High quality draft genome sequence of *Leucobacter chironomi* strain MM2LB^T^ (DSM 19883^T^) isolated from a *Chironomus* sp. egg mass

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**Abstract**

*Leucobacter chironomi* strain MM2LB^T^ (Halpern et al., Int J Syst Evol Microbiol 59:665-70 2009) is a Gram-positive, rod shaped, non-motile, aerobic, chemoorganotroph bacterium. *L. chironomi* belongs to the family *Microbacteriaceae*, a family within the class *Actinobacteria*. Strain MM2LB^T^ was isolated from a chironomid (Diptera; Chironomidae) egg mass that was sampled from a waste stabilization pond in northern Israel. In a phylogenetic tree based on 16S rRNA gene sequences, strain MM2LB^T^ formed a distinct branch within the radiation encompassing the genus *Leucobacter*. Here we describe the features of this organism, together with the complete genome sequence and annotation. The DNA GC content is 69.90%. The chromosome length is 2,964,712 bp. It encodes 2,690 proteins and 61 RNA genes. *L. chironomi* genome is part of the Genomic Encyclopedia of Type Strains, Phase I: the one thousand microbial genomes (KMG) project.

**Keywords:** *Leucobacter chironomi, Microbacteriaceae, Chironomid, Chironomus, Egg mass, Hexavalent chromium*

**Introduction**

Strain MM2LB^T^ (=DSM 19883^T^ = JCM 17022^T^ = LMG 24399^T^), is the type strain of the species *Leucobacter chironomi* [1]. The genus *Leucobacter* was formed by Takeuchi et al. [2] and currently includes 15 species. Members of this genus were found in a verity of environments including air [2,3], nematodes [4,5], sediments with chromium contamination [6], cow dung [7], compost [8], fermented seafood [9], phyllosphere [10] and chironomids (Diptera) [1].

*L. chironomi* MM2LB^T^, was isolated from an insect egg mass (*Chironomus* sp.) that was sampled from a waste stabilization pond in northern Israel [1]. Chironomids (Insecta; Diptera; Chironomidae; *Chironomus* sp.), also known as the non biting midges, are aquatic insects. They undergo a complete metamorphosis of four life stages; egg, larva, pupa and adult that emerges into the air. The eggs are deposited by the adult female at the water’s edge in egg masses which contain hundreds of eggs [11]. Chironomid egg masses were found as natural reservoirs of *Vibrio cholerae* and *Aeromonas* species [11-17]. Strain MM2LB^T^ was isolated in the course of a study that explored the endogenous bacterial communities in chironomid egg masses [1]. Using 454-pyrosequencing technique, Senderovich & Halpern [18], showed that the prevalence of *Leucobacter* in chironomid egg masses and larval endogenous bacterial communities is 0.1% and 0.2%, respectively.

Here we describe a summary classification and a set of the features of *L. chironomi*, together with the genome sequence description and annotation.

**Organism Information**

**Classification and features**

A taxonomic study using a polyphasic approach placed *L. chironomi* strain MM2LB^T^ in the genus *Leucobacter* within the family *Microbacteriaceae* (order; *Actinomycetales*, class; *Actinobacteria*, phylum; *Actinobacteria* (Figure 1). The family *Microbacteriaceae* comprises more than 40 genera and a large variety of species and phenotypes.
**L. chironomi** strain MM2LB\(^T\) is a Gram-positive, aerobic, chemo-organotrophic, non-motile single cell rod (Figure 2). After 48 h incubation on LB agar at 30°C, colonies are opaque, circular, with entire margins and yellow-coloured [1]. Growth is observed at 17–37°C (optimum 30°C), with 0–7% (w/v) NaCl (optimum 0–1.0% NaCl) and at pH 4.0–9.5 (optimum pH 6.0–8.0) (Table 1). Oxidase reaction is negative; catalase reaction is weakly positive. Strain MM2LB\(^T\) produces acetoin and reduces nitrate to nitrogen; \(\text{H}_2\text{S}\) and indole are not produced; urea and gelatin are not hydrolyzed; citrate is not utilized; \(\beta\)-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activities are absent; Putrescine and glycerol are utilized [1].

**Chemotaxonomic data**

The dominant cellular fatty acids are anteiso-C\(_{15:0}\), anteiso-C\(_{17:0}\) and iso-C\(_{16:0}\). Cell-wall amino acids are alanine, glycine, threonine, \(\text{DAB}\), \(\gamma\)-aminobutyric acid and glutamic acid. Strain MM2LB\(^T\) has a B-type crosslinked peptidoglycan. The major menaquinone is MK-11; MK-10 and MK-12 occur in minor amounts [1]. Strain MM2LB\(^T\) is able to grow in the presence of up to 18.0 mM Cr(VI) [1].

**Genome sequencing information**

**Genome project history**

*L. chironomi* MM2LB\(^T\), was selected for sequencing due to its phylogenetic position [19-21], and is part of Genomic Encyclopedia of Type Strains, Phase I: the one thousand microbial genomes (KMG) study [22] which aims not only to increase the sequencing coverage of key reference microbial genomes [23] but also to generate a large genomic basis for the discovery of genes encoding novel enzymes [24]. The sequencing project is accessible in the Genomes OnLine Database [25] and the genome sequence is deposited in GenBank. Sequencing, finishing and annotation were accomplished by the DOE Joint Genome Institute (JGI) [26] using state of the art approaches.

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**Figure 1** Phylogenetic tree highlighting the position of *Leucobacter chironomi* relative to the type strains of the other species within the genus *Leucobacter*. The sequence alignments were performed by using the CLUSTAL W program and the tree was generated using the maximum likelihood method in MEGA 5 software [41]. Bootstrap values (from 1,000 replicates) greater than 50% are shown at the branch points. The bar indicates a 1% sequence divergence.

**Figure 2** Electron micrograph of negatively stained cells of strain MM2LB\(^T\). Cells are nonflagellated rods. Bar, 200 nm.
The project information is summarized in Table 2.

**Growth conditions and genomic DNA preparation**

*L. chironomi* MM2LB\textsuperscript{T}, DSM 19883, was grown in Trypticase Soy Yeast Extract medium (DSMZ medium 92) at 28°C \[27\]. DNA was isolated from 0.5-1.0 g of cell paste using Masterpure DNA purification kit (Epicentre MGP04100) following the standard protocol as recommended by the manufacturer with additional 7.5 units of each of the following enzymes achromopeptidase, lysostaphin, mutanolysin and 2100 units of lysozyme, incubated for one hour at 37°C, followed by addition of 1 \( \mu \)l proteinase K and incubation for 20 min at 70°C for cell lysis. DNA is available through the DNA Bank Network \[28\].

**Genome sequencing and assembly**

The draft genome of *L. chironomi* DSM 19883\textsuperscript{T} was generated at the DOE Joint genome Institute (JGI) using the Illumina technology \[27\]. An Illumina standard shotgun library was constructed and sequenced using the Illumina HiSeq 2000 platform which generated 13,901,154 reads totaling 2,085.2 Mb. All general aspects of library construction and sequencing performed at the JGI can be found at the Institute web site \[25\]. All raw Illumina sequence data was passed through DUK, a filtering program developed at JGI, which removes known Illumina sequencing and library preparation artifacts (Mingkun L, et al., unpublished, 2011). Following steps were then performed for assembly: (1) filtered Illumina reads were assembled using Velvet \[31\], (2) 1–3 kb simulated paired end reads

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### Table 1: Classification and general features of *Leucobacter chironomi* strain MM2LB\textsuperscript{T} according to the MIGS recommendations \[42\], published by the Genome Standards Consortium \[43\] and the Names for Life database \[44\]

| MIGS ID | Property | Term | Evidence code\textsuperscript{a} |
|---------|----------|------|----------------------------------|
| Current classification | Domain | Bacteria | TAS [44,45] |
| Phylum | Actinobacteria | TAS [46] |
| Class | Actinobacteria | TAS [47] |
| Order | Actinomycetales | TAS [47-50] |
| Family | Microbacteriaceae | TAS [47;48;51;52] |
| Genus | Leucobacter | TAS [2] |
| Species | Leucobacter chironomi | TAS [1] |
| Type strain | MM2LB\textsuperscript{T} | TAS [1] |
| Gram stain | positive | TAS [1] |
| Cell shape | rod | TAS [1] |
| Motility | Non-motile | TAS [1] |
| Sporulation | Non-sporulating | IDS |
| Temperature range | 17-37°C | TAS [1] |
| Optimum Temperature | 30°C | TAS [1] |
| pH range | 4.0-9.5 | TAS [1] |
| Optimum pH | 6.0-8.0 | TAS [1] |
| Carbon source | Putrescine and Glycerol\textsuperscript{b} | TAS [1] |
| Habitat | Aquatic/Insect host | TAS [1] |
| Salinity | 0-7.0% NaCl (w/v) | TAS [1] |
| Oxygen requirement | Aerobic | TAS [1] |
| Biotic relationship | Commensal (Insect, chironomid) | TAS [1] |
| Pathogenicity | None | NAS |
| Geographic location | Northern Israel | TAS [1] |
| Sample collection | July 2006 | TAS [1] |
| Latitude | 32.669167 | IDS |
| Longitude | 35.128639 | IDS |
| Altitude | 40 m | TAS [1] |

\textsuperscript{a}Evidence codes - IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). Evidence codes are from the Gene Ontology project \[53\].

\textsuperscript{b}The only carbon source that was positive for this strain, out of all carbon sources that were tested (strain MM2LB\textsuperscript{T} does not use carbohydrates, not even glucose) \[1\].

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were created from Velvet contigs using wgsim [32], (3) Illumina reads were assembled with simulated read pairs using Allpaths–LG [33]. Parameters for assembly steps were: (1) Velvet (velveth: 63 –shortPaired and velvetg: very clean yes –exportFiltered yes –min contig lgth 500 –scaffolding no –cov cutoff 10) (2) wgsim (–e0 –1 100 –2 100 –r 0 –R 0 –X 0) (3) Allpaths–LG (PrepareAllpathsInputs: PHRED 64 = 1 PLOIDY = 1 FRAG COVERAGE = 125 JUMP COVERAGE = 25 LONG JUMP COV = 50, RunAllpathsLG: THREADS = 8 RUN = std shredpairs TARGETS = standard VAPI WARN ONLY = True OVERWRITE = True). The final draft assembly contained 27 contigs in 27 scaffolds and is based on 361.8 Mb of Illumina data, which provides an average 122.1X coverage of the genome.

Genome annotation

Genes were detected using the Prodigal software [34] at the DOE-JGI Genome Annotation pipeline [35,36]. The CDSs predicted were translated and searched against the Integrated Microbial Genomes (IMG) non-redundant database, UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction and functional annotation analysis was carried out in the Integrated Microbial Genomes (IMG-ER) platform [37].

Genome properties

The assembly of the draft genome sequence consists of 27 scaffolds amounting to 2,964,712 bp, and the G + C content is 69.9% (Table 3). Of the 2,751 genes predicted, 2,690 were protein-coding genes, and 61 RNAs. The majority of the protein-coding genes (79.5%) 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 2 Genome sequencing project information

| MIGS ID | Property                | Term                                      |
|---------|-------------------------|-------------------------------------------|
| MIGS 31.1 | Sequencing quality     | Level 2: High-Quality Draft               |
| MIGS-28 | Libraries used          | Illumina Std. shotgun library             |
| MIGS 29 | Sequencing method       | Illumina HiSeq 2000                      |
| MIGS 31.2 | Fold coverage          | 122.1X                                   |
| MIGS 30 | Assemblers              | Velvet (v. 1.1.04), ALLPATHS –LG (v. r42328) |
| MIGS 32 | Gene calling method     | Prodigal 2.5                             |
|        | Locus Tag               | H629                                      |
|        | Genbank ID              | ATXU000000000                            |
|        | Genbank Date of Release | 12-DEC-2013                              |
|        | GOLD ID                 | Gp0013907                                |
|        | BIOPROJECT              | PRNA188922                               |
| MIGS-13 | Source Material Identifier | DSM 198831                             |
|        | Project relevance       | GBEA-KMG, Tree of: Life                  |

Table 3 Genome statistics

| Attribute                  | Value  | % of total |
|----------------------------|--------|------------|
| Genome size (bp)           | 2,964,712 | 100.00    |
| DNA coding (bp)            | 2,686,984 | 90.60      |
| DNA G + C (bp)             | 2,072,411 | 69.90      |
| DNA scaffolds              | 27     | 100.00     |
| Total genes                | 2,751  | 100.00     |
| Protein coding genes       | 2,690  | 97.78      |
| RNA genes                  | 61     | 2.22       |
| Pseudo genes               | 0      | 0          |
| Genes in internal clusters | 2,248  | 81.7       |
| Genes with function prediction | 2,188 | 66.96      |
| Genes assigned to COGs     | 1,842  | 66.96      |
| Genes with Pfam domains    | 2,249  | 81.7       |
| Genes with signal peptides | 158    | 5.74       |
| Genes with transmembrane helices | 755   | 27.44      |
| CRISPR repeats             | 0      | 0          |

Insights from the genome sequence

Senderovich and Halpern [18,38], demonstrated that endogenous bacteria in chironomids have a role in protecting their insect host from toxic metals. L. chironomi strain MM2LB T, which was isolated from a chironomid egg mass was found to tolerate up to 18 mM Cr(VI) [1]. Other Leucobacter species like L. alluvii, L. aridicollis, L. chromireducens, L. chromiresistens, L. komagatae, L. luti and L. salisicius, have also been found to be resistant to hexavalent chromium [1,2,5,39,40]. A chromate membrane transport protein A (ChrA) was detected in the genome of the chromate-resistant bacterium, L. salisicius M1-8 T [40]. However, this gene or other genes with chromium reduction predicted functions were not identified in L. chironomi MM2LB T genome. Nevertheless, three genes for ABC-type metal ion transport system (permease, ATPase and periplasmic components), were detected in the genome of strain MM2LB T. These genes may have a role in L. chironomi chromium tolerance.

More genes that may indicate the potential of strain MM2LB T to tolerate or detoxify metals, were also detected. Among them are genes for arsenical resistance: arsenical-resistance protein (arsB); arsenite efflux pump ACR3 and related permeases. Other genes suggest the potential of L. chironomi to survive in the presence of other toxic metals: copper chaperone; copper-(or silver)-translocating P-type ATPase; heavy metal-(Cd/Co/Hg/Pb/Zn)-translocating P-type ATPase and transcriptional regulator (ArsR family) which is involved in stress-response to heavy metal ions.

Three genes encoding drug resistance transporters are found in strain MM2LB T genome: drug resistance transporter Bcr/CflA subfamily; multidrug resistance
Table 4 Number of genes associated with the general COG functional categories

| Code | Value | % age | Description |
|------|-------|-------|-------------|
| J    | 161   | 8.39  | Translation, ribosomal structure and biogenesis |
| A    | 1     | 0.05  | RNA processing and modification |
| K    | 173   | 8.39  | Transcription |
| L    | 108   | 5.24  | Replication, recombination and repair |
| B    | 1     | 0.05  | Chromatin structure and dynamics |
| D    | 19    | 0.92  | Cell cycle control, cell division, chromosome partitioning |
| V    | 34    | 1.65  | Defense mechanisms |
| T    | 77    | 3.74  | Signal transduction mechanisms |
| M    | 90    | 4.37  | Cell wall/membrane biogenesis |
| N    | 0     | 0     | Cell motility |
| U    | 23    | 1.12  | Intracellular trafficking, secretion and vesicular transport |
| O    | 60    | 2.91  | Posttranslational modification, protein turnover, chaperones |
| C    | 109   | 5.29  | Energy production and conversion |
| G    | 111   | 5.39  | Carbohydrate transport and metabolism |
| E    | 285   | 13.83 | Amino acid transport and metabolism |
| F    | 70    | 3.40  | Nucleotide transport and metabolism |
| H    | 88    | 4.27  | Coenzyme transport and metabolism |
| I    | 83    | 4.03  | Lipid transport and metabolism |
| P    | 142   | 6.89  | Inorganic ion transport and metabolism |
| Q    | 52    | 2.52  | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 240   | 11.64 | General function prediction only |
| S    | 144   | 6.99  | Function unknown |
| -    | 909   | 33.04 | Not in COGs |

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

The genome sequences of three more Leucobacter isolates have recently been published: L. chromiiresistens, isolated from a soil sample [40]; Leucobacter sp. UCD-THU isolated from a residential toilet [54]; and Leucobacter salsicius isolated from Korean salt-fermented seafood [39]. Chromate resistance was reported for some of these species (L. chironomi, L. chromiiresistens and L. salsicius) [1,39,40]. The genome analysis of L. salsicius detected chromate transport protein A (ChrA) that confers heavy metal tolerance via chromate ion efflux from the cytoplasm [39]. In contrast, this gene is not present in the genome of L. chironomi and L. chromiiresistens. However, in both strains, other genes for metals tolerance or ion efflux, are present. Interestingly, we have detected a chromate transporter (Chr) gene in the genome of Leucobacter sp. UCD-THU, although no evidence for chromate resistance was reported in vivo for this strain [54]. Another interesting feature is the differences in the horizontal gene transfer found in all four Leucobacter species genomes. While no horizontal gene transfer from Eukaryotes was detected for Leucobacter sp. UCD-THU, horizontal gene transfer from fungi belonging to the phyla Basidiomycota and Ascomycota in L. salsicius and L. chromiiresistens genomes, respectively. For L. chromiiresistens, which was isolated from seafood, genes transfer from the phylum Chordata was also found. Horizontal gene transfer from insects was detected for L. chironomi in the current study, confirming the fact that chironomid insects are L. chironomi hosts.

Conclusions

In the current study, we characterized the genome of L. chironomi strain MM2LB\textsuperscript{T} that was isolated from a chironomid egg mass [1]. Recently, we have demonstrated that endogenous bacteria in chironomids have a role in protecting their insect host from toxic metals [18,38]. Genes indicating the potential role of strain L. chironomi to tolerate or detoxify metals, where detected in its genome, demonstrating that indeed, L. chironomi which inhabits...
chironomids has a part in protecting its host from toxicants. Genes for ABC-type proline/glycine betaine transport system that were found in the genome may explain the salt tolerance properties of *Leucobacter* species. Evidence of horizontal transfer of genes from *Diploptera* origin to *Leucobacter* implies toward an ancient relationships between *Leucobacter* and its chironomid host.

**Abbreviations**

KMG: One thousand microbial genomes, GBEA: Genomic encyclopedia of Bacteria and Archaea; MGs: Minimum information about a genome sequence; DOE JGI: Department of Energy, Joint Genome Institute; TAS: Traceable; NAS: Non-traceable.

**Competing interests**
The authors declare that they have no competing interests.

**Authors’ contributions**
MH and RP isolated and characterized strain MM2LB; SL, MH, HPK and NCK drafted the manuscript. AL, AC, TBKR, MH, AP, NNI, VMW and TW sequenced, assembled and annotated the genome. All authors read and approved the final manuscript.

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**References**

1. Halpern M, Shaked T, Pukall R, Schumann P. Leucobacter chironomi sp. nov., a chorate resistant bacterium isolated from a chironomid egg mass. Int J Syst Evol Microbiol. 2009;59:665–70.
2. Takeuchi M, Weiss N, Schuman P, Yokota A. Leucobacter komagatae gen. nov., sp. nov., a new aerobic gram-positive, nonsporulating rod with 2,4-diaminobutyric acid in the cell wall. Int J Syst Bacteriol. 1996;46:967–71.
3. Martin E, Ladders N, Jackel U, Schuman P, Kampfer P. Leucobacter aerolatus sp. nov., from the air of a duck barn. Int J Syst Evol Microbiol. 2010;60:2838–42.
4. Somvanshi VS, Lang E, Schumann P, Pukall R, Kroppestendt RM, Ganguly S, et al. Leucobacter sanarellii gen. nov., sp. nov., in the family Microbacteriaceae. Int J Syst Evol Microbiol. 2007;57:682–6.
5. Muir RE, Tan MW. Leucobacter chironimoederucens subsp. solipictus subsp. nov., a pigmented bacterium isolated from the nematode Caenorhabditis elegans, and emended description of *L. chironomudreucens*. Int J Syst Evol Microbiol. 2007;57:2770–6.
6. Morais PV, Paula C, Francisco R, Branco R, Paula Chung A, Da Costa MS. Leucobacter lutii sp. nov., and Leucobacter alliuvi sp. nov., two new species of the genus *Leucobacter* isolated under chromium stress. Syst Appl Microbiol. 2006;29:414–21.
7. Weon HY, Anantham R, Tamura T, Hamada M, Kim SJ, Kim YS, et al. Leucobacter dentrifricus sp. nov., isolated from cow dung. J Microbiol. 2012;50:161–5.
8. Ue H. Leucobacter exalbidus sp. nov., an actinobacterium isolated from a mixed culture of compost. J Gen Appl Microbiol. 2011;57:27–33.
9. Shin NR, Kim MS, Jung MJ, Roh SW, Nam YD, Park EJ, et al. Leucobacter celer sp. nov., isolated from Korean fermented seaweed. Int J Syst Evol Microbiol. 2011;61:2353–7.
10. Behrendt U, Ulrich A, Schuman P. Leucobacter tardus sp. nov., isolated from the phyllosphere of *Solomon tuberosum*. Int J Syst Evol Microbiol. 2008;58:2574–8.
11. Halpern M, Landsberg D, Raats D, Rosenberg E. Culturable and VBNC *Vibrio cholerae*. Interactions with Chironomid egg masses and their bacterial population. Microb Ecol. 2007;53:285–93.
12. Broza M, Halpern M. Chironomid egg masses and Vibrio cholerae. Nature. 2007;441:80.
13. Halpern M, Broza YB, Mittler S, Arakawa E, Broza M. Chironomid egg masses as a natural reservoir of *Vibrio cholerae* non-O1 and non-O139 in fresh water habitats. Microb Ecol. 2004;47:341–9.
14. Halpern M, Raats D, Laviron R, Mittler S. Dependent population dynamics between chironomids (non-biting midges) and *Vibrio cholerae*. FEBS Microbiol Lett. 2006;559–104.
15. Senderovich Y, Genstein Y, Halewa E, Halpern M. *Vibrio cholerae* and *Aeromonas*: do they share a mutual host? ISME J. 2008;2:276–83.
16. Figueras MJ, Beaz-Hidalgo R, Senderovich Y, Lavid Y, Halpern M. Re-identification of *Aeromonas* isolates from chironomid egg masses as the potential pathogenic bacterium *Aeromonas salmonicida*. Environ Microb Res. 2011;23:49–54.
17. Beaz-Hidalgo R, Shaked T, Lavid Y, Halpern M, Figueras M. Chironomid egg masses harbour the classic species *Aeromonas salmonicida* and *Aeromonas sanarelli*. FEBS Microbiol Lett. 2012;327:48–54.
18. Senderovich Y, Halpern M. The protective role of endogenous bacterial communities in chironomid egg masses and larvae. ISME J. 2013;27:2147–58.
19. Goker M, Goker HP. En route to a genome-based classification of *Archaea and Bacteria*. Syst Appl Microbiol. 2010;33:175–82.
20. Goker M, Klein HP. Phylogeny-driven target selection for large-scale genome-sequencing (and other) projects. Stand Genomic Sci. 2013;8:360–73.
21. Wu D, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, et al. A phylogeny-driven Genomic Encyclopaedia of *Bacteria and Archaea*. Nature. 2009;462:1056–60.
22. Kyrdnes NC, Woyke T, Eisein JA, Gaity G, Lillburn TG, Beck BJ, et al. Genomic Encyclopaedia of Type Strains, Phase I: the one thousand microbial genomes (KMG-I): project, Stand Genomic Sci. 2013;6:29–34.
23. Kyrdnes NC, Hugenholtz P, Eisen JA, Woyke T, Goker T, Goker M, Parker CT, et al. Genomic Encyclopaedia of *Bacteria and Archaea*: sequencing a myriad of type strains. PLoS Biol. 2014;8:e1001920.
24. Pao H, Froura J, Du C, Kim TW, Hawley E, Bauer S, et al. Identification of novel biomass-degrading enzymes from microbial dark matter: populating genome sequencing space with functional annotation. Biotechnol Bioeng. 2014;111:1550–65.
25. Reddy TB, Thomas AD, Starnatis D, Bertsch J, Isbarn M, Jansson J, et al. The Genomes OnLine Database (GOLD) v5: a metadata management system based on a four level (meta) genome project classification. Nucleic Acids Res. 2015;43:D1099–106.
26. DOE Joint Genome Institute. http://www.jgi.doe.gov
27. Mavromatis K, Land ML, Brettin TS, Quest DJ, Copeland A, Clum A, et al. The fast changing landscape of sequencing technologies and their impact on microbial genome assemblies and annotation. PLoS One. 2012;7:e48837.
28. List of growth media used at the DSMZ. [http://www.dsmz.de/catalogues/culture-technologies/mediums-list-of-media-for-microorganisms.html](http://www.dsmz.de/catalogues/culture-technologies/mediums-list-of-media-for-microorganisms.html)
29. Gemeinholzer B, Dröge G, Zetzsche H, Haszprunar G, Klenk HP, Güntsch AM, et al. The DNA Bank Network: the start from a German initiative. Biopreserv Biobank. 2011;9:51–5.
30. Bennett S. Solexa Ltd. Pharmacogenomics. 2004;5:433–8.
31. Žerbin D, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. 2008;18:821–9.
32. Wgsim. https://github.com/lh3/wgsim
33. Gnerre S, MacCallum I. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. Proc Natl Acad Sci U S A. 2011;108(4):1513–8.
34. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: Prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics. 2010;11:119.
35. Mavromatis K, Ivanova NN, Chen IM, Szeto E, Markowitz VM, Kyrpides NC. The DOE-JGI Standard operating procedure for the annotations of microbial genomes. Stand Genomic Sci. 2009;1:63–7.
36. Chen IM, Markowitz VM, Chu K, Anderson I, Mavromatis K, Kyrpides NC, et al. Improving microbial genome annotations in an integrated database context. PLoS One. 2013;8:e54859.
37. Markowitz VM, Mavromatis K, Ivanova NN, Chen IM, Chu K, Kyrpides NC. IMG ER: a system for microbial genome annotation expert review and curation. Bioinformatics. 2009;25:2271–8.
38. Senderovich T, Halpern M. Bacterial community composition associated with chironomid egg masses. J Insect Sci. 2012;12:148.
39. Yun J-H, Cho Y-J, Chun J, Hyun D-W, Bae J-W. Genome sequence of the chromate-resistant bacterium Leucobacter salsiclus type strain M1-8T. Stand Genomic Sci. 2014;9:495–504.
40. Sturm G, Buchta K, Kurz T, Rensing SA, Gescher J. Draft genome sequence of Leucobacter chromiresistens, an extremely chromium-tolerant strain. J Bacteriol. 2012;194:540–1.
41. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGAS: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol Biol Evol. 2011;28:2731–9.
42. Field D, Garrity GM, Gray T, Morrison N, Selengut J, Sterk P, et al. The minimum information about a genome sequence (MGIS) specification. Nat Biotechnol. 2008;26:541–7.
43. Stackebrandt ERF, Ward-Rainey NL. Proposal for a new hierarchic classification system, Actinobacteria classis nov. Int J Syst Bacteriol. 1997;47:479–91.
44. 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 Evol Microbiol. 2009;59:608–12.
45. Skerman VBD, McGowan V, Sneath PHA. Approved Lists of Bacterial Names. Int J Syst Bacteriol. 1980;30:225–420.
46. Buchanan RE. Studies in the nomenclature and classification of bacteria. II. The primary subdivisions of the Schizomycetes. J Bacteriol. 1917;2:155–64.
47. Stackebrandt ERF. Concept of a new hierarchic classification system, Actinobacteria classis nov. Int J Syst Bacteriol. 1997;47:479–91.
48. 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 Evol Microbiol. 2009;59:608–12.
49. Skerman VBD, McGowan V, Sneath PHA. Approved Lists of Bacterial Names. Int J Syst Bacteriol. 1980;30:225–420.
50. Buchanan RE. Studies in the nomenclature and classification of bacteria. II. The primary subdivisions of the Schizomycetes. J Bacteriol. 1917;2:155–64.
51. Pubmed. Validation of the publication of new names and new combinations previously effectively published outside the IJSS. List No. 53. Int J Syst Bacteriol. 1995;45:418–9.
52. Park YH, Suzuki K, Yim DG, Lee KY, Bae J-W. Genome sequence of the chromate-resistant bacterium Leucobacter salsiclus type strain M1-8T. Stand Genomic Sci. 2014;9:505–14.
53. Skerman VBD, McGowan V, Sneath PHA. Approved Lists of Bacterial Names. Int J Syst Bacteriol. 1980;30:225–420.
54. Buchanan RE. Studies in the nomenclature and classification of bacteria. II. The primary subdivisions of the Schizomycetes. J Bacteriol. 1917;2:155–64.
55. Pubmed. Validation of the publication of new names and new combinations previously effectively published outside the IJSS. List No. 53. Int J Syst Bacteriol. 1995;45:418–9.
56. Park YH, Suzuki K, Yim DG, Lee KY, Kim E, Yoon J, et al. Supragenetic classification of peptidoglycan group B actinomycetes by nucleotide sequencing of 5S ribosomal RNA. Antonie Van Leeuwenhoek. 1993;66:307–13.
57. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. Gene Ontology Consortium Nat Genet. 2000;25:25–9.