Genome sequence of the pink to light reddish-pigmented Rubellimicrobium mesophilum type strain (DSM 19309T), a representative of the Roseobacter group isolated from soil, and emended description of the species

Thomas Riedel, Stefan Spring, Anne Fiebig, Jörn Petersen, Markus Göker, Hans-Peter Klenk

To cite this version:

Thomas Riedel, Stefan Spring, Anne Fiebig, Jörn Petersen, Markus Göker, et al.. Genome sequence of the pink to light reddish-pigmented Rubellimicrobium mesophilum type strain (DSM 19309T), a representative of the Roseobacter group isolated from soil, and emended description of the species. Standards in Genomic Sciences, BioMedCentral, 2014, 9 (3), pp.902-913. 10.4056/sigs.5621012 . hal-01230502

HAL Id: hal-01230502
https://hal.sorbonne-universite.fr/hal-01230502

Submitted on 18 Nov 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License
Genome sequence of the pink to light reddish-pigmented *Rubellimicrobium mesophilum* type strain (DSM 19309\(^T\)), a representative of the *Roseobacter* group isolated from soil, and emended description of the species

Thomas Riedel\(^1\), Stefan Spring\(^3\), Anne Fiebig\(^3\), Jörn Petersen\(^3\), Markus Göker\(^3\)*, Hans-Peter Klenk\(^3\)

\(^1\) Sorbonne Universités, UPMC Univ Paris 06, USR3579, LBBM, Observatoire Océanologique, Banyuls/Mer, France
\(^2\) CNRS, USR3579, LBBM, Observatoire Océanologique, Banyuls/Mer, France
\(^3\) Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

*Correspondence: Markus Göker (mgo08@dsmz.de)

**Keywords:** Irregular rod-shaped, motile, aerobic, stenohaline, chemoorganotroph, *Roseobacter* group, *Roseobacter* group, *Rhodobacteraceae*, Alphaproteobacteria.

**Rubellimicrobium mesophilum** Dastager et al. 2008 is a mesophilic and light reddish-pigmented representative of the *Roseobacter* group within the alphaproteobacterial family *Rhodobacteraceae*. Representatives of the *Roseobacter* group play an important role in the marine biogeochemical cycles and were found in a broad variety of marine environments associated with algal blooms, different kinds of sediments, and surfaces of invertebrates and vertebrates. Roseobacters were shown to be widely distributed, especially within the total bacterial community found in coastal waters, as well as in mixed water layers of the open ocean. Here we describe the features of *R. mesophilum* strain MSL-20\(^T\) together with its genome sequence and annotation generated from a culture of DSM 19309\(^T\). The 4,927,676 bp genome sequence consists of one chromosome and probably one extrachromosomal element. It contains 5,082 protein-coding genes and 56 RNA genes. As previously reported, the G+C content is significantly different from the actual genome sequence-based G+C content and as the type strain tests positively for oxidase, the species description is emended accordingly. The genome was sequenced as part of the activities of the Transregional Collaborative Research Centre 51 (TRR51) funded by the German Research Foundation (DFG).

**Introduction**

Strain MSL-20\(^T\) (= DSM 19309\(^T\) = KCTC 22012\(^T\)) is the type strain of the species *Rubellimicrobium mesophilum* [1], one of four species with validly published names in the genus *Rubellimicrobium* [2,3]; the other three species in the genus are *R. thermophilum* [3], *R. aerolatum* [4] and *R. roseum* [5]. *Rubellimicrobium* belongs to the abundant marine *Roseobacter* group [6]. The species epithet *mesophilum* refers to the Greek adjective *mesos*, middle, as well as from the Neo-Latin adjective ‘philus –a –um’, friend/loving [1], the middle (temperature-) loving. Strain MSL-20\(^T\) was isolated from soil located at Bigeum Island, Republic of Korea [1], whereas the other type strains within the genus *Rubellimicrobium* were isolated from a paper mill (*R. thermophilum* [3]), air (*R. aerolatum* [4]) and forest soil (*R. roseum* [5]), which indicates rather diverse habitats for *Rubellimicrobium*. Current PubMed records do not indicate any follow-up research with strain MSL-20\(^T\) since the initial description of *R. mesophilum* [1]. Here we present a summary classification and a set of features for *R. mesophilum* MSL-20\(^T\), together with the description of the complete genomic sequencing and annotation.

**Classification and features**

**16S rRNA gene analysis**

Figure 1 shows the phylogenetic neighborhood of *R. mesophilum* in a 16S rRNA gene sequence-based
The sequence of the single 16S rRNA gene in the DSM 19309\textsuperscript{T} genome does not differ from the previously published 16S rRNA gene sequence (EF547368), which contains four ambiguous base calls.

The genomic 16S rRNA gene sequence of \textit{R. mesophilum} DSM 19309\textsuperscript{T} was compared with the Greengenes database for determining the weighted relative frequencies of taxa and (truncated) keywords as previously described \cite{7}. The most frequently occurring genera were \textit{Paracoccus} (45.3\%), \textit{Loktanella} (30.3\%), \textit{Rubellimicrobium} (14.0\%), \textit{Methylarcula} (8.4\%) and \textit{'Pararubellimicrobium'} (2.0\%) (58 hits in total). Regarding the five hits to sequences from other members of the genus, the average identity within HSPs was 94.9\%, whereas the average coverage by HSPs was 99.3\%. Among all other species, the one yielding the highest score was \textit{'Pararubellimicrobium aerilata'} (EU338486), which corresponded to an identity of 96.3\% and a HSP coverage of 98.0\%. (Note that the Greengenes database uses the INSDC (=EMBL/NCBI/DDBJ) annotation, which is not an authoritative source for nomenclature or classification). The highest-scoring environmental sequence was JF417792 (Greengenes short name 'microbial structures coalbeds located Eerduosi Basin China coalbed clone QQSB73'), which showed an identity of 98.7\% and a HSP coverage of 99.6\%. The most frequently occurring keywords within the labels of all environmental samples which yielded hits were 'skin' (10.6\%), 'fossa' (5.9\%), 'poplit' (4.2\%), 'forearm, volar' (3.3\%) and 'sea' (2.8\%) (192 hits in total). Environmental samples which yielded hits of a higher score than the highest scoring species were not found, indicating that \textit{R. mesophilum} has rarely been detected in the environment.

\begin{figure}[!h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Phylogenetic tree highlighting the position of \textit{R. mesophilum} relative to the type strains of the other species within the genus \textit{Rubellimicrobium} and the neighboring genera \textit{Citreicella} and \textit{Wenxinia}. The tree was inferred from 1,381 aligned characters of the 16S rRNA gene sequences under the maximum likelihood (ML) criterion as previously described \cite{7}. The branches are scaled in terms of the expected number of substitutions per site. Numbers adjacent to the branches are support values from 1,000 ML bootstrap replicates (left) and from 1,000 maximum-parsimony bootstrap replicates (right) if larger than 60\% \cite{7}. Lineages with type strain genome sequencing projects registered in GOLD \cite{8} are labeled with one asterisk \cite{9}.}
\end{figure}

http://standardsingenomics.org
Rubellimicrobium mesophilum

Morphology and physiology
Cells of strain MSL-20T stain Gram-negative, are described to be motile (without a flagellum) [1], and ovoid or rod-shaped, 1.6-3.4 µm in length and 0.4-0.7 µm in width (Figure 2 and Table 1). On Reasoner’s 2A (R2A) agar they form pink to light red-pigmented colonies. According to [1], cells are negative for oxidase (but see below) and nitrate reduction activities, but show only weak catalase activity. They hydrolyze starch and Tween 80, assimilate cellulose, histidine, leucine and fructose, but do not utilize citrate and propionate. Cells test positive for leucine arylamidase, naphthol-AS-BI-phosphohydrolase and α-glucosidase. Growth is observed in a temperature range of 20-37°C with an optimum at 28°C. The pH range for growth is between pH 7-11 with an optimum at pH 7.0 ± 0.2. No growth occurs in the presence of NaCl in concentrations of 0.5% and above. Cells of strain MSL-20T do not utilize the carbohydrates cellobiose, D-mannose, salicin, D-xylose, α-melibiose, D-sorbitol, L-malate and D-ribose, which are utilized by its close relative R. thermophilum DSM 16684T (all data from [1]).

Chemotaxonomy
The principal cellular fatty acids of strain MSL-20T are C16:0 (36.9%), C18:1ω7c (36.5%), 11-methyl C18:1ω7c (12.4%), C18:0 (3.6%), C10:0 (1.3%), C12:0 (1.3%) and C17:0 (1.2%) and differ significantly from those detected in R. thermophilum. The major respiratory lipoquinone is ubiquinone Q-10, which is a common feature of alphaproteobacterial representatives (all data from [1]).

Genome sequencing and annotation
Genome project history
The genome of strain R. mesophilum DSM 19309T was first selected for genome sequencing in phase I of the one thousand microbial genomes (KMG-I) project [20], an extension of the Genomic Encyclopaedia of Bacteria and Archaea (GEBA) [21], but ultimately sequenced within the DFG funded project “Ecology, Physiology and Molecular Biology of the Roseobacter clade: Towards a Systems Biology Understanding of a globally Important Clade of Marine Bacteria”. The strain was chosen for genome sequencing according to a phylogeny-driven target selection procedure for large scale genome-sequencing (and other) projects as routinely used for the KMG-I project [20,22]. The project information can be found in the Genome OnLine Database [8]. The Whole Genome Shotgun (WGS) sequence is deposited in GenBank and the Integrated Microbial Genomes database (IMG) [23]. A summary of the project information is shown in Table 2.

Figure 2. Micrograph of R. mesophilum DSM 19309T.
Table 1. Classification and general features of *R. mesophilum* MSL-20\(^{T}\) according the MIGS recommendations [10] published by the Genome Standards Consortium [11].

| MIGS ID | Property | Term | Evidence code |
|---------|----------|------|---------------|
|         |          | Domain *Bacteria* | TAS [12] |
|         |          | Phylum *Proteobacteria* | TAS [13] |
|         |          | Class *Alphaproteobacteria* | TAS [14,15] |
|         |          | Order *Rhodobacterales* | TAS [15,16] |
|         |          | Family *Rhodobacteraceae* | TAS [15,17] |
|         |          | Genus *Rubellimicrobium* | TAS [3] |
|         |          | Species *Rubellimicrobium mesophilum* | TAS [1] |
|         |          | Strain MSL-20\(^{T}\) | TAS [1] |
| Current classification | Gram stain | negative | TAS [1] |
|         | Cell shape | irregular rod-shaped | TAS [1] |
|         | Motility | motile | TAS [1] |
|         | Sporulation | non-sporulating | NAS |
|         | Temperature range | 20-37°C | TAS [1] |
|         | Optimum temperature | 28°C | TAS [1] |
|         | Salinity | stenohaline | TAS [1] |
| MIGS-22 | Oxygen requirement | aerobic | TAS [1] |
|         | Carbon source | carbohydrates, amino acids | TAS [1] |
|         | Energy metabolism | chemoorganotroph | NAS |
| MIGS-6 | Habitat | soil | TAS [1] |
| MIGS-15 | Biotic relationship | free living | TAS [1] |
| MIGS-14 | Pathogenicity | none | NAS |
|         | Biosafety level | 1 | TAS [18] |
| MIGS-23 | Isolation | soil | TAS [1] |
| MIGS-4 | Geographic location | Bigeum island (Republic of Korea) | TAS [1] |
| MIGS-5 | Sample collection time | April 2006 | NAS |
| MIGS-4.1 | Latitude | 34.739 | NAS |
| MIGS-4.2 | Longitude | 125.920 | NAS |
| MIGS-4.3 | Depth | not reported | NAS |
| MIGS-4.4 | Altitude | not reported | NAS |

Evidence codes - 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 of the Gene Ontology project [19].
Rubellimicrobium mesophilum

Table 2. Genome sequencing project information

| MIGS ID | Property                  | Term                                                                 |
|---------|---------------------------|----------------------------------------------------------------------|
| MIGS-31 | Finishing quality         | Non-contiguous finished                                              |
|         |                           | Two genomic libraries: one Illumina PE library (420 bp insert size), one 454 PE library (3 kb insert size) |
| MIGS-28 | Libraries used            | Illumina GA IIx, Illumina MiSeq, 454 GS-FLX+Titanium                  |
| MIGS-29 | Sequencing platforms      |                                                                      |
| MIGS-31.2| Sequencing coverage      | 129×                                                                |
| MIGS-30 | Assemblers                | Velvet version 1.1.36, Newbler version 2.3, Consed 20.0             |
| MIGS-32 | Gene calling method       | Prodigal 1.4                                                         |
|         | INSDC ID                  | AOSK00000000                                                        |
| INSDC ID| GenBank Date of Release   | pending publication                                                  |
| GOLD ID |                           |                                                                      |
| NCBI project ID |                       | 188767                                                             |
| Database: IMG |                         | 2523533591                                                        |
| MIGS-13 | Source material identifier| DSM 19309T                                                          |
|         | Project relevance         | Tree of Life, biodiversity                                           |

Growth conditions and DNA isolation

A culture of DSM 19309T was grown aerobically in DSMZ medium 830 (R2A medium) [24] at 28°C. Genomic DNA was isolated using Jetflex Genomic DNA Purification Kit (GENOMED 600100) following the standard protocol provided by the manufacturer, but modified by an incubation time of 60 min, an overnight incubation on ice on a shaker, the use of additional 50 µl proteinase K, and the addition of 100 µl protein precipitation buffer. DNA is available from DSMZ through the DNA Bank Network [25].

Genome sequencing and assembly

The genome was sequenced using a combination of two libraries (Table 2). The paired-end library contained inserts of an average of 420 bp in length. Illumina sequencing was performed on a GA IIx platform with 150 cycles. The first run on the Illumina GA IIx platform delivered 3.6 million reads. In order to increase the sequencing depth, a second Illumina run was performed, providing another 7.0 million reads. Error correction and clipping were performed by fastq-mcf [26] and quake [27]. The data was assembled using Velvet [28]. The first draft assembly from 5,400,234 filtered reads (median read length of 132 nt) resulted in more than 143 unordered contigs. To gain information about the contig arrangement an additional 454 run was performed. The paired-end jumping library of 3 kb insert size was sequenced on 1/8 of a lane. Pyrosequencing resulted in 102,695 reads with an average read length of 199 bp, assembled with Newbler (Roche Diagnostics). The resulting assembly consisted of 261 scaffolds. Both draft assemblies (Illumina and 454 sequences) were fractionated into artificial Sanger reads of 1,000 nt in length plus 75 bp overlap on each site. These artificial reads served as an input for the phred/phrap/consed package [29]. By manual editing, 138 contigs could be assembled on 127 scaffolds. The combined sequences provided a 129× coverage of the genome.

Genome annotation

Genes were identified using Prodigal [30] as part of the JGI genome annotation pipeline. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) non-redundant database, UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Identification of RNA genes was carried out by using HMMER 3.0rc1 [31] (rRNAs) and tRNAscan-SE 1.23 [32] (tRNAs). Other non-coding genes were predicted using INFERNAL 1.0.2 [33]. Additional gene prediction analysis and functional annotation was performed within the Integrated Microbial Genomes - Expert Review (IMG-ER) platform [34]. CRISPR elements were detected using CRT [35] and PILER-CR [36].
Genome properties
The genome statistics are provided in Table 3 and Figure 3. The genome of strain DSM 19309\textsuperscript{T} has a total length of 4,927,676 bp and a G+C content of 69.7%. Of the 5,138 genes predicted, 5,082 were identified as protein-coding genes, and 56 as RNAs. The majority of the protein-coding genes (56.7%) 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.

**Table 3. Genome statistics**

| Attribute                                      | Value     | % of Total |
|------------------------------------------------|-----------|------------|
| Genome size (bp)                               | 4,927,676 | 100.00     |
| DNA coding region (bp)                         | 4,254,404 | 86.34      |
| DNA G+C content (bp)                           | 3,431,981 | 69.65      |
| Number of scaffolds MIGS-9                     | 127       |            |
| Extrachromosomal elements MIGS-10              | 1         |            |
| Total genes                                    | 5,138     | 100.00     |
| RNA genes                                      | 56        | 1.09       |
| rRNA operons                                    | 1         |            |
| tRNA genes                                      | 45        | 0.88       |
| Protein-coding genes                           | 2,915     | 56.73      |
| Genes with function prediction (proteins)      | 2,167     | 42.18      |
| Genes in paralog clusters                      | 4,172     | 81.20      |
| Genes assigned to COGs                         | 3,818     | 74.31      |
| Genes assigned Pfam domains                    | 3,977     | 77.40      |
| Genes with signal peptides                     | 384       | 7.47       |
| Genes with transmembrane helices               | 966       | 18.80      |
| CRISPR repeats                                 | 0         |            |

**Figure 3.** Graphical map of the largest, 267,932 bp long scaffold. From bottom to the top: Genes on forward strand (colored by COG categories), Genes on reverse strand (colored by COG categories), RNA genes (tRNAs green), GC content (black), GC skew (purple/olive).
Rubellimicrobium mesophilum

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

| Code | Value | %age | Description                                           |
|------|-------|------|-------------------------------------------------------|
| J    | 186   | 4.4  | Translation, ribosomal structure and biogenesis       |
| A    | 3     | 0.1  | RNA processing and modification                       |
| K    | 279   | 6.6  | Transcription                                         |
| L    | 250   | 5.9  | Replication, recombination and repair                 |
| B    | 4     | 0.1  | Chromatin structure and dynamics                      |
| D    | 35    | 0.8  | Cell cycle control, cell division, chromosome partitioning |
| Y    | 0     | 0.0  | Nuclear structure                                     |
| V    | 34    | 0.8  | Defense mechanisms                                   |
| T    | 176   | 4.2  | Signal transduction mechanisms                       |
| M    | 223   | 5.3  | Cell wall/membrane/envelope biogenesis               |
| N    | 27*   | 0.6  | Cell motility                                         |
| Z    | 0     | 0.0  | Cytoskeleton                                          |
| W    | 0     | 0.0  | Extracellular structures                              |
| U    | 51    | 1.2  | Intracellular trafficking and secretion, and vesicular transport |
| O    | 148   | 3.5  | Posttranslational modification, protein turnover, chaperones |
| C    | 251   | 6.0  | Energy production and conversion                     |
| G    | 453   | 10.7 | Carbohydrate transport and metabolism                |
| E    | 478   | 11.3 | Amino acid transport and metabolism                  |
| F    | 94    | 2.2  | Nucleotide transport and metabolism                  |
| H    | 152   | 3.6  | Coenzyme transport and metabolism                    |
| I    | 143   | 3.4  | Lipid transport and metabolism                        |
| P    | 182   | 4.3  | Inorganic ion transport and metabolism               |
| Q    | 124   | 2.9  | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 508   | 12.0 | General function prediction only                      |
| S    | 421   | 10.0 | Function unknown                                     |
| -    | 1,320 | 25.7 | Not in COGs                                          |

*Only one gene each for flagellar motor and flagellar hook capping, no structural genes for flagella.

Insights into the genome

Plasmids

The identification of plasmids is difficult because typical replication modules comprising the characteristic replicase and the adjacent parAB partitioning operon are missing [36]. However, comprehensive BLASTP searches with plasmid replicases from Rhodobacterales revealed the presence of one RepB gene (rumeso_01479), whereas RepA-, RepABC-type and DnaA-like replicases are absent from the genome. The localization of the chromosomal replication initiator DnaA documents that scaffold 15 is part of the chromosome (Table 5).

The 119 kb RepB type plasmid contains a post-segregational killing system (PSK) consisting of a typical operon with two small genes encoding a stable toxin and an unstable antitoxin (rumesco_01477/78 [37]).

Table 5. General genomic location and features of the chromosomal and one extrachromosomal replicon from R. mesophilum strain DSM 19309T.

| Replicon | Scaffold | Replicase | Length (bp) | GC (%) | Topology | No. Genes |
|----------|----------|-----------|-------------|--------|----------|-----------|
| Chromosome¹ | 15       | DnaA      | 102,082     | 71     | linear*  | 105       |
| Plasmid  | 9        | RepB      | 119,205     | 68     | linear*  | 141       |

*circularity not experimentally validated
²deduced from automatic annotation
¹partial sequence including the replicase dnaA (rumeso_02152).
Phages
Phages are widely distributed and abundant in marine and freshwater environments [38-40] and are known to be horizontal gene transfer agents that drive bacterial diversity [40,41]. Temperate phage genomes can be integrated in the host genome as prophages and perform a symbiotic relationship with their hosts [42].

Several phage-associated gene sequences were detected in the genome sequence of strain DSM 19309T, particularly in “genomic islands” (e.g., rumeso_00405, rumeso_00407 rumeso_01586 to rumeso_01600).

Quorum Sensing
Several Gram-negative bacteria produce and release chemical signal molecules called autoinducers. In correlation to the population density they detect those signal molecules and respond with an alteration of gene expression and therefore with diverse behaviors (e.g., luminescence, virulence, antibiotic resistance, changes in morphology and cell division) [43-46].

Genome analysis of strain DSM 19309T revealed the presence of gene-encoding sequences associated with the mechanism of quorum sensing e.g. N-homoserine-lactone synthetase, rumeso_02218 (LuxI homologue); probably involved in response and transcriptional regulators, rumeso_02217 (luxR homologue).

Metabolic plasticity
Unlike many representatives of the Roseobacter group [6], R. mesophilum DSM 19309T encodes no genes involved in the harvesting of light and phototrophic growth, which reflect its occurrence in niches within soil that are characterized by the absence of light. Nevertheless, the annotated genome sequence reveals a high metabolic versatility that was not expected by the phenotypic characterization presented in the species description [1].

The genome encodes a large number of diverse ABC transporters facilitating the uptake of various substrates like carbohydrates (e.g., rumeso_04497 to 04500), polyamines (e.g., rumeso_04716 to 04719), peptides (e.g., rumeso_00087 to 00090), amino acids (e.g., rumeso_00231 to 00234) and sulfonates (e.g., rumeso_00558 to 05059). Sulfonates could represent unexpected but common substrates for this species. The organic sulfonates taurine and cysteic acid are widely distributed in animal tissue and can enter soil by feces. In some soil bacteria, these compounds are used as sole source of carbon, nitrogen and sulfur [47]. Indeed, a complete degradation pathway for taurine was detected in the genome of strain DSM 19309T. Taurine is first converted by a taurine-pyruvate aminotransferase (rumeso_05057) to sulfoacetaldehyde, which in turn is cleaved by the enzyme sulfoacetaldehyde acetyltransferase (rumeso_03970) into sulfite and acetyl-phosphate. Acetyl-phosphate can be either converted to acetyl-CoA by a phosphotransacetylase (rumeso_03968) and funneled into the intermediary metabolism or is used for the generation of ATP by the enzyme acetate kinase (rumeso_03967). The potentially toxic compound sulfite can be oxidized to sulfate by various sulfate oxidases (e.g., rumeso_03951).

In addition, the utilization of electron acceptors seems to be variable and not restricted to oxygen. Genes encoding at least two predicted cytochrome c oxidases, one of the cbb3-type (rumeso_00470 to 00472) and the other of the aa3-type (rumeso_02204 to 02206), which terminate the electron transport chain with oxygen, were detected. However, according to the species description strain MSL-20T should be oxidase negative [1], we have found that the oxidase test for this strain is positive, which is in line with the results of the genome analysis.

Under periodic anoxic conditions that frequently occur in wet soils, nitrate could be used as alternative electron acceptor. According to the genome sequence, the denitrification pathway of this strain is probably incomplete and terminates with the greenhouse gas nitrous oxide (N2O), as has been previously demonstrated for Ottowia thiooxydans [48]. Only genes encoding a respiratory nitrate reductase (rumeso_02471 to 02474), nitrite reductase (rumeso_02669) and nitric oxide reductase (rumeso_00142 to 00145) were detected, whereas no genes for the terminal nitrous oxide reductase were found.

Comparison of Rubellimicrobium genomes
Recently the genome sequence of the type strain for second representative of the genus Rubellimicrobium, R. thermophilum DSM 16684T became available [9]. Lifestyle, habitat and preferred temperature range of R. thermophilum differ significantly from the ones of R. mesophilum [3]. The genome sequences of both strains were compared using the digital DNA-DNA hybridiza-
Rubellimicrobium mesophilum

The number of pairwise genes was inferred from the phylogenetic profiler tool of the IMG platform. Homologous genes were detected with an E-value cutoff of $10^{-5}$ and a minimum identity of 30%. Proportions of 56% and 45% of the gene count in *W. marina* and *R. mesophilum*, respectively, are shared between all three genomes. In the case of *R. thermophilum*, a fraction of homologous genes of 70% is present in the other two genomes. Very few genes are shared only between *R. thermophilum* and *W. marina*.

Figure 4. Venn diagram depicting the intersections of proteins sets (total numbers in parentheses) of the two *Rubellimicrobium* species and *W. marina*.

Although both genomes differ significantly in size (3.2 Mbp for *R. mesophilum* and 4.9 Mbp for *R. thermophilum*), the proportions of genes per COG category is very similar (Table 3 and [9]). The IMG Abundance Profile [34] demonstrated some differences, however. Enzymes for transport and utilization of amino acids and polyamines (COG1173, COG0747, COG3842) were present in higher abundance in *R. thermophilum*, which is in agreement with the results from wet-lab substrate tests [1,3]. Huge differences in the abundance of proteins can be found within the class of transposases (COG2801, COG 3436, COG2936, COG0665, COG0404). While *R. thermophilum* codes for two transposase genes, more than 30 transposase genes were identified in *R. mesophilum*. Combined with the presence of the site-specific recombinase XerD (involved in the recombination of plasmids [20]) this indicates a high level of genetic recombination within *R. mesophilum*. Furthermore, 23 genes coding for RTX toxins and Ca$^+$-binding proteins (COG 2931) were found. These proteins are structurally diverse, playing an important role in the colonization of various habitats and surfaces [50]. Additionally, 14 proteins of the xenobiotic-degrading glutathion-S-transferases were present in *R. mesophilum*. The occurrence of these proteins may enable the bacteria to grow in polluted areas.

**Taxonomic note**

The G+C content of the genomic DNA of strain MSL-20T is given in the species description as 72.3 mol% [1], which represents a discrepancy of more than 2% from the value of 69.7 mol% deduced from the genome sequence. In addition to the deviant oxidase test this calls for an emendation of the species description according to the proposal of Meier-Kolthoff et al. [47].

**Emended description of *Rubellimicrobium mesophilum* Dastager et al. 2008**

The description of the species *Rubellimicrobium mesophilum* is the one given by Dastager et al. 2008 [1], with the following modifications. Oxidase test is positive. The G+C content, rounded to zero decimal places, is 70%.
Acknowledgements
The authors gratefully acknowledge Evelyne Brambilla (DSMZ) for DNA extraction and quality control. This work was performed under the auspices of the German Research Foundation (DFG) Transregio-SFB 51 Roseobacter grant.

References
1. Dastager SG, Lee JC, Ju YJ, Park DJ, Kim CJ. Rubellimicrobium mesophilum sp. nov., a mesophilic, pigmented bacterium isolated from soil. *Int J Syst Evol Microbiol* 2008; 58:1797-1800. PubMed http://dx.doi.org/10.1099/ijs.0.65590-0
2. Ezpeleta JP. List of prokaryotic names with standing in nomenclature. www.bacterio.net/qr/rubellimicrobium.html
3. Denner EB, Kolari M, Hoornstra D, Tisiko I, Kappener P, Busse HJ, Salkinoja-Salonen M. Rubellimicrobium thermophilum gen. nov., sp. nov., a red-pigmented, moderately thermophilic bacterium isolated from coloured slime deposits in paper machines. *Int J Syst Evol Microbiol* 2006; 56:1355-1362. PubMed http://dx.doi.org/10.1099/ijs.0.63751-0
4. Weon HY, Son JA, Yoo SH, Hong SB, Jeon YA, Kwon SW, Koo BS. Rubellimicrobium aerolatum sp. nov., isolated from an air sample in Korea. *Int J Syst Evol Microbiol* 2009; 59:406-410. PubMed http://dx.doi.org/10.1099/ijs.0.65856-0
5. Cao YR, Jiang Y, Wang Q, Tang SK, He WX, Xue QH, Xu LH, Jiang CL. Rubellimicrobium roseum sp. nov., a Gram-negative bacterium isolated from the forest soil sample. *Antoni van Leeuwenhoek* 2010; 98:389-394. PubMed http://dx.doi.org/10.1007/s10482-010-9452-2
6. Buchan A, González JM, Moran MA. Overview of the marine Roseobacter lineage. *Appl Environ Microbiol* 2005; 71:5665-5677. PubMed http://dx.doi.org/10.1128/AEM.71.10.5665-5677.2005
7. Göker M, Cleland D, Saunders E, Lapidus A, Nolan M, Lucas S, Hammon N, Deshpande S, Cheng JF, Tapia R, et al. Complete genome sequence of *Isosphaera pallida* type strain (IS1B). *Stand Genomic Sci* 2011; 4:63-71. PubMed http://dx.doi.org/10.4056/sigs.1533840
8. Pagani I, Liolios K, Jansson J, Chen IM, Smirnova T, Nosrat B, Markowitz VM, Kyrpides NC. The Genomes OnLine Database (GOLD) v.4: status of genomic and metagenomic projects and their associated metadata. *Nucleic Acids Res* 2012; 40:D571-D579. PubMed http://dx.doi.org/10.1093/nar/gkr1100
9. Fiebig A, Riedel T, Gronow S, Petersen J, Klenk HP, Göker M. Genome sequence of the reddish-pigmented *Rubellimicrobium thermophilum* type strain (DSM 16684T), a member Roseobacter clade. *Stand Genomic Sci* 2013; 7:107-119.
10. 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 http://dx.doi.org/10.1038/nbt1360
11. Field D, Amaral-Zettler L, Cochrane G, Cole JR, Dywynn PD, Garrity GM, Gilbert J, Glöckner FO, Hirschman L, Karsch-Mizrachi I, et al. Clarifying Concepts and Terms in Biodiversity Informatics. *PLoS Biol* 2013; 9:e1001088. PubMed http://dx.doi.org/10.1371/journal.pbio.1001088
12. Woese CR, Kandler O, Woelch 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 http://dx.doi.org/10.1073/pnas.87.12.4576
13. Garrity GM, Bell JA, Lilburn T. Phylum XIV. Proteobacteria phyl nov. In: Brenner DJ, Krieg NR, Stanley JT, Garrity GM (eds), Bergey’s Manual of Systematic Bacteriology, second edition. Vol. 2 (The Proteobacteria), part B (The Gammaproteobacteria), Springer, New York, 2005, p. 1.
14. Garrity GM, Bell JA, Lilburn T. Class I. Alphaproteobacteria class. nov. In: Brenner DJ, Krieg NR, Stanley JT, Garrity GM (eds), Bergey’s Manual of Systematic Bacteriology, second edition. Vol. 2 (The Proteobacteria), part C (The Alph-, Bet-, Delta-, and Epsilonproteobacteria), Springer, New York, 2005, p. 1.
15. Validation List No. 107. List of new names and new combinations previously effectively, but not validly, published. *Int J Syst Evol Microbiol* 2006; 56:1-6. PubMed http://dx.doi.org/10.1099/ijs.0.64188-0
16. Garrity GM, Bell JA, Lilburn T. Order III. Rhodobacterales ord. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds), Bergey’s Manual of Systematic Bacteriology, second edition. vol. 2 (The Proteobacteria), part C (The Alph-, Beta-,
Rubellimicrobium mesophilum

Delta-, and Epsilonproteobacteria, Springer, New York, 2005, p. 161.

17. Garrity GM, Bell JA, Lilburn T. Family I. Rhodobacteraceae fam. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds), Bergey’s Manual of Systematic Bacteriology, second edition. vol. 2 (The Proteobacteria), part C (The Alpha-, Beta-, Delta-, and Epsilonproteobacteria), Springer, New York, 2005, p. 161.

18. BAuA. Classification of Bacteria and Archaea in risk groups. TRBA 2010; 466:93.

19. 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. The Gene Ontology Consortium. Nat Genet 2000; 25:25-29. PubMed http://dx.doi.org/10.1038/75556

20. Kyrpides NC, Woyke T, Eisen JA, Garrity GM, Whitman WB, Klenk HP. Genomic encyclopedia of type strains, phase I: the one thousand microbial genomes (KNG-I) project. Stand Genomic Sci 2013; 9:628-634. http://dx.doi.org/10.4056/sigs.5068949

21. 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 Encyclopaedia of Bacteria and Archaea. Nature 2009; 462:1056-1060. PubMed http://dx.doi.org/10.1038/nature08656

22. Göker M, Klenk HP. Phylogeny-driven target selection for large scale genome-sequencing (and other) projects. Stand Genomic Sci 2013; 8:360-374. PubMed http://dx.doi.org/10.4056/sigs.3446951

23. Markowitz VM, Chen IMA, Palaniappan K, Chu K, Szeto E, Grechkin Y, Ratner A, Jacob B, Huang J, Williams P, et al. IMG: the integrated microbial genomes database and comparative analysis system. Nucleic Acids Res 2012; 40:D115-D122. PubMed http://dx.doi.org/10.1093/nar/gkr1044

24. List of growth media used at the DSMZ: http://www.dmsz.de/catalogues/catalogue-microorganisms/culture-technology/list-of-media-for-microorganisms.html.

25. Gemeinholzer B, Dröge G, Zetzsche H, Haszprunar G, Klenk HP, Güntsch A, Berendsohn WG, Wägele JW. The DNA Bank Network: the start from a German initiative. Biopreserv Biobank 2011; 9:51-55. http://dx.doi.org/10.1089/bio.2010.0029

26. Aronesty E. ea-utils: Command-line tools for processing biological sequencing data; 2011, http://code.google.com/p/ea-utils.

27. Kelley DR, Schatz MC, Salzberg SL. Quake: quality-aware detection and correction of sequencing errors. Genome Biol 2010; 11:R116. PubMed http://dx.doi.org/10.1186/gb-2010-11-11-r116

28. Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 2008; 18:821-829. PubMed http://dx.doi.org/10.1101/gr.074492.107

29. Gordon D, Abajian C, Green P. Consed: a graphical tool for sequence finishing. Genome Res 1998; 8:195-202. PubMed http://dx.doi.org/10.1101/gr.8.3.195

30. 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. PubMed http://dx.doi.org/10.1186/1471-2105-11-119

31. Finn DR, Clements J, Eddy SR. HHMER web server: interactive sequence similarity searching. Nucleic Acids Research 2011, Web Server Issue 39:W29-W37.

32. Lowe TM, Eddy SR. tRNAscan-SE: A Program for Improved Detection of Transfer RNA Genes in Genomic Sequence. Nucleic Acids Res 1997; 25:955-964. PubMed http://dx.doi.org/10.1093/nar/25.5.0955

33. Nawrocki EP, Kolbe DL, Eddy SR. Infernal 1.0: Inference of RNA alignments. Bioinformatics 2009; 25:1335-1337. PubMed http://dx.doi.org/10.1093/bioinformatics/btp157

34. 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 http://dx.doi.org/10.1093/bioinformatics/btp393

35. Bland C, Ramsey TL, Sabree F, Lowe M, Brown K, Kyrpides NC, Hugenholtz P. CRISPR recognition tool (CRT): a tool for automatic detection of clustered regularly interspaced palindromic repeats. BMC Bioinformatics 2007; 8:209. PubMed http://dx.doi.org/10.1186/1471-2105-8-209

36. Petersen J. Phylogeny and compatibility: plasmid classification in the genomics era. Arch Microbiol 2011; 193:313-321. PubMed

37. Zielenskiewicz U, Cegłowski P. Mechanisms of plasmid stable maintenance with special focus on plasmid addiction systems. Acta Biochim Pol 2001; 48:1003-1023. PubMed
38. Wommack KE, Colwell RR. Viroplankton: viruses in aquatic ecosystems. *Microbiol Mol Biol Rev* 2000; 64:69-114. PubMed [http://dx.doi.org/10.1128/MMBR.64.1.69-114.2000](http://dx.doi.org/10.1128/MMBR.64.1.69-114.2000)

39. Proctor LM, Fuhrman JA, Ledbetter MC. Marine bacteriophages and bacterial mortality. *Eos* 1988; 69:1111-1112.

40. Paul JH. Prophages in marine bacteria: dangerous molecular bombs or the key to survival in the seas? *ISME J* 2008; 2:579-589. PubMed [http://dx.doi.org/10.1038/ismej.2008.35](http://dx.doi.org/10.1038/ismej.2008.35)

41. Cancayra C, Proux C, Fournous G, Bruttin A. Prophage genomics. *Microbiol Mol Biol Rev* 2003; 67:238-276. PubMed [http://dx.doi.org/10.1128/MMBR.67.2.238-276.2003](http://dx.doi.org/10.1128/MMBR.67.2.238-276.2003)

42. Ackermann HW, DuBow MS. Viruses of Prokaryotes. Vol I. General Properties of Bacteriophages. CRC Press 1987; Inc.: Boca Raton, FL.

43. Miller MB, Bassler BL. Quorum sensing in bacteria. *Annu Rev Microbiol* 2001; 55:165-199. PubMed [http://dx.doi.org/10.1146/annurev.micro.55.1.165](http://dx.doi.org/10.1146/annurev.micro.55.1.165)

44. Bassler BL. How bacteria talk to each other: regulation of gene expression by quorum sensing. *Curr Opin Microbiol* 1999; 2:582-587. PubMed [http://dx.doi.org/10.1016/S1369-5274(99)00025-9](http://dx.doi.org/10.1016/S1369-5274(99)00025-9)

45. Waters CM, Bassler BL. Quorum Sensing: Cell-to-Cell Communication in Bacteria. *Annu Rev Cell Dev Biol* 2005; 21:319-246. PubMed [http://dx.doi.org/10.1146/annurev.cellbio.21.012704.131001](http://dx.doi.org/10.1146/annurev.cellbio.21.012704.131001)

46. Patzelt D, Wang H, Buchholz I, Rohde M, Gröbe L, Pradella S, Neumann A, Schulz S, Heyber S, Münch K, et al. You are what you talk: quorum sensing induces individual morphologies and cell division modes in *Dinoroseobacter shibae*. *ISME J* 2013; 7:2274-2286. PubMed [http://dx.doi.org/10.1038/ismej.2013.107](http://dx.doi.org/10.1038/ismej.2013.107)

47. Stapley EO, Starkey RL. Decomposition of cysteic acid and taurine by soil microorganisms. *J Gen Microbiol* 1970; 64:77-84. PubMed [http://dx.doi.org/10.1099/00221287-64-1.77](http://dx.doi.org/10.1099/00221287-64-1.77)

48. Spring S, Jäckel U, Wagner M, Kämpfer P. *Ottowia thiooxydans* gen. nov., sp. nov., a novel facultatively anaerobic, N₂O-producing bacterium isolated from activated sludge, and transfer of *Hylemonella gracilis* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 2004; 54:99-106. PubMed [http://dx.doi.org/10.1099/ijs.0.02727-0](http://dx.doi.org/10.1099/ijs.0.02727-0)

49. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013; 14:60. PubMed [http://dx.doi.org/10.1186/1471-2105-14-60](http://dx.doi.org/10.1186/1471-2105-14-60)

50. Auch AF, Klenk HP, Göker M. Standard operating procedure for calculating genome-to-genome distances based on high-scoring segment pairs. *Stand Genomic Sci* 2010; 2:142-148. PubMed [http://dx.doi.org/10.4056/sigs.541628](http://dx.doi.org/10.4056/sigs.541628)

51. Riedel T, Fiebig A, Han J, Huntemann M, Spring S, Petersen J, Ivanova NN, Markowitz V, Göker M, et al. Genome sequence of the *Wenxinia marina* type strain (DSM 24838T), a representative of the *Roseobacter* clade isolated from oilfield sediments. *Stand Genomic Sci* (under review).

52. Edgar RC. PILER-CR Fast and accurate identification of CRISPR repeats. *BMC Bioinformatics* 2007; 8:18. PubMed [http://dx.doi.org/10.1186/1471-2105-8-18](http://dx.doi.org/10.1186/1471-2105-8-18)

53. Meier-Kolthoff JP, Klenk HP, Göker M. Taxonomic use of the G+C content and DNA:DNA hybridization in the genomic age. *Int J Syst Evol Microbiol* 2014; 64:352-356. PubMed [http://dx.doi.org/10.1099/ijs.0.056994-0](http://dx.doi.org/10.1099/ijs.0.056994-0)