heating at 60°C for 15 mi-
utes [1]. Some strains of T. paurometabola pro-
duce acid from fructose, galactose, glucose, glyc-
erol, inositol, manitol, mannose, sorbitol, sucrose,
and trehalose [1]. Acid is not produced from L-
arabinose, L-rhamnose, or D-xylene [1]. Some
strains of T. paurometabola grow on ethanol, fruc-
tose, galactose, glucose, inositol, manitol, man-
lose, melizitose, sorbitol, sucrose, trehalose, xy-
llose, n-butanol, isobutanol, 2,3-butylene glycol,
propanol, propylene glycol, citrate, fumarate, ma-
late, pyruvate, and succinate [1]. The organism
does not grow on adonitol, arabinose, inulin, lac-
tose, raffinose, or rhamnose [1]. Acetamide and
nicotinamide are used as sole nitrogen sources but
not benzamide [1]. Acetamide, glutamate, gluco-
samine, monoethanolamine, and serine are used
as sole sources of carbon and nitrogen [1]. T. pau-
rometabola is able to degrade Tween 20, Tween
40, Tween 60, and Tween 80, but not adenine,
casein, or elastin [1]. Some strains of T. paumo-
etabolum degrade xanthine and tyrosine [1]. The
organism produces β-galactosidase and urease,
but not arylsulfatase or α-esterase [1]. T. pauro-
metabolum is resistant to ethambutol (5 µg/ml),
5-fluorouracil (20 µg/ml), mitomycin C (10 µg/ml),
and picric acid (0.2% w/v) [1]. The organ-
ism is susceptible to bleomycin (5 µg/ml) [1].

Figure 2. Scanning electron micrograph of T. paurometabola no. 33T
Table 1. Classification and general features of *T. paurometabola* no. 33' according to the MIGS recommendations [22] and the NamesforLife database [23]

| MIGS ID | Property                   | Term                                      | Evidence code |
|---------|----------------------------|-------------------------------------------|---------------|
|         | Current classification     | Domain *Bacteria*                         | TAS [24]      |
|         |                            | Phylum “*Actinobacteria*”                 | TAS [25]      |
|         |                            | Class *Actinobacteria*                    | TAS [26]      |
|         |                            | Subclass *Actinobacteridae*               | TAS [26,27]   |
|         |                            | Order *Actinomycetales*                   | TAS [26-29]   |
|         |                            | Suborder *Corynebacterineae*              | TAS [26,27]   |
|         |                            | Family *Tsukamurellaceae*                 | TAS [26,27]   |
|         |                            | Genus *Tsukamurella*                      | TAS [1]       |
|         |                            | Species *Tsukamurella paurometabola*      | TAS [1]       |
|         |                            | Type strain no. 33                        | TAS [2]       |
|         | Gram stain                 | positive                                  | TAS [2]       |
|         | Cell shape                 | short rods occurring singly, in pairs or in masses | TAS [2] |
|         | Motility                   | none                                      | TAS [2]       |
|         | Sporulation                | none                                      | TAS [2]       |
|         | Temperature range          | 10°C–35°C, not at 45°C                    | NAS [1]       |
|         | Optimum temperature        | not reported                              |               |
|         | Salinity                   | not reported                              |               |
| MIGS-22 | Oxygen requirement         | obligately aerobic                        | TAS [1]       |
|         | Carbon source              | carbohydrates                             | TAS [1]       |
|         | Energy metabolism          | chemoorganotroph                          | TAS [1]       |
| MIGS-6  | Habitat                    | soil, human sputum, insect microbiome     | TAS [2,4]     |
| MIGS-15 | Biotic relationship        | free-living                               | NAS           |
| MIGS-14 | Pathogenicity              | infection of the lung, lethal meningitis, and necrotizing tenosynovitis | TAS [4] |
|         | Biosafety level            | 1+                                        | TAS [30]      |
| MIGS-4  | Isolation                  | ovaries of *Cimex lectularius* (bedbug)   | TAS [2,4]     |
| MIGS-5  | Geographic location        | most probably close to Columbus, Ohio     | NAS           |
| MIGS-4.1| Sample collection time     | 1941 or before                            | TAS [2]       |
| MIGS-4.1| Latitude                   | not reported                              |               |
| MIGS-4.2| Longitude                  | not reported                              |               |
| MIGS-4.3| Depth                      | not reported                              |               |
| MIGS-4.4| 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 of the Gene Ontology project [31]. If the evidence code is IDA, the property was directly observed by one of the authors or an expert mentioned in the acknowledgements.

**Chemotaxonomy**

The major cell wall sugars of *T. paurometabola* are arabinose and galactose [1], but ribose and traces of glucose have also been observed (unpublished data, DSMZ). The diagnostic amino acid of peptidoglycan is *meso*-diaminopimelic acid (variation A1); the glycan moiety of cell walls contains N-glycolyl residues [1]. Arabinogalactan is covalently attached to the peptidoglycan [32]. Long-chain highly unsaturated mycolic acids (62 to 78 carbon atoms) are present and contain one to six double bonds [1]. Fatty acid esters released on pyrolysis of mycolic acids have 20 to 22 carbon atoms [1,21]. The major polar lipids of *T. paurometabola* are di-phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and mono- and diacylated phosphatidylinositol dimannosides [1,21]. Some strains of *T. paurometabola* produce glycolipids [1]. The long-chain cellular fatty acids are predominantly straight-chain saturated, mono-unsaturated, and 10-methyl branched acids [1].

http://standardsingenomics.org
Tsukamurella paurometabola type strain (no. 33T)

Menaquinones are the sole respiratory quinones, with MK-9 predominating [1]: 80% MK-9 (H₃), 6.8% MK-8 (H₂), 3.5% MK-7 (H₀), 2.3% MK-10 (H₀) and 6.7% MK-8 (H₂) (unpublished data, DSMZ).

**Genome sequencing and annotation**

**Genome project history**

This organism was selected for sequencing on the basis of its phylogenetic position [33], and is part of the *Genomic Encyclopedia of Bacteria and Archaea* project [34]. The genome project is deposited in the Genome Online Database [18] and the complete genome sequence is deposited in GenBank. 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: Sanger 8 kb pMCL200 library, 40 kb          |
|         |                           | (fosmid, pcc1Fos) library, 454 pyrosequence standard library         |
| MIGS-29 | Sequencing platforms      | ABI3730, 454 GS FLX Titanium                                         |
| MIGS-31.2| Sequencing coverage       | 8.25 × Sanger; 37.9 × pyrosequence                                   |
| MIGS-30 | Assemblers                | Newbler version 1.1.02.15, phrap                                        |
| MIGS-32 | Gene calling method       | Prodigal 1.4, GenePRIMP                                               |
|         |                           | CP001966 (chromosome)                                                 |
|         |                           | CP001967 (plasmid Tpau01)                                             |
| INSDC ID| Genbank Date of Release   | May 17, 2010                                                          |
| GOLI D  |                           | Gc01341                                                              |
| NCBI project ID | Database: IMG-GEBA | 646564587                                      |
| MIGS-13 | Source material identifier| DSM 20162                                                             |
|         | Project relevance         | Tree of Life, GEBA                                                    |

**Growth conditions and DNA isolation**

*T. paurometabola* no. 33T, DSM 2016, was grown in medium 535 (Trypticase soy broth medium) [35] at 28°C. DNA was isolated from 0.5-1 g of cell paste using MasterPure Gram Positive DNA Purification Kit (Epicentre MGP04100) following the standard protocol as recommended by the manufacturer, with modification st/LALMice for cell lysis as described in [24]. DNA is available through the DNA Bank Network [36].

**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 can be found at the JGI website [37]. Pyrosequencing reads were assembled using the Newbler assembler (Roche). Large Newbler contigs were broken into 4,920 overlapping fragments of 1,000 bp and entered into assembly as pseudoreads. The sequences were assigned quality scores based on Newbler consensus q-scores with modifications to account for overlap redundancy and adjust inflated q-scores. A hybrid 454/Sanger assembly was made using the parallel phrap assembler [38]. Possible mis-assemblies were corrected with Dupfinisher or transposon bombing of bridging clones [39]. Gaps between contigs were closed by editing in Consed, custom primer walk or PCR amplification. A total of 516 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, the combination of the Sanger and 454 sequencing platforms provided 46.15 × coverage of the genome. The final assembly contains 42,170 Sanger reads and 745,985 pyrosequencing reads.
Genome annotation

Genes were identified using Prodigal [40] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI GenePRIMP pipeline [41]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) non-redundant database, UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and functional annotation was performed within the Integrated Microbial Genomes - Expert Review (IMG-ER) platform [42].

Genome properties

The genome consists of a 4,379,918 bp long chromosome and a 99,806 bp long plasmid, both with a G+C content of 68.4% (Figure 3 and Table 3). Of the 4,391 genes predicted, 4,335 were protein-coding genes, and 56 RNAs; 93 pseudogenes were also identified. The majority of the protein-coding genes (68.7%) 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.

Figure 3. Graphical circular map of the chromosome. 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.
Table 3. Genome Statistics

| Attribute                  | Value     | % of Total |
|----------------------------|-----------|------------|
| Genome size (bp)           | 4,479,724 | 100.00%    |
| DNA coding region (bp)     | 4,108,044 | 91.70%     |
| DNA G+C content (bp)       | 3,064,083 | 68.40%     |
| Number of replicons        | 2         |            |
| Extrachromosomal elements  | 1         |            |
| Total genes                | 4,391     | 100.00%    |
| RNA genes                  | 56        | 1.28%      |
| rRNA operons               | 2         |            |
| Protein-coding genes       | 4,335     | 98.72%     |
| Pseudo genes               | 93        | 2.12%      |
| Genes with function prediction | 3,017   | 68.71%     |
| Genes in paralog clusters  | 691       | 15.74%     |
| Genes assigned to COGs     | 3,025     | 68.89%     |
| Genes assigned Pfam domains| 3,376     | 76.88%     |
| Genes with signal peptides | 1,031     | 23.48%     |
| Genes with transmembrane helices | 1,114  | 25.37%     |
| CRISPR repeats             | N.D.      |            |

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

| Code | Value | %age  | Description                                                                 |
|------|-------|-------|-----------------------------------------------------------------------------|
| J    | 169   | 5.0   | Translation, ribosomal structure and biogenesis                            |
| A    | 1     | 0.0   | RNA processing and modification                                             |
| K    | 310   | 9.2   | Transcription                                                               |
| L    | 198   | 5.9   | Replication, recombination and repair                                         |
| B    | 1     | 0.0   | Chromatin structure and dynamics                                             |
| D    | 31    | 0.9   | Cell cycle control, cell division, chromosome partitioning                  |
| Y    | 0     | 0.0   | Nuclear structure                                                            |
| V    | 39    | 1.2   | Defense mechanisms                                                           |
| T    | 131   | 3.9   | Signal transduction mechanisms                                               |
| M    | 135   | 4.0   | Cell wall/membrane/envelope biogenesis                                       |
| N    | 3     | 0.1   | Cell motility                                                               |
| Z    | 0     | 0.0   | Cytoskeleton                                                                |
| W    | 0     | 0.0   | Extracellular structures                                                    |
| U    | 29    | 0.9   | Intracellular trafficking, secretion, and vesicular transport                |
| O    | 102   | 3.0   | Posttranslational modification, protein turnover, chaperones                |
| C    | 217   | 6.4   | Energy production and conversion                                             |
| G    | 220   | 6.5   | Carbohydrate transport and metabolism                                        |
| E    | 274   | 8.1   | Amino acid transport and metabolism                                         |
| F    | 85    | 2.5   | Nucleotide transport and metabolism                                          |
| H    | 165   | 4.9   | Coenzyme transport and metabolism                                           |
| I    | 231   | 6.8   | Lipid transport and metabolism                                               |
| P    | 169   | 5.0   | Inorganic ion transport and metabolism                                      |
| Q    | 172   | 5.1   | Secondary metabolites biosynthesis, transport and catabolism               |
| R    | 430   | 12.7  | General function prediction only                                             |
| S    | 269   | 8.0   | Function unknown                                                            |
| -    | 1,366 | 31.1  | Not in COGs                                                                 |

Tsukamurella paurometabola type strain (no. 33T)
Acknowledgements
We would like to gratefully acknowledge the help of Marlen Jando (DSMZ) for growing cultures of *T. paurometabola*. This work was performed under the auspices of the US Department of Energy Office of Science, Biological and Environmental Research Program, and by the University of California, Lawrence Berkeley National Laboratory under contract No. DE-AC02-05CH1231, Lawrence Livermore National Laboratory under Con-tract No. DE-AC52-07NA27344, and Los Alamos Na-tional Laboratory under contract No. DE-AC02-06NA25396, UT-Battelle and Oak Ridge National Lab-oratory under contract DE-AC05-00R22725, as well as German Research Foundation (DFG) INST 599/1-1 and Thailand Research Fund Royal Golden Jubilee Ph.D. Program No. PHD/0019/2548 for MY.

References
1. Collins MD, Smida J, Dorsch M, Stackebrandt E. *Tsukamurella* gen. nov. harboring *Corynebacterium paurometabolum* and *Rhodococcus aurantiacus*. *Int J Syst Bacteriol* 1988; 38:385-391. doi:10.1099/00207713-38-4-385
2. Steinhaus EA. A study of the bacteria associated with thirty species of insects. *J Bacteriol* 1941; 42:757-790. PubMed
3. Euzéby JP. List of Bacterial Names with Standing in Nomenclature: a folder available on the internet. *Int J Syst Bacteriol* 1997; 47:590-592. PubMed doi:10.1099/00207713-47-2-590
4. Collins MD, Cummins CS. Genus *Corynebacterium* Lehmann and Neumann 1896. In: Sneth PHA, Mair NS, Sharpe ME, Holt JG (eds), Bergey's Manual of Systematic Bacteriology, Gram-positive Bacteria other than *Actinomycetales*, 1st Edition, Volume 2, Williams & Wilkins, Baltimore, 1986, p. 1275.
5. Tsukamura M, Mizuna S. A new species, *Gordonia aurantiaca*, occurring in sputa of patients with pulmonary disease. [in Japanese]. *Kekkaku* 1971; 46:93-98. PubMed
6. Tsukamura M, Yano I. *Rhodococcus sputi* sp. nov., nom. rev., and *Rhodococcus aurantiacus* sp. nov., nom. rev. *Int J Syst Bacteriol* 1985; 35:364-368. doi:10.1099/00207713-35-3-364
7. Euzéby JP. Taxonomic note: necessary correction of specific and subspecific epithets according to Rules 12c and 13b of the International Code of Nomenclature of Bacteria (1990 Revision). *Int J Syst Bacteriol* 1998; 48:1073-1075. doi:10.1099/00207713-48-3-1073
8. Schwartz MA, Tabet SR, Collier AC, Wallis CK, Carlson LC, Nguyen TT, Kattar MM, Coyle MB. Central venous catheter-related bacteremia due to *Tsukamurella* species in the immunocompromised host: a case series and review of the literature. *Clin Infect Dis* 2002; 35:e72-e77. PubMed doi:10.1086/342561
9. Rey D, Fraisse P, Riegel P, Piemont Y, Lang JM. *Tsukamurella* infections. Review of the literature apropos of a case. *Pathol Biol (Paris)* 1997; 45:60-65. PubMed
10. Shapiro CL, Haft RF, Gantz NM, Doern GV, Christenson JC, O'Brien R, Overall JC, Brown BA, Eallace RJ, Jr. *Tsukamurella paurometabola*: a novel pathogen causing catheter-related bacteremia in patients with cancer. *Clin Infect Dis* 1992; 14:200-203. PubMed doi:10.1093/clinids/14.1.200
11. Lai KK. A cancer patient with central venous catheter-related sepsis caused by *Tsukamurella paurometabolum* (*Gordona aurantiaca*). *Clin Infect Dis* 1992; 18:830-832. PubMed doi:10.1093/clinids/18.5.830
12. Bouza E, Pérez-Parra A, Rosal M, Martín-Rabadán P, Rodríguez-Créixems M, Marin M. *Tsukamurella*: a cause of catheter-related bloodstream infections. *Eur J Clin Microbiol Infect Dis* 2009; 28:203-210. PubMed doi:10.1007/s10096-008-0607-2
13. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 2000; 17:540-552. PubMed
14. Lee C, Grasso C, Sharlow MF. Multiple sequence alignment using partial order graphs. *Bioinformatics* 2002; 18:452-464. PubMed doi:10.1093/bioinformatics/18.3.452
15. Stamatakis A, Hoover P, Rougemont J. A rapid bootstrap algorithm for the RAxML web servers. *Syst Biol* 2008; 57:758-771. PubMed doi:10.1080/10635150802429642
**Tsukamurella paurometabola** type strain (no. 33T)

17. Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A. How many bootstrap replicates are necessary? *Lect Notes Comput Sci* 2009; **5541**:184-200. doi:10.1007/978-3-642-02008-7_13

18. Liolios K, Chen IM, Mavromatis K, Tavernarakis N, Hugenholtz P, Markowitz VM, Kyropides NC. The Genomes On Line Database (GOLD) in 2009: status of genomic and metagenomic projects and their associated metadata. *Nucleic Acids Res* 2010; **38**:D346-D354. PubMed doi:10.1093/nar/gkp848

19. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie E, Keller K, Huber T, Dalevi D, Hu P, Andersen G. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 2006; **72**:5069-5072. PubMed doi:10.1128/AEM.03006-05

20. Porter MF. An algorithm for suffix stripping. *Program: electronic library and information systems*. 1980; **14**:130-137.

21. Group 22, Nocardioform Actinomycetes, Genus *Tsukamurella*. In: Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (eds), Bergey’s Manual of Determinative Bacteriology, 9th Edition, Williams & Wilkins, Baltimore, 1994, p. 627.

22. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Thomson N, Allen MJ, Angioli SV, *et al.* The minimum information about a genome sequence (MIGS) specification. *Nat Biotechnol* 2008; **26**:541-547. PubMed doi:10.1038/nbt1360

23. Garrity G. NamesforLife. BrowserTool takes expertise out of the database and puts it right in the browser. *Microbiol Today* 2010; **37**:9.

24. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc Natl Acad Sci USA* 1990; **87**:4576-4579. PubMed doi:10.1073/pnas.87.12.4576

25. Garrity GM, Holt JG. The road map to the Manual. In: Garrity GM, Boone DR, Castenholz R (eds), Bergey’s Manual of Systematic Bacteriology, 2 ed, vol. 1. Springer, New York, 2001, p. 119–169.

26. Stackebrandt E, Rainey FA, Ward-Rainey NL. Proposal for a new hierarchical classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* 1997; **47**:479-491. doi:10.1099/00207713-47-2-479

27. Zhi XY, Li WJ, Stackebrandt E. An update of the structure and 16S RNA gene sequence-based definition of higher ranks of the class Actinobacteria, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. *Int J Syst Evol Microbiol* 2009; **59**:589-608. PubMed doi:10.1099/ijs.0.065780-0

28. Buchanan RE. Studies in the nomenclature and classification of the bacteria II. The primary subdivisions of the schizomycetes. *J Bacteriol* 1917; **2**:155-164. PubMed

29. Skerman VBD, McGowan V, Sneath PHA. Approved Lists of Bacterial Names. *Int J Syst Bacteriol* 1980; **30**:225-420. doi:10.1099/00207713-30-1-225

30. BAuA. 2010. Classification of bacteria and archaea in risk groups. TRBA 466 p. 187.

31. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, *et al.* Gene Ontology: tool for the unification of biology. *Nat Genet* 2000; **25**:25-29. PubMed doi:10.1038/75556

32. Tropis M, Lemassu A, Vincent V, Daïlé M. Structural elucidation of the predominant motifs of the major cell wall arabinogalactan antigens from the borderline species *Tsukamurella paurometabolum* and *Mycobacterium fallax*. *Glycobiology* 2005; **15**:677-686. PubMed doi:10.1093/glycob/cwi052

33. Klenk HP, Göker M. An update of the gene–classification of *Archaea* and Bacteria? *Syst Appl Microbiol* 2010; **33**:175-182. PubMed doi:10.1016/j.syapm.2010.03.003

34. Wu D, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, Kunin V, Goodwin L, Wu M, Tindall BJ, *et al.* A phylogeny-driven genomic encyclopedia of *Bacteria* and *Archaea*. *Nature* 2009; **462**:1056-1060. PubMed doi:10.1038/nature08656

35. List of growth media used at DSMZ: [http://www.dsmz.de/microorganisms/media_list.php](http://www.dsmz.de/microorganisms/media_list.php).

36. Gemeintholzer B, Dröge G, Zetsche H, Haszprunar G, Klenk HP, Güntsch A, Berendsohn WG, Wägele JW. The DNA Bank Network: the start from a German initiative. *Biopreservation and Biobanking* 2011; **9**:51-55. doi:10.1089/bio.2010.0029

37. JGI website. [http://www.jgi.doe.gov](http://www.jgi.doe.gov)

38. The Phred/Phrap/Consed software package. [http://www.phrap.com](http://www.phrap.com)
39. Sims D, Brettin T, Detter J, Han C, Lapidus A, Copeland A, Glavina Del Rio T, Nolan M, Chen F, Lucas S, et al. Complete genome sequence of Ky tococcus sedentarius type strain (541T). Stand Genomic Sci 2009; 1:12-20. PubMed doi:10.4056/sigs.761

40. Hyatt D, Chen GL, LoCascio PF, Land ML, Lari mer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 2010; 11:119. PubMed doi:10.1186/1471-2105-11-119

41. Pati A, Ivanova NN, Mikhailova N, Ovchinnikova G, Hooper SD, Lykidis A, Kyrpides NC. GenePRIMP: a gene prediction improvement pipeline for prokaryotic genomes. Nat Methods 2010; 7:455-457. PubMed doi:10.1038/nmeth.1457

42. Markowitz VM, Ivanova NN, Chen IMA, Chu K, Kyrpides NC. IMG ER: a system for microbial genome annotation expert review and curation. Bioinformatics 2009; 25:2271-2278. PubMed doi:10.1093/bioinformatics/btp393