Complete genome sequence of *Gordonia bronchialis* type strain (3410T)

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*Gordonia bronchialis* Tsukamura 1971 is the type species of the genus. *G. bronchialis* is a human-pathogenic organism that has been isolated from a large variety of human tissues. Here we describe the features of this organism, together with the complete genome sequence and annotation. This is the first completed genome sequence of the family Gordoniaceae. The 5,290,012 bp long genome with its 4,944 protein-coding and 55 RNA genes is part of the Genomic Encyclopedia of Bacteria and Archaea project.

Introduction

Strain 3410T (= DSM 43247 = ATCC 25592 = JCM 3198) is the type strain of the species *Gordonia bronchialis*, which is the type species of the genus. The genus *Gordonia* (formerly *Gordona*) was originally proposed by Tsukamura in 1971 [1]. The generic name *Gordona* has been chosen to honor Ruth E. Gordon, who studied extensively 'Mycobacterium' rhodochrous (included later as a member of *Gordona*) [1]. In 1977, it was subsumed into the genus *Rhodococcus* [2], but revived again in 1988 by Stackebrandt *et al.* [3]. At the time of writing, the genus contained 28 validly published species [4]. The genus *Gordonia* is of great interest for its bioremediation potential [5]. Some species of the genus have been used for the decontamination of polluted soils and water [6,7]. Other species were isolated from industrial waste water [8], activated sludge foam [9], automobile tire [10], mangrove rhizosphere [11], tar-contaminated oil [12], soil [13] and an oil-producing well [7]. Further industrial interest in *Gordonia* species stems from their use as a source of novel enzymes [14,15]. There are, however, quite a number of *Gordonia* species that are associated with human and animal diseases [16], among them *G. bronchialis*. Here we present a summary classification and a set of features for *G. bronchialis* 3410T, together with the description of the complete genomic sequencing and annotation.
Classification and features

Strain 3410^T was isolated from the sputum of a patient with pulmonary disease (probably in Japan) [1]. Further clinical strains in Japan have been isolated from pleural fluid, tumor in the eyelid, granuloma, leukorrhea, skin tissue and pus [17]. In other cases, G. bronchialis caused bacteremia in a patient with a sequestrated lung [18] and a recurrent breast abscess in an immunocompetent patient [19]. Finally, G. bronchialis was isolated from sternal wound infections after coronary artery bypass surgery [20]. G. bronchialis shares 95.8-98.7% 16S rRNA gene sequence similarity with the other type strains of the genus Gordonia, and 95.3-96.4% with the type strains of the neighboring genus Williamsia.

Figure 1 shows the phylogenetic neighborhood of for G. bronchialis 3410^T in a 16S rRNA based tree. The sequences of the two 16S rRNA gene copies in the genome of G. bronchialis 3410^T, differ from each other by one nucleotide, and differ by up to 5 nucleotides from the previously published 16S rRNA sequence from DSM 43247 (X79287). These discrepancies are most likely due to sequencing errors in the latter sequence.

In a very comprehensive study, Tsukamura analyzed a set of 100 quite diverse characters for 41 G. bronchialis strains isolated from sputum of patients with pulmonary disease, including the type strain [1]. Unfortunately, this study does not present the characteristics of the type strain 3410^T as such. We nevertheless first present these data, as this study gives a good overview of the species itself. In order to summarize the data here, we regard positive reactions in more than 34 strains as positive, and positive reactions in only 13 or less strains as negative. Most characters, however, are either clearly positive (40 or 41 strains) or clearly negative (0 or 1 strains). The detailed methods are reported elsewhere [25,26].

G. bronchialis is Gram-positive (Table 1) and shows slight but not strong acid-fastness. A mycelium is not observed.

G. bronchialis strains are non-motile and produce neither conidia nor endospores [1,3]. G. bronchialis is an obligately aero-
bic chemoorganotroph with an oxidative-type metabolism [3]. The cells are rod-shaped and show compact grouping (like a cord) (Figure 2), and provide a rough colonial morphology with pinkish-brown colony pigmentation [1]. Photochromogenicity was not observed. *G. bronchialis* grows quite rapidly [1], with visible colonies appearing within 1-3 days [1,36]. *G. bronchialis* is positive for catalase and nitrate reduction, but arylsulphatase (3 days and 2 weeks), salicylate and PAS degradation was not observed [1]. Growth occurs on 0.2% salicylate and PAS degradations were not observed [1]. Growth occurs in 0.2% sodium p-aminosalicylate and 62.5 and 125 µg NH₂OH·HCl/ml, but not with 250 or 500 µg. *G. bronchialis* is tolerant to both 0.1 and 0.2% picric acid. *G. bronchialis* grows at 28°C and 37°C, but not at 45°C or 52°C [1]. *G. bronchialis* is positive for acetamidase, urease, nicotinamidase and pyrazinamidase, but negative for benzamidase, isonicotinamidase, salicylamidase, allanoinase, succinamidase, and malonamidase [1]. *G. bronchialis* utilizes acetate, succinate, malate, pyruvate, fumarate, glycerol, glucose, mannose, trehalose, inositol, fructose, sucrose, ethanol, propanol, and propylene glycol as a carbon source for growth, but not citrate, benzoate, malonate, galactose, arabinose, xylose, rhamnose, raffinose, manninitol, sorbitol, or various forms of butylene glycol (1,3-; 1,4-; 2,3-) [1]. *G. bronchialis* utilizes t-glutamate and acetamide as a N-C source, but not t-serine, benzamide, monoethanolamine or trimethylene diamine. Glucosamine is utilized by 18 strains [1]. *G. bronchialis* utilizes as nitrogen source t-glutamate, t-serine, t-methionine, acetamide, urea, pyrazinamide, isonicotinamide, nicotinamide, succinamide, but not benzamide and nitrite. Nitrate is utilized by 25 strains as nitrogen source [1]. *G. bronchialis* strains do not produce nicotinic acid. *G. bronchialis* strains do not grow on TCH medium (10 µg/ml) or on salicylate medium (0.05% and 0.01%) [1].

In the following, characteristics of the type strain 3410T are presented: strain 3410T reduces nitrate and hydrolyses urea, but it does not hydrolyze aesculin, allantoin or arbutin [37]. It decomposes (% w/v) starch (1) and uric acid (0.5), but not hypoxanthine (0.4), tributyrin (0.1), tween 80 (1), tyrosine (0.5) and xanthine (0.4) [37]. It grows on glycerol (1) and sodium fumarate (1) as sole carbon sources (% w/v), but not on arbutin (1), D-cellobiose (1), N-acetyl-D-glucosamine (0.1), adipic acid (0.1), betaine (0.1), oxalic acid (0.1), propan-1-ol (0.1) [37]. Strain 3410T grows in the presence (% w/v) of oleic acid (0.1) and zinc chloride (0.001) [37].

In an API ZYM test, strain 3410T reacts positively for alkaline phosphatase, butyrate esterase, leucine arylamidase and naphtol-AS-BI-phosphohydrolase, but not for caprylate esterase, cystine arylamidase, β-glucosidase, myristate lipase, and valine arylamidase [13]. Complementary to the results of Tsukamura [1], strain 3410T utilizes as sole carbon source D(+) cellobiose, D(+) galactose, D(+) mannose, meso-inositol, L(+) rhamnose and sodium succinate, but not D(-)lactose, D(-) ribose, sodium benzoate and sodium citrate [38]. The use of D(+) galactose [38] might contrast above reported results from Tsukamura [1]. l-threonine and l-valine are used as sole nitrogen source by strain 3410T, but not l-asparagine, l-proline and l-serine [38]. Interestingly, Tsukamura reports that 40 out of 41 strains utilize L-serine as sole nitrogen source [1], and it is not clear if the only negative strain in the Tsukamura study could be the type strain 3410T [1].

In the BiOLOG system, strain 3410T reacts positively for α-cyclodextrin, β-cyclodextrin, dextrin, glycogen, maltose, maltotriose, D-mannose, 3-methyl glucose, palatinose, l-raffinose, salicin, turanose, D-xylose, L-lactic acid, methyl succinate, N-acetyl-L-glutamic acid [12], but not for N-acetylglucosamine, amygdalin, D-arabitol, L-rhamnose, D-ribose, D-sorbitol, D-trehalose, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, α-ketoglutaric acid, α-ketovaleric acid, L-lactic acid methyester, L-malic acid, propionic acid, succinic acid, alaninamide, L-alanine and glycerol [12]. Further carbon source utilization results are published elsewhere [8].

Drug susceptibility profiles of 13 *G. bronchialis* strains from clinical samples have been examined in detail [17], but they are too complex to summarize here. No significant matches with any 16S rRNA sequences from environmental genomic samples and surveys are reported at the NCBI BLAST server (November 2009).

**Chemotaxonomy**

The cell-wall peptidoglycan is based upon meso-diaminopimelic acid (variation Aly). The glycan moiety of the peptidoglycan contains N-glycolylmuramic acid. The wall sugars are arabinose and galactose. Mycolic acids are present with a range of ca. 48-66 carbon atoms. The predominant menaquinone is MK-9(H2), with only low amounts of MK-9(H0), MK-8(H2), and MK-7(H2) [3,8,39-41]. Moreover, the cell envelope of *G. bronchialis* 3410T.
contains a lipoarabinomannan-like lipoglycan [42]. The same study also observed a second amphiphilic fraction with properties suggesting a phosphatidylinositol mannoside [42]. The cellular fatty acid composition (%) is C<sub>16:0</sub> (23), tuberculostearic acid (20), C<sub>16:1cis9</sub> (16), C<sub>16:1cis7</sub> (11), C<sub>18:1</sub> (10), and 10-methyl C<sub>17:0</sub> (7). All other fatty acids are at 3% or below [8].

Table 1. Classification and general features of *G. bronchialis* 3410<sup>T</sup> according to the MIGS recommendations [27]

| MIGS ID | Property               | Term                                      | Evidence code |
|---------|------------------------|-------------------------------------------|---------------|
|         | Current classification |                                           |               |
|         | Domain                  | Bacteria                                  | TAS [28]      |
|         | Phylum                  | Actinobacteria                            | TAS [29]      |
|         | Class                   | Actinobacteria                            | TAS [30]      |
|         | Order                   | Actinomycetales                           | TAS [30]      |
|         | Suborder                | Corynebacterineae                         | TAS [30,31]   |
|         | Family                  | Gordoniaceae                              | TAS [30]      |
|         | Genus                   | Gordonia                                  | TAS [3,30,32] |
|         | Species                 | Gordonia bronchialis                      | TAS [1]       |
|         | Type strain             | 3410                                      | TAS [1]       |
|         | Gram stain              | positive                                  | TAS [1]       |
|         | Cell shape              | short rods in compact grouping (cord-like)| TAS [1]       |
|         | Motility                | non-motile                                | TAS [1]       |
|         | Sporulation             | non-sporulating                           | TAS [1]       |
|         | Temperature range        | grows at 28°C and 37°C, not at 45°C       | TAS [1]       |
|         | Optimum temperature     | probably between 28°C and 37°C            | TAS [1]       |
|         | Salinity                | 2.5%                                      | TAS [33]      |
| MIGS-22 | Oxygen requirement      | obligate aerobe                           | TAS [1]       |
| MIGS-6  | Habitat                 | human                                     | TAS [1]       |
| MIGS-15 | Biotic relationship     | free living                               | NAS           |
| MIGS-14 | Pathogenicity           | opportunistic pathogen                    | TAS [1,17-20] |
|         | Biosafety level         | 2                                         | TAS [34]      |
|         | Isolation               | sputum from human with pulmonary disease in (probably) Japan | TAS [1] |
| MIGS-4  | Geographic location     | global                                    | TAS [1,17-20] |
| MIGS-5  | Sample collection time  | 1971 or before                            | TAS [1]       |
| 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 the Gene Ontology project [35]. If the evidence code is IDA, then 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 [http://standardsingenomics.org](http://standardsingenomics.org)
**Gordonia bronchialis** type strain (3410T)

Genome OnLine Database [24] 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.

![Scanning electron micrograph of *G. bronchialis* 3410T](image)

**Figure 2.** Scanning electron micrograph of *G. bronchialis* 3410T

**Table 2.** Genome sequencing project information

| MIGS ID | Property                        | Term                                           |
|---------|--------------------------------|------------------------------------------------|
| MIGS-31 | Finishing quality              | Finished                                       |
| MIGS-29 | Libraries used                 | Two Sanger libraries: 8kb pMCL200, fosmid pcc1Fos |
|         |                                | One 454 pyrosequence standard library          |
| MIGS-28 | Sequencing platforms           | ABI3730, 454 GS FLX                            |
| MIGS-30 | Sequencing coverage            | 7.98× Sanger; 23.2× pyrosequence               |
| MIGS-31.2| Assemblers                    | Newbler, phrap                                 |
| MIGS-32 | Gene calling method            | Prodigal, GenePRIMP                             |
|         | INSDC ID                       | CP001802                                       |
|         | GenBank Date of Release        | October 28, 2009                               |
|         | GOLD ID                        | Gc01134                                        |
|         | NCBI project ID                | 29549                                          |
|         | Database: IMG-GEBA             | 2501939625                                     |
| MIGS-13 | Source material identifier     | DSM 43247                                      |
|         | Project relevance              | Tree of Life, GEBA                              |

**Growth conditions and DNA isolation**

*G. bronchialis* 3410T, DSM 43247, was grown in DSMZ 535 [43] at 28°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 with modification st/LALMP for cell lysis according to Wu *et al.* [44].

**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 on the JGI [website](#). 454 Pyrosequencing reads were assembled using the Newbler assembler version.
1.1.02.15 (Roche). Large Newbler contigs were broken into 5,776 overlapping fragments of 1,000 bp 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 [45] or transposon bombing of bridging clones (Épicentre Biotechnologies, Madison, WI). Gaps between contigs were closed by editing in Consed, custom primer walk or PCR amplification. A total of 876 primer walk reactions, 12 transposon bombs, and 1 pcr shatter libraries were necessary 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 51.2× coverage of the genome. The final assembly contains 52,329 Sanger and 508,130 pyrosequence reads.

Genome annotation
Genes were identified using Prodigal [46] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI GenePRIMP pipeline [47]. 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 manual functional annotation was performed within the Integrated Microbial Genomes Expert Review (IMG-ER) platform [48].

Genome properties
The genome consists of a 5.2 Mbp long chromosome and a 81,410 bp plasmid (Table 3 and Figure 3). Of the 4,999 genes predicted, 4,944 were protein coding genes, and 55 RNAs; 264 pseudogenes were also identified. The majority of the protein-coding genes (69.1%) were assigned with a putative function while those remaining were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 4.

| Table 3. Genome Statistics | Value | % of Total |
|----------------------------|-------|------------|
| Genome size (bp)           | 5,290,012 | 100.00%    |
| DNA coding region (bp)     | 4,897,508 | 92.58%     |
| DNA G+C content (bp)       | 3,546,559 | 67.04%     |
| Number of replicons        | 1     |            |
| Extrachromosomal elements  | 1     |            |
| Total genes                | 4,999 | 100.00%    |
| RNA genes                  | 55    | 1.10%      |
| rRNA operons               | 2     |            |
| Protein-coding genes       | 4,944 | 98.90%     |
| Pseudo genes               | 264   | 5.28%      |
| Genes with function prediction | 3,453  | 69.07%     |
| Genes in paralog clusters  | 804   | 16.08%     |
| Genes assigned to COGs     | 3,335 | 66.71%     |
| Genes assigned Pfam domains | 3,508  | 70.17%     |
| Genes with signal peptides | 1,038 | 20.76%     |
| Genes with transmembrane helices | 1,209 | 24.18%     |
| CRISPR repeats             | 0     |            |
**Figure 3.** Graphical circular map of the chromosome and the plasmid. 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 4.** Number of genes associated with the general COG functional categories

| Code | value | %age | Description                                           |
|------|-------|------|-------------------------------------------------------|
| J    | 164   | 3.3  | Translation, ribosomal structure and biogenesis      |
| A    | 1     | 0.0  | RNA processing and modification                      |
| K    | 357   | 7.2  | Transcription                                         |
| L    | 238   | 4.8  | Replication, recombination and repair                 |
| B    | 1     | 0.0  | Chromatin structure and dynamics                      |
| D    | 28    | 0.6  | Cell cycle control, mitosis and meiosis              |
| Y    | 0     | 0.0  | Nuclear structure                                     |
| V    | 50    | 1.0  | Defense mechanisms                                   |
| T    | 158   | 3.2  | Signal transduction mechanisms                        |
| M    | 133   | 2.7  | Cell wall/membrane biogenesis                        |
| N    | 2     | 0.0  | Cell motility                                         |
| Z    | 1     | 0.0  | Cytoskeleton                                          |
| W    | 0     | 0.0  | Extracellular structures                              |
| U    | 30    | 0.6  | Intracellular trafficking and secretion               |
Table 4 (cont.) Number of genes associated with the general COG functional categories

| Code | value | %age  | Description                                                                 |
|------|-------|-------|----------------------------------------------------------------------------|
| O    | 123   | 2.5   | Posttranslational modification, protein turnover, chaperones                |
| C    | 261   | 5.3   | Energy production and conversion                                            |
| G    | 197   | 4.0   | Carbohydrate transport and metabolism                                       |
| E    | 283   | 5.7   | Amino acid transport and metabolism                                         |
| F    | 90    | 1.8   | Nucleotide transport and metabolism                                         |
| H    | 172   | 3.5   | Coenzyme transport and metabolism                                          |
| I    | 270   | 5.5   | Lipid transport and metabolism                                              |
| P    | 202   | 4.1   | Inorganic ion transport and metabolism                                      |
| Q    | 210   | 4.2   | Secondary metabolites biosynthesis, transport and catabolism                |
| R    | 505   | 10.2  | General function prediction only                                            |
| S    | 286   | 5.8   | Function unknown                                                            |
| -    | 1664  | 33.7  | Not in COGs                                                                 |

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While the manuscript was in editorial processing a 29th species in the genus *Gordonia* was published: *G. hankookensis* [49], which is not featured in Figure 1.

References
1. Tsukamura M. Proposal of a new genus, *Gordona*, for slightly acid-fast organisms occurring in sputa of patients with pulmonary disease and in soil. *J Gen Microbiol* 1971; 68:15-26. PubMed
2. Goodfellow M, Alderson G. The Actinomycete-genus *Rhodococcus*: A Home for the 'rhodochrous' Complex. *J Gen Microbiol* 1977; 100:99-122. PubMed
3. Stackebrandt E, Smida J, Collins MD. Evidence of phylogenetic heterogeneity within the genus *Rhodococcus*: revival of the genus *Gordona* (Tsukamura). *J Gen Microbiol* 1988; 34:341-348. doi:10.2323/jgm.34.341
4. Euzéby JP. List of bacterial names with standing in nomenclature. http://www.bacterio.cict.fr/g/gordonia.html
5. Yoon JH, Lee J, Kang S, Takeuchi M, Shin Y, Lee S, Kang K, Park Y. *Gordonia nitida* sp. nov., a bacterium that degrades 3-ethylpyridine and 3-methylpyridine. *Int J Syst Evol Microbiol* 2000; 50:1203-1210. PubMed
6. Bell KS, Philp JC, Aw DWJ, Christofi N. The genus *Rhodococcus*. *J Appl Microbiol* 1998; 85:195-210. PubMed doi:10.1046/j.1365-2672.1998.00525.x
7. Xue Y, Sun X, Zhou P, Liu R, Liang F, Ma Y. *Gordonia paraffinivorans* sp. nov., a hydrocarbon-degrading actinomycete isolated from an oil-producing well. *Int J Syst Evol Microbiol* 2003; 53:1643-1646. PubMed doi:10.1099/ijs.0.02605-0
8. Kim KK, Lee CS, Kroppenstedt RM, Stackebrandt E, Lee ST. *Gordonia sihwensis* sp. nov., a novel nitrate-reducing bacterium isolated from a wastewater-treatment bioreactor. *Int J Syst Evol Microbiol* 2003; 53:1427-1433. PubMed doi:10.1099/ijs.0.02224-0
9. Soddell JA, Stainsby FM, Eales KL, Seviour RJ, Goodfellow M. *Gordonia delluvii* sp. nov., an ac-
10. Linos A, Steinbüchel A, Spröer C, Kroppenstedt RM. *Gordonia polysoprenivorans* sp. nov., a rubber-degrading actinomycete isolated from an automobile tyre. *Int J Syst Bacteriol* 1999; **49:**1785-1791. PubMed

11. Takeuchi M, Hatano K. *Gordonia rhizosphera* sp. nov. isolated from the mangrove rhizosphere. *Int J Syst Bacteriol* 1998; **48:**907-912. PubMed

12. Kummer C, Schumann P, Stackebrandt E. *Gordonia alkanivorans* sp. nov., isolated from tar-contaminated soil. *Int J Syst Bacteriol* 1999; **49:**1513-1522. PubMed

13. Shen FT, Goodfellow M, Jones AL, Chen YP, Arun AB, Lai WA, Rekha PD, Young CC. *Gordonia soli* sp. nov., a novel actinomycete isolated from soil. *Int J Syst Evol Microbiol* 2006; **56:**2597-2601. PubMed doi:10.1099/ijs.0.64492-0

14. Kim SB, Brown R, Oldfield C, Gilbert S, Iliarionov S, Goodfellow M. *Gordonia amicalis* sp. nov., a novel dibenzothiophene-desulphurizing actinomycete. *Int J Syst Evol Microbiol* 2000; **50:**2031-2036. PubMed

15. Kim SB, Brown R, Oldfield C, Gilbert SC, Goodfellow M. *Gordonia desulfuricans* sp. nov., a benzo-thiophene-desulphurizing actinomycete. *Int J Syst Bacteriol* 1999; **49:**1845-1851. PubMed

16. Goodfellow M, Maldonado LA. 2006. The families Dietziaceae, Gordoniaceae, Nocardiaceae and Tsukamurellaceae. In: F Dworkin, S Falkow, KH Schleifer E Stackebrandt (eds), *The Prokaryotes,* vol. 3. Springer, New York.

17. Aoyama K, Kang Y, Yazawa K, Gonoi T, Kamei K, Mikami Y. Characterization of clinical isolates of *Gordonia* species in Japanese clinical samples during 1998-2008. *Mycopathologia* 2009; **168:**175-183. PubMed doi:10.1007/s11046-009-9213-9

18. Sng LH, Koh TH, Toney SR, Floyd M, Butler WR, Tan BH. *Bacteremia* caused by *Gordonia bronchialis* in a patient with sequestered lung. *J Clin Microbiol* 2004; **42:**2870-2871. PubMed doi:10.1128/JCM.42.6.2870-2871.2004

19. Werno AM, Anderson TP, Chambers ST, Laird HM, Murdoch DR. Recurrent breast abscess caused by *Gordonia bronchialis* in an immunocompetent patient. *J Clin Microbiol* 2005; **43:**3009-3010. PubMed doi:10.1128/JCM.43.6.3009-3010.2005

20. Richet HM, Craven PC, Brown JM, Lasker BA, Cox CD, McNeil MM, Tice AD, Jarvis WR, Tablan OC. A cluster of *Rhodococcus* (*Gordona*) *bronchialis* sternal-wound infections after coronary-artery bypass surgery. *N Engl J Med* 1991; **324:**104-109. PubMed

21. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 2000; **17:**540-552. PubMed

22. 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

23. Stamatakis A, Hoover P, Rougemont J. A Rapid Bootstrap Algorithm for the RAxML Web Servers. *Syst Biol* 2008; **57:**758-771. PubMed doi:10.1086/1063510802429642

24. Liolios K, Mavromatis K, Tavernarakis N, Kyrpides NC. The Genomes On Line Database (GOLD) in 2007: status of genomic and metagenomic projects and their associated metadata. *Nucleic Acids Res* 2008; **36:**D475-D479. PubMed doi:10.1093/nar/gkm884

25. Tsukamura M. Identification of mycobacteria. *Tubercle, London* 1967; **48:**311-338. doi:10.1016/S0041-3879(67)80040-0

26. Tsukamura M. Adansonian classification of mycobacteria. *J Gen Microbiol* 1966; **45:**253-273. PubMed

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

28. 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

29. Garrity GM, Holt JG. The Road Map to the Manual. In: Garrity GM, Boone DR, Castenholz RW (eds), *Berger’s Manual of Systematic Bacteriology,* Second Edition, Volume 1, Springer, New York, 2001, p. 119-169.

30. Stackebrandt E, Rainey FA, Ward-Rainey NL. Proposal for a new hierarchic classification sys-
tem, Actinobacteria classis nov. Int J Syst Bacteriol 1997; 47:479-491.

31. Zhi XY, Li WJ, Stackebrandt E. An update of the structure and 16S rRNA 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 Bacteriol 1997; 47:479-491.

32. Zhi XY, Li WJ, Stackebrandt E. An update of the structure and 16S rRNA 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 Bacteriol 1997; 47:479-491. PubMed doi:10.1099/ijs.0.65780-0

33. Zhi XY, Li WJ, Stackebrandt E. An update of the structure and 16S rRNA 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 Bacteriol 1997; 47:479-491. PubMed doi:10.1099/ijs.0.65780-0

34. Zhi XY, Li WJ, Stackebrandt E. An update of the structure and 16S rRNA 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 Bacteriol 1997; 47:479-491. PubMed doi:10.1099/ijs.0.65780-0

35. Zhi XY, Li WJ, Stackebrandt E. An update of the structure and 16S rRNA 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 Bacteriol 1997; 47:479-491. PubMed doi:10.1099/ijs.0.65780-0

36. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

37. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

38. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

39. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

40. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

41. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

42. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

43. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

44. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

45. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

46. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

47. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

48. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

49. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

50. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

51. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

52. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

53. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

54. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

55. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

56. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

57. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

58. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

59. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

60. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

61. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

62. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

63. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

64. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

65. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.

66. Editor L. Validation List No. 30. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:371.