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# Genome sequence of the reddish-pigmented *Rubellimicrobium thermophilum* type strain (DSM 16684<sup>T</sup>), a member of the *Roseobacter* clade

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*Rubellimicrobium thermophilum* Denner et al. 2006 is the type species of the genus *Rubellimicrobium*, a representative of the *Roseobacter* clade within the *Rhodobacteraceae*. Members of this clade were shown to be abundant especially in coastal and polar waters, but were also found in microbial mats and sediments. They are metabolically versatile and form a physiologically heterogeneous group within the *Alphaproteobacteria*. Strain C-Ivk-R2A-2<sup>T</sup> was isolated from colored deposits in a pulp dryer; however, its natural habitat is so far unknown. Here we describe the features of this organism, together with the draft genome sequence and annotation and novel aspects of its phenotype. The 3,161,245 bp long genome contains 3,243 protein-coding and 45 RNA genes.

## Introduction

Strain C-Ivk-R2A-2<sup>T</sup> (= DSM 16684 = CCUG 51817 = HAMBI 2421) is the type strain of the species *Rubellimicrobium thermophilum* [1]. The genus name *Rubellimicrobium* was derived from the Neo-Latin adjective '*rubellus*', red or reddish, and the Neo-Latin noun '*microbium*', microbe, referring to its reddish pigmentation. The species epithet was derived from the Greek noun '*thermê*', heat, as well as from the Neo-Latin adjective '*philus -a -um*', friend/loving, referring to its growth temperature [1]. C-Ivk-R2A-2<sup>T</sup> was isolated from colored deposits in a pulp dryer in Finland, so the natural habitat is so far unknown [1]. At the time of writing, PubMed records did not indicate any follow-up research with strain C-Ivk-R2A-2<sup>T</sup> after the initial description and valid publication of the new species *Rubellimicrobium thermophilum* [1]. Here we present a summary classification and a set of features for *R. thermophilum* C-Ivk-R2A-2<sup>T</sup>, together with the description of the genomic sequencing and annotation. We also describe novel aspects of its phenotype.

## Features of the organism

### 16S rRNA gene analysis

The single genomic 16S rRNA gene sequence of *R. thermophilum* DSM 16684<sup>T</sup> was compared using NCBI BLAST [2,3] under default settings (e.g., considering only the high-scoring segment pairs (HSPs) from the best 250 hits) with the most recent release of the Greengenes database [4] and the relative frequencies of taxa and keywords (reduced to their stem [5]) were determined, weighted by BLAST scores. The most frequently occurring genera were *Rubellimicrobium* (26.9%), *Oceanicola* (18.5%), *Rhodobacter* (12.4%), *Methylophilum* (10.4%) and *Loktanella* (10.1%) (37 hits in total). Regarding the five hits to sequences from members of the species, the average identity within HSPs was 99.9%, whereas the average coverage by HSPs was 99.2%. Among all other species, the one yielding the highest score was '*Pararubellimicrobium aerilata*' (EU338486), which corresponded to an identity of 94.2% and an HSP coverage of 97.8%. (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 AJ489269 (Greengenes short name 'food *Echinamoeba thermarum* clone'), which showed an identity of 99.9% and an HSP coverage of 99.1%. The most frequently occurring keywords within the labels of all environmental samples which yielded hits were 'skin' (10.1%), 'fossa' (6.0%), 'poplit' (3.6%), 'forearm, volar' (3.6%) and 'water' (2.5%) (213 hits in total). The most frequently occurring keywords within the labels of

those environmental samples which yielded hits of a higher score than the highest scoring species were 'biofilm' (18.2%), 'echinamoeba, food, thermarum' (9.1%) and 'color, machin, moder, paper, paper-machin, thermophil' (9.1%) (2 hits in total). Figure 1 shows the phylogenetic neighborhood of *R. thermophilum* in a 16S rRNA sequence based tree. The sequence of the single 16S rRNA gene copy in the genome does not differ from the previously published 16S rDNA sequence (AJ844281).

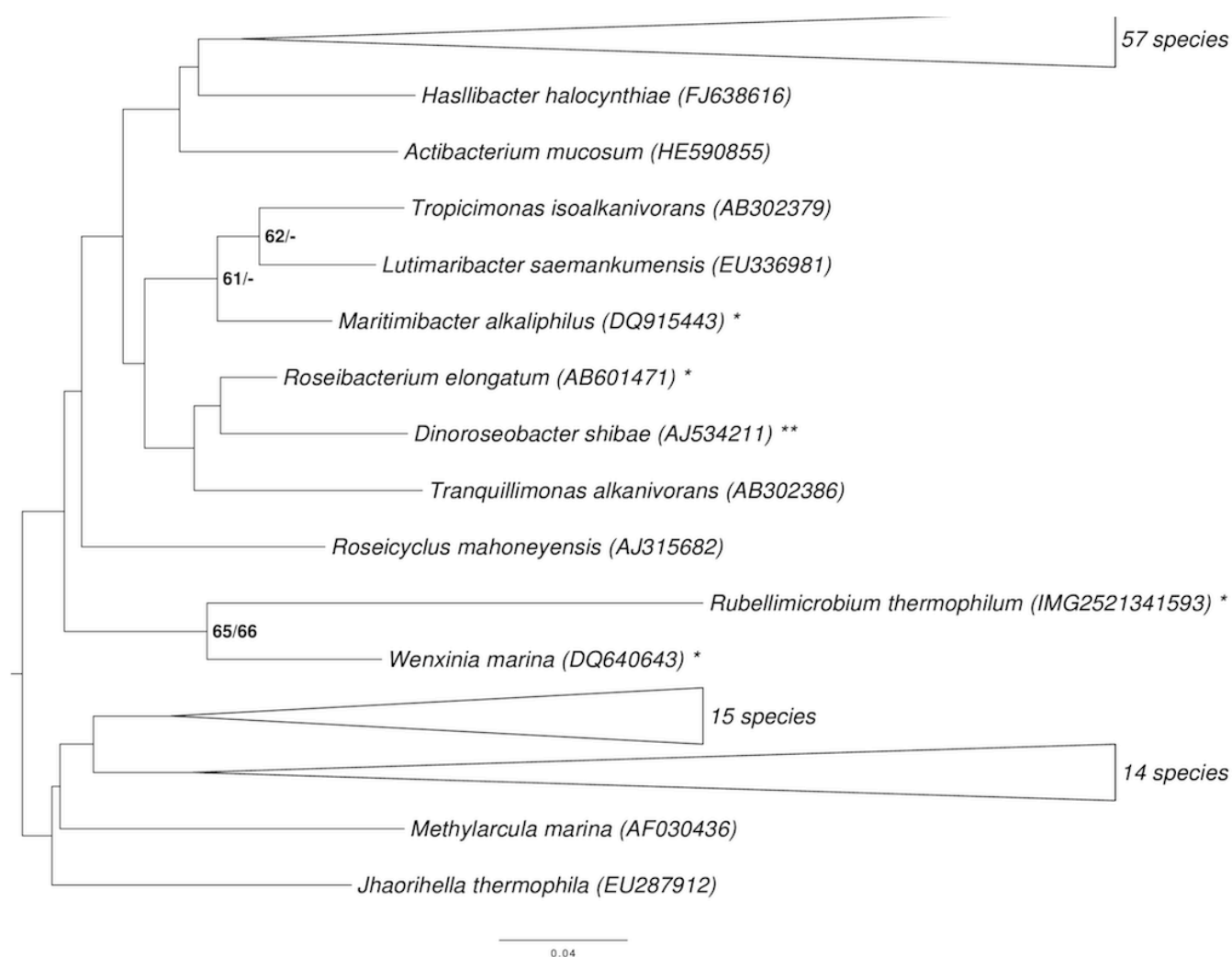


Figure 1 Phylogenetic tree highlighting the position of *R. thermophilum* relative to the type strains of the type species of the other genera within the family *Rhodobacteraceae*. The tree was inferred from 1,330 aligned characters [6,7] of the 16S rRNA gene sequence under the maximum likelihood (ML) criterion [8]. Rooting was done initially using the midpoint method [9] and then checked for its agreement with the current classification (Table 1). The branches are scaled in terms of the expected number of substitutions per site. Numbers adjacent to the branches are support values from 650 ML bootstrap replicates [10] (left) and from 1,000 maximum-parsimony bootstrap replicates [11] (right) if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [12] are labeled with one asterisk, those also listed as 'Complete and Published' with two asterisks [13].

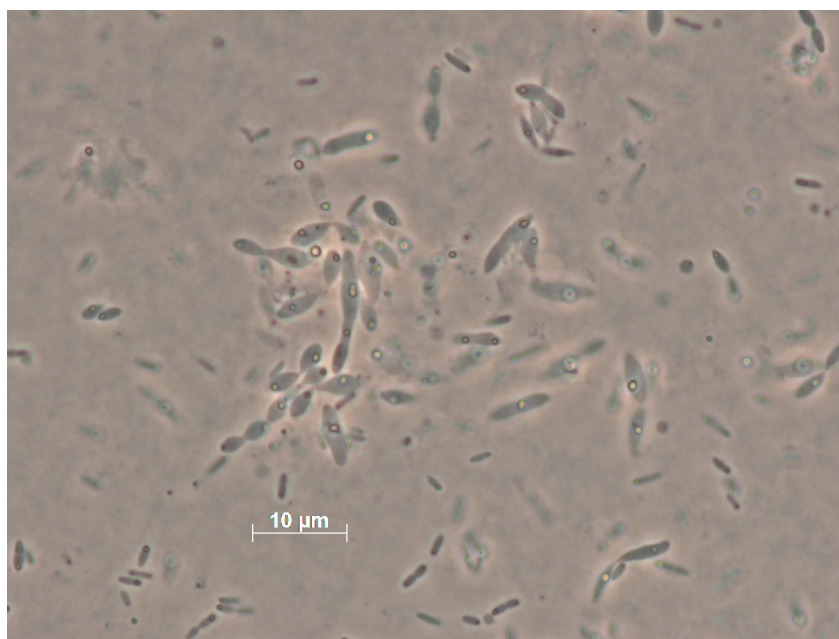
## Morphology and physiology

Cells of strain C-Ivk-R2A-2<sup>T</sup> stain Gram-negative are rod shaped and 0.6-0.8 µm in width and 2.0-4.0 µm in length (Figure 2) [1]. Cells are motile and possess one to three polar flagella [1]. C-Ivk-R2A-2<sup>T</sup> is moderately thermophilic and grows over a temperature range of 28–56°C with an optimum between 45°C and 52°C, whereas no growth occurs at room temperature or at temperatures higher than 57°C. Colonies grown on Reasoner's 2A agar (R2A) at 45°C for 2 days are translucent, entire, convex and smooth. Cells are red-pigmented (carotenoids); the pigment absorbance spectrum reveals three distinct peaks: one major peak at 495 nm and to others at 465 nm and 525 nm [1]. Cells are strictly aerobic. Growth does not occur under anaerobic conditions whether or not the cultures are grown in the dark or the light. Bacteriochlorophyll *a* is not synthesized. Cells are cytochrome *c*-oxidase positive, weakly positive for catalase as well as urease-positive. Nitrate is not reduced [1]. Intracellular inclusion bodies containing polyphosphate and polyhydroxyalkanoates are produced [1].

The cells of strain C-Ivk-R2A-2<sup>T</sup> assimilate the following compounds: L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose, α-D-melibiose, D-rhamnose, D-ribose, sucrose, salicin, D-trehalose, D-xylose, adonitol, *myo*-inositol, maltitol, D-mannitol, D-sorbitol, acetate, 4-aminobutyrate, glutarate, DL-3-hydroxybutyrate, DL-lactate, L-

malate, oxoglutarate, pyruvate, L-alanine, L-ornithine and L-proline. Cells do not produce acid from D-glucose, lactose, sucrose, L-arabinose, L-rhamnose, maltose, D-xylose, cellobiose, D-mannitol, dulcitol, salicin, adonitol, *myo*-inositol, sorbitol, raffinose, trehalose, methyl α-D-glucoside, erythritol, melibiose, D-arabitol or D-mannose [1]. The strain does not assimilate the following compounds: *N*-acetyl-D-glucosamine, putrescine, propionate, *cis*- and *trans*-aconitate, adipate, azelate, citrate, fumarate, itaconate, mesaconate, suberate, β-alanine, L-aspartate, L-histidine, L-leucine, L-phenylalanine, L-serine, L-tryptophan, 3- and 4-hydroxybenzoate and phenylacetate [1]. The following compounds are hydrolyzed by strain C-Ivk-R2A-2<sup>T</sup>: *p*-nitrophenyl (pNP) α-d-glucopyranoside, pNP β-D-glucopyranoside, bis-pNP phosphate, pNP phenylphosphonate and L-alanine *p*-nitroanilide (pNA), whereas aesculin, pNP β-D-galactopyranoside, pNP β-D-glucuronide, pNP phosphorylcholine, 2-deoxythymidine-5'-pNP phosphate, L-glutamate-γ-3-carboxy pNA, L-proline pNA, Tween 80, starch and casein are not hydrolyzed [1].

Strain C-Ivk-R2A-2<sup>T</sup> was also found to be susceptible to ampicillin, chloramphenicol, colistin sulfate, gentamicin, kanamycin, lincomycin, neomycin, nitrofurantoin, penicillin G, polymyxin B, streptomycin, tetracycline and vancomycin [1].



**Figure 2.** Micrograph of *R. thermophilum* DSM 16684<sup>T</sup>.

The physiology of *R. thermophilum* DSM 16684<sup>T</sup> was investigated in this study using Generation-III microplates in an OmniLog phenotyping device (BIOLOG Inc., Hayward, CA, USA). The microplates were inoculated at 28°C and 37°C, respectively, with a cell suspension at a cell density of 95-96% turbidity and dye IF-A. Further additives were vitamin, micronutrient and sea-salt solutions. The plates were sealed with parafilm to avoid a loss of fluid. The exported measurement data were further analyzed with the opm package for R [23,24], using its functionality for statistically estimating parameters from the respiration curves such as the maximum height, and automatically translating these values into negative and positive reactions.

At 28°C, the strain was positive for D-turanose, pH 6, 1% NaCl, 4% NaCl, D-galactose, 3-O-methyl-D-glucose, D-fucose, L-fucose, L-rhamnose, 1% sodium lactate, *myo*-inositol, rifamycin SV, L-aspartic acid, L-glutamic acid, L-histidine, L-serine, D-glucuronic acid, quinic acid, L-lactic acid, citric acid,  $\alpha$ -keto-glutaric acid, D-malic acid, L-malic acid, nalidixic acid, potassium tellurite, acetoacetic acid and sodium formate. The strain was negative for dextrin, D-maltose, D-trehalose, D-cellobiose,  $\beta$ -gentiobiose, sucrose, stachyose, pH 5, D-raffinose,  $\alpha$ -D-lactose, D-melibiose,  $\beta$ -methyl-D-galactoside, D-salicin, *N*-acetyl-D-glucosamine, *N*-acetyl- $\beta$ -D-mannosamine, *N*-acetyl-D-galactosamine, *N*-acetyl-neuraminic acid, 8% NaCl, D-glucose, D-mannose, D-fructose, inosine, fusidic acid, D-serine, D-sorbitol, D-mannitol, D-arabitol, glycerol, D-glucose-6-phosphate, D-fructose-6-phosphate, D-aspartic acid, D-serine, troleandomycin, minocycline, gelatin, glycyl-L-proline, L-alanine, L-arginine, L-pyroglutamic acid, lincomycin, guanidine hydrochloride, niaproof, pectin, D-galacturonic acid, L-galactonic acid- $\gamma$ -lactone, D-gluconic acid, glucuronamide, mucic acid, D-saccharic acid, vancomycin, tetrazolium violet, tetrazolium blue, *p*-hydroxy-phenylacetic acid, methyl pyruvate, D-lactic acid methyl ester, bromo-succinic acid, lithium chloride, tween 40,  $\gamma$ -amino-n-butyric acid,  $\alpha$ -hydroxy-butyric acid,  $\beta$ -hydroxy-butyric acid,  $\alpha$ -keto-butyric acid, propionic acid, acetic acid, aztreonam, butyric acid and sodium bromate.

At 37°C, the strain was positive for D-maltose, D-trehalose, D-cellobiose,  $\beta$ -gentiobiose, sucrose, D-turanose, stachyose, pH 6, D-raffinose, D-melibiose,  $\beta$ -methyl-D-galactoside, D-salicin, 1% NaCl, 4% NaCl, D-glucose, D-mannose, D-fructose, D-galactose, 3-O-methyl-D-glucose, D-fucose, L-fucose, L-rhamnose, inosine, 1% sodium lactate, D-sorbitol, D-mannitol, D-arabitol, *myo*-inositol, glycerol, rifamycin SV, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-serine, pectin, D-gluconic acid, D-glucuronic acid, glucuronamide, quinic acid, methyl pyruvate, L-lactic acid, citric acid,  $\alpha$ -keto-glutaric acid, D-malic acid, L-malic acid, nalidixic acid, potassium tellurite, tween 40,  $\gamma$ -amino-n-butyric acid,  $\beta$ -hydroxy-butyric acid, propionic acid, acetic acid and sodium formate. No reactions could be observed for dextrin, pH 5,  $\alpha$ -D-lactose, *N*-acetyl-D-glucosamine, *N*-acetyl- $\beta$ -D-mannosamine, *N*-acetyl-D-galactosamine, *N*-acetyl-neuraminic acid, 8% NaCl, fusidic acid, D-serine, D-glucose-6-phosphate, D-fructose-6-phosphate, D-aspartic acid, D-serine, troleandomycin, minocycline, gelatin, glycyl-L-proline, L-pyroglutamic acid, lincomycin, guanidine hydrochloride, niaproof, D-galacturonic acid, L-galactonic acid- $\gamma$ -lactone, mucic acid, D-saccharic acid, vancomycin, tetrazolium violet, tetrazolium blue, *p*-hydroxy-phenylacetic acid, D-lactic acid methyl ester, bromo-succinic acid, lithium chloride,  $\alpha$ -hydroxy-butyric acid,  $\alpha$ -keto-butyric acid, acetoacetic acid, aztreonam, butyric acid and sodium bromate.

According to [1], *R. thermophilum* is able to metabolize a wide range of carbon sources. This observation is not fully confirmed by the OmniLog measurements at 28°C. For instance, more than eleven sugars were not metabolized under the given cultivation conditions in the Generation-III microplates. This is apparently caused by distinct cultivation conditions, because the behavior is in high agreement with [1] if a temperature of 37°C is chosen, which is closer to the reported optimum temperature [1]. Particularly the optimal growth temperature of 45°C highly differs from the one that had to be used in the OmniLog assays (28°C). Conversely, in contrast to [1] the OmniLog measurements yielded positive reactions for citrate, L-histidine and L-serine at 28°C and additionally for propionate at 37°C. This may be due to the higher sensitivity of respiratory measurements compared to growth measurements [24,25].



## Chemotaxonomy

The principal cellular fatty acids of strain C-Ivk-R2A-2<sup>T</sup> are C<sub>19:0 cyclo</sub> ω<sub>7c</sub> (43.9 %), C<sub>16:0</sub> (22.3 %), C<sub>18:0</sub> (22.0 %), C<sub>18:1 ω<sub>7c</sub></sub> (4.5 %), C<sub>10:0 3-OH</sub> (1.2 %), C<sub>18:1 ω<sub>7c</sub></sub> 11-methyl (0.9 %), C<sub>20:2 ω<sub>6,9c</sub></sub> (0.7 %), C<sub>17:0 cyclo</sub> (0.5 %) C<sub>17:0</sub> (0.4 %) and summed feature 2 containing C<sub>16:1 iso I</sub> and/or C<sub>14:0 3-OH</sub> (1.2 %). Two unknown fatty acids are identified by their equivalent chain length (ECL): ECL 11.799 (2.3 %) as well as ECL 17.322 (0.7 %) [1].

Additionally, ubiquinone Q-10 is the predominant respiratory lipoquinone, but ubiquinone Q-9 was also detected in minor amounts [1].

The polyamine pattern is characterized by the major compounds spermidine (11.5 μmol/g dry

weight), *sym*-homospermidine (9.7 μmol/g dry weight) and putrescine (8.9 μmol/g dry weight) [1]. Minor polyamine pattern compounds are spermine (1.9 μmol/g dry weight), *sym*-norspermidine (0.4 μmol/g dry weight), cadaverine (0.1 μmol/g dry weight) and diaminopropane in trace amounts [1]. Interestingly, this polyamine composition is different from other previously reported members of the family *Rhodobacteraceae*. Whereas members of *Paracoccus*, *Rhodobacter*, *Rhodovulum* and *Roseomonas* mainly contain spermidine and putrescine, the polyamine compound *sym*-homospermidine is not detectable in these representatives [1].

**Table 1.** Classification and general features of *R. thermophilum* C-Ivk-R2A-2<sup>T</sup> according to the MIGS recommendations [14].

MIGS ID	Property	Term	Evidence code
MIGS-7	Current classification	Domain <i>Bacteria</i>	TAS [15]
		Phylum <i>Proteobacteria</i>	TAS [16]
		Class <i>Alphaproteobacteria</i>	TAS [17]
		Order <i>Rhodobacterales</i>	TAS [18,19]
		Family <i>Rhodobacteraceae</i>	TAS [20]
		Genus <i>Rubellimicrobium</i>	TAS [1]
		Species <i>Rubellimicrobium thermophilum</i>	TAS [1]
		Type strain C-Ivk-R2A-2 <sup>T</sup>	TAS [1]
	Gram stain	negative	TAS [1]
	Cell shape	rod-shaped	TAS [1]
	Motility	motile	TAS [1]
	Sporulation	non-sporulating	TAS [1]
	Temperature range	thermophile (28°C – 56°C)	TAS [1]
	Optimum temperature	45-52°C	TAS [1]
	Salinity	stenohaline	NAS
MIGS-22	Relationship to oxygen	aerobic	TAS [1]
	Carbon source	mono- and polysaccharides	TAS [1]
MIGS-6	Habitat	not reported	
MIGS-6.2	pH	not reported	
MIGS-15	Biotic relationship	free living	NAS
	Biosafety level	1	TAS [21]
MIGS-23.1	Isolation	colored deposits in a pulp dryer	TAS [1]
MIGS-4	Geographic location	Finland	TAS [1]
MIGS-4.1	Latitude	not reported	
MIGS-4.2	Longitude	not reported	
MIGS-4.3	Depth	not reported	

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 the Gene Ontology project [22].

## Genome sequencing and annotation

### Genome project history

The genome was sequenced within the project “Ecology, Physiology and Molecular Biology of the *Roseobacter* clade: Towards a Systems Biology Understanding of a Globally Important Clade of Marine Bacteria” funded by the German Research Council (DFG). The strain was chosen for genome sequencing according the *Genomic Encyclopedia of Bacteria and Archaea* (GEBA) criteria [26,27]. Project information is stored at the Genomes On-Line Database [12]. The Whole Genome Shotgun (WGS) sequence is deposited in Genbank and the Integrated Microbial Genomes database (IMG) [28]. A summary of the project information is shown in Table 2.

### Growth conditions and DNA isolation

A culture of DSM 16684<sup>T</sup> was grown aerobically in DSMZ medium 830 (R2A medium) [29] at 45°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, the incubation on ice over night 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 [30].

### Genome sequencing and assembly

The genome was sequenced using a combination of Illumina and 454 libraries (Table 2). Illumina sequencing was performed on a GA IIx platform with 150 cycles. The paired-end library contained inserts of 456 nt length in average. To correct sequencing errors and improve quality of the reads, clipping was performed using fastq-mcf [31] and quake [32]. The remaining 4,190,250 reads with an average length of 106 nt were assembled using Velvet [33]. To gain information on the contig arrangement an additional 454 run was performed. The paired-end jumping library of 3 kb insert size was sequenced on a 1/8 lane. Pyrosequencing resulted in 115,925 reads, with an average read length of 451 nt, assembled with Newbler (Roche Diagnostics) into a draft assembly comprising 36 scaffolds. Both draft assemblies (Illumina and 454 sequences) were fractionated into artificial Sanger reads of 1000 nt in length plus 75 nt overlap on each site. These artificial reads served as an input for the phred/phrap/consed package [34]. By manual editing the number of contigs was reduced to 44 organized in ten scaffolds. The combined sequences provided a 203 × coverage of the genome.

**Table 2.** Genome sequencing project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	Non-contiguous finished
MIGS-28	Libraries used	Two genomic libraries: one Illumina PE library (456 bp insert size), one 454 PE library (3kb insert size)
MIGS-29	Sequencing platforms	Illumina GAIIx, 454 GS-FLX + Titanium (Roche)
MIGS-31.2	Sequencing coverage	203 ×
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	AAOLV000000000
	GenBank Date of Release	July 31, 2013
	GOLD ID	Gi11864
	NCBI project ID	178147
	Database: IMG	2521172529
MIGS-13	Source material identifier	DSM 16684
	Project relevance	Tree of Life, biodiversity

## Genome annotation

Genes were identified using Prodigal [35] as part of the JGI genome annotation pipeline [36]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) non-redundant database, UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Identification of RNA genes were carried out by using HMMER 3.0rc1 [37] (rRNAs) and tRNAscan-SE 1.23 [38] (tRNAs). Other non-coding genes were predicted using INFERNAL 1.0.2 [39]. Additional gene prediction analysis and functional annotation was performed within the Integrated Microbial Genomes - Expert Review

(IMG-ER) platform [40]. CRISPR elements were detected using CRT [41] and PILER-CR [42].

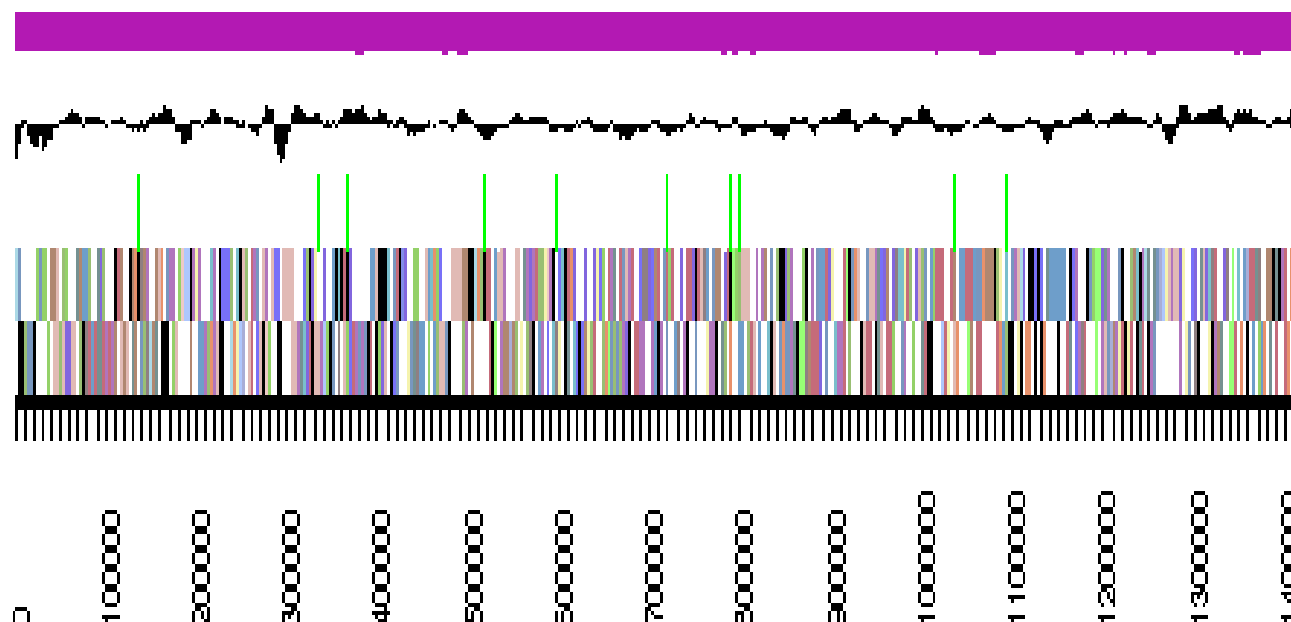
## Genome properties

The genome statistics are provided in Table 3 and Figure 3. The genome has a total length of 3,161,245 bp and a G+C content of 69.1%. Of the 3,288 genes predicted, 3,243 were protein-coding genes, and 45 RNAs. The majority of the protein-coding genes (80.4%) 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)	3,161,245	100.00
DNA coding region (bp)	2,813,333	88.99
DNA G+C content (bp)	2,185,501	69.13
Number of scaffolds	10	
Total genes	3,288	100.00
RNA genes	45	1.37
rRNA operons	1	
tRNA genes	37	1.13
Protein-coding genes	3,243	98.63
Genes with function prediction (proteins)	2,645	80.44
Genes in paralog clusters	2,688	81.75
Genes assigned to COGs	2,599	79.05
Genes assigned Pfam domains	2,689	81.78
Genes with signal peptides	235	7.15
Genes with transmembrane helices	664	20.19
CRISPR repeats	1	





**Figure 3.** Map of the largest scaffold. From bottom to top: 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 (purple/olive).

## Insights into the genome

The ten scaffolds of the draft genome sequence of strain C-Ivk-R2A-2<sup>T</sup> were screened with BLAST for the presence of the four abundant plasmid replicases from the *Rhodobacterales*, representing DnaA-like, RepABC-, RepA- and RepB-type replicons [43]. None of these typical extrachromosomal elements was detected.

Prophage-like structures have been found in many bacteria and they are known to drive the diversity of bacteria by facilitating lateral gene transfer [44]. Genome analysis of strain DSM 16684<sup>T</sup> revealed the presence of several genes encoding proteins associated with prophages (ruthe\_00218 to 00220, ruthe\_00605, ruthe\_00607 to 00610, ruthe\_00612, ruthe\_00614, ruthe\_00617, ruthe\_00618, ruthe\_00620, ruthe\_2061, ruthe\_2066, ruthe\_02072, ruthe\_02185, ruthe\_02480, ruthe\_02482 to 02484, ruthe\_02495, ruthe\_02499, ruthe\_02502, ruthe\_02972, ruthe\_02974, ruthe\_02976, ruthe\_02977, ruthe\_02984, ruthe\_02988, and ruthe\_02991 to 03295).

The *soxB* gene (ruthe\_01788) encodes a component of the thiosulfate-oxidizing Sox enzyme complex, which is known to be part of the genomes of various groups of bacteria [45]. Several other genes involved in this process were also detected (e.g. ruthe\_01784, ruthe\_01785 and ruthe\_01786).

Genome analysis of strain *R. thermophilum* DSM 16684<sup>T</sup> further revealed the presence of several genes encoding proteins associated with the utilization of urease (ruthe\_02149 to 02151, ruthe\_02153 to 02156). Several genes encoding proteins involved in the transport of Fe<sup>3+</sup>-siderophores and Fe<sup>3+</sup>-hydroxamate via ABC-transporters were also detected (e.g. ruthe\_03167 to 03172).

Additionally, several gene sequences associated with CRISPRs (ruthe\_02227 to 02230, ruthe\_02232 to 02234, ruthe\_02250, ruthe\_02251, ruthe\_02253 and ruthe\_02255), cytochrome c oxidase activity (ruthe\_00413 to 00417), cytochrome *cbb3* oxidase activity (ruthe\_01647 to 01654) as well as cytochrome *bd-I* ubiquinol oxidase activity (ruthe\_01776, ruthe\_01777) were found.

Additional gene sequences of interest encode a predicted ring-cleavage extradiol dioxygenase (ruthe\_00477), which indicates a possible degradation of aromatic compounds. A sensor of blue light using FAD (BLUF, ruthe\_01818) was also found, indicating possible blue-light dependent signal transduction.

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

Code	value	%age	Description
J	149	5.2	Translation, ribosomal structure and biogenesis
A	3	0.1	RNA processing and modification
K	162	5.7	Transcription
L	117	4.1	Replication, recombination and repair
B	3	0.1	Chromatin structure and dynamics
D	25	0.9	Cell cycle control, cell division, chromosome partitioning
Y	0	0.0	Nuclear structure
V	30	1.1	Defense mechanisms
T	86	3.0	Signal transduction mechanisms
M	165	5.8	Cell wall/membrane/envelope biogenesis
N	32	1.1	Cell motility
Z	1	0.0	Cytoskeleton
W	0	0.0	Extracellular structures
U	51	1.8	Intracellular trafficking and secretion, and vesicular transport
O	118	4.1	Posttranslational modification, protein turnover, chaperones
C	170	5.9	Energy production and conversion
G	306	10.7	Carbohydrate transport and metabolism
E	334	11.7	Amino acid transport and metabolism
F	74	2.6	Nucleotide transport and metabolism
H	128	4.5	Coenzyme transport and metabolism
I	99	3.5	Lipid transport and metabolism
P	145	5.1	Inorganic ion transport and metabolism
Q	85	3.0	Secondary metabolites biosynthesis, transport and catabolism
R	331	11.5	General function prediction only
S	254	8.9	Function unknown
-	689	21.0	Not in COGs

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## References

- Denner EBM, Kolari M, Hoornstra D, Tsitko I, Kämpfer 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](https://pubmed.ncbi.nlm.nih.gov/16511111/) <http://dx.doi.org/10.1099/ijs.0.63751-0>
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol* 1990; **215**:403-410. [PubMed](https://pubmed.ncbi.nlm.nih.gov/2231812/)
- Korf I, Yandell M, Bedell J. BLAST, O'Reilly, Sebastopol, 2003.
- DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. Greengenes, a Chimera-Checked 16S

- rRNA Gene Database and Workbench Compatible with ARB. *Appl Environ Microbiol* 2006; **72**:5069-5072. [PubMed](#)  
<http://dx.doi.org/10.1128/AEM.03006-05>
5. Porter MF. An algorithm for suffix stripping. *Program: electronic library and information systems* 1980; **14**:130-137.
  6. Lee C, Grasso C, Sharlow MF. Multiple sequence alignment using partial order graphs. *Bioinformatics* 2002; **18**:452-464. [PubMed](#)  
<http://dx.doi.org/10.1093/bioinformatics/18.3.452>
  7. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 2000; **17**:540-552. [PubMed](#)  
<http://dx.doi.org/10.1093/oxfordjournals.molbev.a026334>
  8. Stamatakis A, Hoover P, Rougemont J. A rapid bootstrap algorithm for the RAxML web-servers. *Syst Biol* 2008; **57**:758-771. [PubMed](#)  
<http://dx.doi.org/10.1080/10635150802429642>
  9. Hess PN, De Moraes Russo CA. An empirical test of the midpoint rooting method. *Biol J Linn Soc Lond* 2007; **92**:669-674.  
<http://dx.doi.org/10.1111/j.1095-8312.2007.00864.x>
  10. Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A. How many bootstrap replicates are necessary? *Lect Notes Comput Sci* 2009; **5541**:184-200.  
[http://dx.doi.org/10.1007/978-3-642-02008-7\\_13](http://dx.doi.org/10.1007/978-3-642-02008-7_13)
  11. Swofford DL. PAUP\*: Phylogenetic Analysis Using Parsimony (\*and Other Methods), Version 4.0 b10. Sinauer Associates, Sunderland, 2002.
  12. 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>
  13. Wagner-Döbler I, Ballhausen B, Berger M, Brinkhoff T, Buchholz I, Bunk B, Cypionka H, Daniel R, Drepper T, Gerdt G, et al. The complete genome sequence of the algal symbiont *Dinoroseobacter shibae* – a hitchhiker's guide to life in the sea. *ISME J* 2010; **4**:61-77. [PubMed](#)  
<http://dx.doi.org/10.1038/ismej.2009.94>
  14. 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>
  15. Woese CR, Kandler O, Weelis ML. Towards a natural system of organisms. Proposal for the domains *Archaea* and *Bacteria*. *Proc Natl Acad Sci USA* 1990; **87**:4576-4579. [PubMed](#)  
<http://dx.doi.org/10.1073/pnas.87.12.4576>
  16. 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.
  17. 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 *Alpha*-, *Beta*-, *Delta*-, and *Epsilonproteobacteria*), Springer, New York, 2005, p. 1.
  18. 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>
  19. Garrity GM, Bell JA, Lilburn T. Order III. *Rhodobacterales* ord. 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 *Alpha*-, *Beta*-, *Delta*-, and *Epsilonproteobacteria*), Springer, New York, 2005, p. 161.
  20. Garrity GM, Bell JA, Lilburn T. Family I. *Rhodobacteraceae* fam. 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 *Alpha*-, *Beta*-, *Delta*-, and *Epsilonproteobacteria*), Springer, New York, 2005, p. 161.
  21. BAUA. Classification of *Bacteria* and *Archaea* in risk groups. *TRBA* 2010; **466**:93.
  22. 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>
  23. Vaas LAI, Sikorski J, Michael V, Göker M, Klenk HP. Visualization and curve-parameter estimation strategies for efficient exploration of phenotype microarray kinetics. *PLoS ONE* 2012; **7**:e34846.

- [PubMed](#)  
<http://dx.doi.org/10.1371/journal.pone.0034846>
24. Vaas LA, Sikorski J, Hofner B, Fiebig A, Buddhuhs N, Klenk HP, Göker M. opm: an R package for analyzing OmniLog® phenotype microarray data. *Bioinformatics* 2013; **29**:1823-1824. [PubMed](#)  
<http://dx.doi.org/10.1093/bioinformatics/btt291>
25. Bochner BR. Global phenotypic characterization of bacteria. *FEMS Microbiol Rev* 2009; **33**:191-205. [PubMed](#) <http://dx.doi.org/10.1111/j.1574-6976.2008.00149.x>
26. Göker M, Klenk HP. Phylogeny-driven target selection for genome-sequencing (and other) projects. *Stand Genomic Sci* 2013; **8**:360-374. [PubMed](#)  
<http://dx.doi.org/10.4056/sigs.3446951>
27. 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>
28. 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>
29. List of growth media used at the DSMZ: <http://www.dsmz.de/catalogues/catalogue-microorganisms/culture-technology/list-of-media-for-microorganisms.html>.
30. 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. [PubMed](#)  
<http://dx.doi.org/10.1089/bio.2010.0029>
31. Aronesty E. *ea-utils*: Command-line tools for processing biological sequencing data. 2011; <http://code.google.com/p/ea-utils>.
32. 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>
33. 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>
34. 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>
35. 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>
36. 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**:63-67. [PubMed](#) <http://dx.doi.org/10.4056/sigs.632>
37. Finn DR, Clements J, Eddy SR. HMMER web server: interactive sequence similarity searching. *Nucleic Acids Res. Web Server Issue* 2011; **39**:W29-W37.
38. 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.
39. 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>
40. 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>
41. 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>
42. 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>
43. Petersen J, Frank O, Göker M, Pradella S. Extrachromosomal, extraordinary and essential--the plasmids of the *Roseobacter* clade. *Appl Microbiol Biotechnol* 2013; **97**:2805-2815. [PubMed](#)  
<http://dx.doi.org/10.1007/s00253-013-4746-8>
44. Ochman H, Lawrence J, Groisman EA. Lateral gene transfer and the nature of bacterial innovation. *Nature* 2000; **405**:299-304. [PubMed](#)  
<http://dx.doi.org/10.1038/35012500>
45. Friedrich CG, Bardischewsky F, Rother D, Quentmeier A, Fischer J. Prokaryotic sulfur oxidation. *Curr Opin Microbiol* 2005; **8**:253-259. [PubMed](#)  
<http://dx.doi.org/10.1016/j.mib.2005.04.005>