Complete genome sequence of the aquatic bacterium  

**Runella slithyformis** type strain (LSU 4T)

Alex Copeland1, Xiaojing Zhang1,2, Monica Misra1,2, Alla Lapidus1, Matt Nolan1, Susan Lucas1, Shweta Deshpande1, Jan-Fang Cheng1, Roxanne Tapia1,2, Lynne A. Goodwin1,2, Sam Pitluck1, Konstantinos Liolios1, Ioanna Pagani1, Natalia Ivanova1, Natalia Mikhailova1, Amrita Pati1, Amy Chen1, Krishna Palaniappan1, Miriam Land1,4, Loren Hauser1,4, Chongle Pan1,4, Cynthia D. Jeffries1,4, John C. Detter1, Evelyne-Marie Brambilla1, Manfred Rohde6, Olivier D. Ngatchou Djao6, Markus Göker5, Johannes Sikorski5, Brian J. Tindall5, Tanja Woyke1, James Bristow1, Jonathan A. Eisen1,7, Victor Markowitz3, Philip Hugenholtz1,8, Nikos C. Kyrpides1, Hans-Peter Klenk5,8, and Konstantinos Mavromatis1

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

*Corresponding author: Hans-Peter Klenk*

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**Introduction**

Strain LSU 4T (= DSM 19594 = ATCC 29530 = NCIMB 11436) is the type strain of the species *Runella slithyformis*, which is the type species of its genus *Runella* [1,2]. The genus currently consists of four validly named species [3]. The genus name is derived from 'rune', a runic letter and the Latin diminutive ending 'ella', yielding the Neo-Latin word *'Runella'* meaning 'that which resembles figures of the runic alphabet' [3]. The species epithet is derived from slithy, a nonsense word from Lewis Carroll's *Jabberwocky* for a fictional organism that is 'slithy' and the Latin word 'suffix' meaning '-like, in the shape of', yielding the Neo-Latin word 'slithyformis' meaning 'slithy in form' [3]. *R. slithyformis* strain LSU 4T was isolated from University Lake near Baton Rouge, Louisiana, USA, and described by Larkin and Williams in 1978 [1]. Another strain of *R. slithyformis*, termed strain 6, was isolated from Elbow Bayou near Baton Rouge [1]. Members of the genus *Runella* colonize diverse environmental habitats, preferentially aquatic ecosystems, including water bodies in Baton Rouge [1], a wastewater treatment plant in South-Korea [4], environmental water samples and their biofilms in...
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Japan [5], and an activated sludge process involved in enhanced biological removal of phosphor in Korea [6]. Another species of this genus was also isolated from the stems of surface-sterilized maize [7]. Here we present a summary classification and a set of features for *R. slithyformis* strain LSU 4T, together with the description of the complete finished genome sequencing and annotation.

### Classification and features

A representative genomic 16S rRNA sequence of *R. slithyformis* LSU 4T was compared using NCBI BLAST [8,9] 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 [10] and the relative frequencies of taxa and keywords (reduced to their stem [11]) were determined, weighted by BLAST scores. The most frequently occurring gene names were *Runella* (31.0%), *Dyadobacter* (30.3%), *Cytophaga* (13.7%), *Cyclobacterium* (7.5%) and *Algoriphagus* (4.0%) (51 hits in total). Regarding the single hit to sequences from members of the species, the average identity within HSPs was 99.2%, whereas the average coverage by HSPs was 96.9%. Regarding the two hits to sequences from other members of the genus, the average identity within HSPs was 95.0%, whereas the average coverage by HSPs was 91.1%. Among all other species, the one yielding the highest score was *R. zeae* (NR_025004), which corresponded to an identity of 95.0% and an HSP coverage of 91.1%. (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 GQ480089 (changes during sewage treated process activated sludge wastewater treatment plant clone BXHB50*), which showed an identity of 96.6% and an HSP coverage of 98.0%. The most frequently occurring keywords within the labels of all environmental samples which yielded hits were 'skin' (5.5%), 'soil' (2.1%), 'sludg' (2.0%), 'biofilm' (1.7%) and 'forearm, volar' (1.7%) (199 hits in total). While few of these keywords fit the aquatic and sludge environments from which strain LSU 4T originated, the majority of the hits point to human and even soil, which were, until now, not considered as habitats for *R. slithyformis*. However, environmental samples which yielded hits of a higher score than the highest scoring species were not found.

Figure 1 shows the phylogenetic neighborhood of *R. slithyformis* LSU 4T in a 16S rRNA based tree. The sequences of the two identical 16S rRNA gene copies in the genome do not differ from the previously published 16S rRNA sequence (M62786), which contains 13 ambiguous base calls.

The cells of strain LSU 4T are generally curved rods, with the degree of curvature of individual cells within a culture varying from nearly straight to crescent shape. Cell diameter varies from 0.5 to 0.9 µm, and the length from 2.0 to 3.0 µm [1]. With its curved rod shape, strain LSU 4T differs from other members of the genus, such as *R. limosa* which has long rods while *R. zeae* is bent rod-shaped [6]. On the MS agar medium used at the time of isolation, *R. slithyformis* rarely formed long spirals. However, Chelius and Triplett [23] reported the formation of long spirals by the strain LSU 4T when cells were allowed to grow in R2A broth medium (see Figure 2). Larkin and Williams [1] reported a possible production of filaments up to 14 µm in length, which are not coiled. This contrasts the findings of Chelius *et al.* [7] who described the cells of the strain LSU 4T as circular with swollen ends that would not form filaments. Rings with an outer diameter of 2.0 to 3.0 µm may also occur [1]. Colonies produced a pale pink, nondiffusible, nonfluorescent pigment on MS agar [1]. The strain LSU 4T is a Gram-negative bacterium (Table 1). Strain LSU 4T is non-motile, aerobic and chemoorganotrophic [1]. It does not grow on media with NaCl concentrations of 1.5% or higher [23]. This feature was similar to that of another member of this genus, *R. zeae* [7]. The temperature range for growth is between 4°C-37°C, with an optimum between 20°C-30°C [6]; the strain being unable to grow at temperatures above 37°C [23]. The sole carbon sources used by the strain LSU 4T for growth on MS agar are glyco- gen, D-arabitol, dulcitol, inositol, mannitol, sorbitol and sorbose, but the growth was weak except in the presence of glycogen [23]. Some of these features are however contradictory to the findings of Chelius *et al.* [7] whose attempt to grow the strain LSU 4T in the presence of glycogen in R2A medium was unsuccessful. Further detailed physiological insight, e.g., carbon source utilization in R2A medium, MS agar medium, or by the API 50 CH test, have been reported previously [7,23]. Also, resistance to a variety of antibiotics has been reported [7,23].
Figure 1. Phylogenetic tree highlighting the position of *R. slithyformis* relative to the type strains of the type species of the other genera within the family *Cytophagaceae*. The tree was inferred from 1,330 aligned characters of the 16S rRNA gene sequence under the maximum likelihood (ML) criterion [14]. Rooting was done initially using the midpoint method [15] 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 400 ML bootstrap replicates [16] (left) and from 1,000 maximum parsimony bootstrap replicates [17] (right) if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [18] are labeled with one asterisk, those also listed as 'Complete and Published' with two asterisks [19-22].

Figure 2. Scanning electron micrograph of *R. slithyformis* LSU 4T
Chemotaxonomy

The principal cellular fatty acids of strain LSU 4T are iso-C_{15:0} 2-oH/C_{16:1}ω7c (32.1%), iso-C_{15:0} (19.8%) and C_{16:1}ω5c (16.5%) [23]. Minor fatty acids include C_{16:0} (7.1%), iso-C_{17:0} 3-oH (7.0%), anteiso-C_{15:0} (4.3%), iso-C_{15:0} 3-oH (4.1%), iso-C_{15:1} G (2.4%), C_{16:0} 3-oH (2.0%), an unknown one (ECL 13.6) (1.83%) and C_{15:0} (1.5%) [23]. Major polar lipids were not reported for strain LSU 4T, but those of the genus Runella could be retrieved from R. defluvii strain EMB13T and R. limosa strain EMB111T [4,6].

Table 1. Classification and general features of R. slithyformis LSU 4T according to the MIGS recommendations [24].

| MIGS ID | Property                        | Term                                      | Evidence code |
|---------|---------------------------------|-------------------------------------------|---------------|
| Dom 1   | Domain                          | Bacteria                                  | TAS [25]      |
| Phyl 2   | Phylum                          | Bacteroidetes                              | TAS [26,27]   |
| Class 3  | Class                           | Cytophagia                                | TAS [27,28]   |
| Order 4  | Order                           | Cytophagales                              | TAS [2,29]    |
| Family 5 | Family                          | Cytophagaceae                             | TAS [2,30]    |
| Genus 6  | Genus                           | Runella                                   | TAS [1,2]     |
| Species 7| Species                         | Runella slithyformis                      | TAS [1,2]     |
| Type strain LSU 4T | Current classification | Order Cytophagales                        | TAS [2,29]    |
| Gram stain | Gram stain                     | negative                                  | TAS [1]       |
| Cell shape | Cell shape                     | curved rod-shaped, rigid                  | TAS [1]       |
| Motility | Motility                        | non-motile                                | TAS [1]       |
| Sporulation | Sporulation                    | none                                      | TAS [1]       |
| Temperature range | Temperature range          | psychrotolerant mesophiles, grows at temperatures as low as 4°C | TAS [23] |
| Optimum temperature | Optimum temperature    | 20°C-30°C                                 | TAS [6]       |
| Salinity | Salinity                        | no growth in the presence of NaCl (1.5%)  | TAS [31]      |
| Relationship to oxygen | Relationship to oxygen | strictly aerobic                          | TAS [1]       |
| Carbon source | Carbon source                | carbohydrates                             | TAS [1,23]    |
| Energy metabolism | Energy metabolism        | chemoorganotroph                          | TAS [1]       |
| Habitat | Habitat                         | fresh water                               | TAS [1]       |
| Biotic relationship | Biotic relationship        | free living                               | NAS           |
| Known pathogenicity | Known pathogenicity       | none                                      | NAS           |
| Specific host | Specific host               | none                                      | NAS           |
| Biosafety level | Biosafety level            | 1                                         | NAS           |
| Isolation | Isolation                     | fresh water lake                          | TAS [1]       |
| Geographic location | Geographic location | University Lake, Baton Rouge, Louisiana, USA | TAS [1]       |
| Time of sample collection | Time of sample collection | 1978 or before                            | TAS [1]       |
| Latitude | Latitude                        | 30.417                                    | NAS           |
| Longitude | Longitude                      | -91.167                                   | NAS           |
| Depth | Depth                           | not reported                              | NAS           |
| Altitude | Altitude                        | 15 m                                      | NAS           |

Evidence codes - IDA: Inferred from Direct Assay (first time in publication); TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project. If the evidence code is IDA, then the property was directly observed for a living isolate by one of the authors or an expert mentioned in the acknowledgements [33].
Genome sequencing and annotation

Genome project history

This organism was selected for sequencing on the basis of its phylogenetic position [34], and is part of the **Genomic Encyclopedia of Bacteria and Archaea** project [35]. The genome project is deposited in the Genomes On Line Database [18] and the complete genome sequence is deposited in GenBank. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI). A summary of the project information is shown in Table 2.

| MIGS ID | Property                  | Term                                                                 |
|---------|---------------------------|----------------------------------------------------------------------|
| MIGS-31 | Finishing quality         | Finished                                                            |
| MIGS-28 | Libraries used            | Four genomic libraries: one 454 pyrosequence standard library, two 454 PE libraries (2 kb and 11 kb insert sizes), one Illumina library |
| MIGS-29 | Sequencing platforms      | Illumina GAii, 454 GS FLX Titanium                                   |
| MIGS-31.2 | Sequencing coverage   | 100.4 × Illumina; 28.2 × pyrosequence                               |
| MIGS-30 | Assemblers                | Newbler version 2.3, Velvet 0.7.63, phrap version SPS - 4.24         |
| MIGS-32 | Gene calling method       | Prodigal 1.4, GenePRIMP                                              |

Genbank Date of Release: August 16, 2011
GOLD ID: Gc01829
NCBI project ID: 49125
Database: IMG-GEBA 2505679030
INSDC ID: CP002859 (chromosome)
CP002860-64 (plasmids RUNSL01-05)

Growth conditions and DNA isolation

*R. slithyformis* strain LSU 4T, DSM 19594, was grown in **DSMZ medium 7** (*Ancyllobacter*-*Spirosoma* medium) [36] at 28°C. DNA was isolated from 0.5-1 g of cell paste using MasterPure Gram-positive DNA purification kit (Epicentre MGP04100) following the standard protocol as recommended by the manufacturer with modification st/DL for cell lysis as described in Wu et al. 2009 [35]. DNA is available through the DNA Bank Network [31].

Genome sequencing and assembly

The genome was sequenced using a combination of Illumina and 454 sequencing platforms. All general aspects of library construction and sequencing can be found at the JGI website [37]. Pyrosequencing reads were assembled using the Newbler assembler (Roche). The initial Newbler assembly consisting of 121 contigs in two scaffolds was converted into a phrap [38] assembly by making fake reads from the consensus, to collect the read pairs in the 454 paired end library. Illumina GAii sequencing data (638.9 Mb) was assembled with Velvet [39] and the consensus sequences were shredded into 2.0 kb overlapped fake reads and assembled together with the 454 data. The 454 draft assembly was based on 206.2 Mb 454 draft data and all of the 454 paired end data. Newbler parameters are -consed -a 50 -l 350 -g -m -ml 20. The Phred/Phrap/Consed software package [38] was used for sequence assembly and quality assessment in the subsequent finishing process. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible mis-assemblies were corrected with gapResolution [37], Dupfinisher [40], or sequencing cloned bridging PCR fragments with subcloning. Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR primer walks (J.-F. Chang, unpublished). A total of 289 additional reactions and 3 shatter libraries

http://standardsingenomics.org
were necessary to close gaps and to raise the quality of the finished sequence. Illumina reads were also used to correct potential base errors and increase consensus quality using a software Polisher developed at JGI [41]. The error rate of the completed genome sequence is less than 1 in 100,000. Together, the combination of the Illumina and 454 sequencing platforms provided 128.6 × coverage of the genome. The final assembly contained 540,807 pyrosequence and 19,068,176 Illumina reads.

**Genome annotation**

Genes were identified using Prodigal [42] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI GenePRIMP pipeline [43]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and functional annotation was performed within the Integrated Microbial Genomes - Expert Review (IMG-ER) platform [44].

**Genome properties**

The genome consists of one circular chromosome with a length of 6,568,739 bp and a G+C content of 47%, and five circular plasmids with 38,784 bp, 44,754 bp, 66,926 bp, 93,527 bp and 106,999 bp length, respectively (Table 3 and Figure 3). Of the 6,025 genes predicted, 5,974 were protein-coding genes, and 51 RNAs; 182 pseudogenes were also identified. The majority of the protein-coding genes (59.7%) 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.

| Attribute                        | Value  | % of Total |
|----------------------------------|--------|------------|
| Genome size (bp)                 | 6,919,729 | 100.00%    |
| DNA coding region (bp)           | 6,063,039 | 87.62%     |
| DNA G+C content (bp)             | 3,212,364 | 46.42%     |
| Number of replicons              | 6      |            |
| Extrachromosomal elements        | 5      |            |
| Total genes                      | 6,025  | 100.00%    |
| RNA genes                        | 51     | 0.85%      |
| rRNA operons                     | 2      |            |
| tRNA genes                       | 43     | 0.71%      |
| Protein-coding genes             | 5,974  | 99.15%     |
| Pseudo genes                     | 182    | 3.02%      |
| Genes with function prediction   | 3,599  | 59.73%     |
| Genes in paralog clusters        | 3,238  | 53.74%     |
| Genes assigned to COGs           | 3,912  | 64.93%     |
| Genes assigned Pfam domains      | 4,008  | 66.52%     |
| Genes with signal peptides       | 1,748  | 29.01%     |
| Genes with transmembrane helices | 1,350  | 22.41%     |
| CRISPR repeats                   | 0      |            |
Figure 3. Graphical map of the circular chromosome (plasmids not shown, but accessible through the img/er pages on the JGI web pages [37]). From outside to center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.
**Table 4. Number of genes associated with the general COG functional categories**

| Code | Value | %age | Description                                           |
|------|-------|------|-------------------------------------------------------|
| J    | 173   | 4.0  | Translation, ribosomal structure and biogenesis       |
| A    | 0     | 0.0  | RNA processing and modification                       |
| K    | 338   | 7.8  | Transcription                                         |
| L    | 216   | 5.0  | Replication, recombination and repair                  |
| B    | 1     | 0.2  | Chromatin structure and dynamics                       |
| D    | 36    | 0.8  | Cell cycle control, cell division, chromosome partitioning |
| Y    | 0     | 0.0  | Nuclear structure                                     |
| V    | 126   | 2.9  | Defense mechanisms                                    |
| T    | 272   | 6.3  | Signal transduction mechanisms                        |
| M    | 372   | 8.6  | Cell wall/membrane/envelope biogenesis                |
| N    | 14    | 0.3  | Cell motility                                         |
| Z    | 1     | 0.0  | Cytoskeleton                                          |
| W    | 0     | 0.0  | Extracellular structures                              |
| U    | 73    | 1.7  | Intracellular trafficking, secretion, and vesicular transport |
| O    | 132   | 3.1  | Posttranslational modification, protein turnover, chaperones |
| C    | 204   | 4.7  | Energy production and conversion                      |
| G    | 351   | 8.1  | Carbohydrate transport and metabolism                 |
| E    | 304   | 7.0  | Amino acid transport and metabolism                   |
| F    | 86    | 2.0  | Nucleotide transport and metabolism                   |
| H    | 161   | 3.7  | Coenzyme transport and metabolism                     |
| I    | 158   | 3.7  | Lipid transport and metabolism                        |
| P    | 226   | 5.2  | Inorganic ion transport and metabolism                |
| Q    | 99    | 2.3  | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 620   | 14.3 | General function prediction only                      |
| S    | 372   | 8.6  | Function unknown                                      |
| -    | 2,113 | 35.1 | Not in COGs                                           |

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