Genome sequence of the dark pink pigmented *Listia bainesii* microsymbiont *Methylobacterium* sp. WSM2598

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**Abstract**

Strains of a pink-pigmented *Methylobacterium* sp. are effective nitrogen- (N₂) fixing microsymbionts of species of the African crotalarioid genus *Listia*. Strain WSM2598 is an aerobic, motile, Gram-negative, non-spore-forming rod isolated in 2002 from a *Listia bainesii* root nodule collected at Estcourt Research Station in South Africa. Here we describe the features of *Methylobacterium* sp. WSM2598, together with information and annotation of a high-quality draft genome sequence. The 7,669,765 bp draft genome is arranged in 5 scaffolds of 83 contigs, contains 7,236 protein-coding genes and 18 RNA-only encoding genes. This rhizobial genome is one of 100 sequenced as part of the DOE Joint Genome Institute 2010 *Genomic Encyclopedia for Bacteria and Archaea-Root Nodule Bacteria* (GEBA-RNB) project.

**Keywords:** Root-nodule bacteria, Nitrogen fixation, Symbiotic specificity, Alphaproteobacteria

**Introduction**

Nodulated legumes are important and established components of Australian agricultural systems: the value of atmospheric nitrogen (N₂) fixed by rhizobia in symbiotic association with these legumes is estimated to be worth more than $2 billion annually [1,2]. The major agricultural region of south-western Australia has a Mediterranean climate, with soils that are often acid, have a low clay content and low organic matter, and tend to be inherently infertile [3,4]. The last forty years, however, have seen a sharp decrease in average winter rainfall by about 15–20% [5]. This, together with the development of dryland salinity [6], has challenged the sustainability of using the commonly sown subterranean clover and annual medics as pasture legumes in these systems. Alternative perennial legume species (and their associated rhizobia) are therefore being sought [2]. We have identified a suite of South African perennial, herbaceous forage legumes, including several species in the crotalarioid genus *Listia* (previously *Lotononis*) [7], that are potentially well-adapted to the arid climate and acid, infertile soils of the target agricultural areas.

*Listia* species are found in seasonally wet habitats throughout southern and tropical Africa [8]. They produce stoloniferous roots [8,9] and form lupinoid nodules rather than the indeterminate type found in other crotalarioid species [7,10]. Rhizobial infection occurs by epidermal entry rather than via root hair curling [7]. *Listia*-rhizobia symbioses are highly specific. The tropically distributed *L. angolensis* forms effective (i.e. N₂-fixing) nodules with newly described species of *Microvirga* [11], while all other studied *Listia* species are only nodulated by strains of pigmented methyllobacteria [7,10,12]. Unlike the methylotrophic *Methylobacterium nodulans*, which specifically nodulates some species of *Crotalaria* [13], the *Listia* methyllobacteria are unable to utilize methanol as a sole carbon source [14]. In Australia, strains of pigmented methyllobacteria have been used as commercial inoculants for *Listia bainesii* and are able to persist in acidic, sandy, infertile soils, while remaining symbiotically and serologically stable [10,15].

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Figure 1 Images of Methylobacterium sp. strain WSM2598 using scanning (Left) and transmission (Center) electron microscopy as well as light microscopy to visualize colony morphology on solid ½LA [10] (Right).

| Table 1 Classification and general features of Methylobacterium sp. strain WSM2598 according to the MIGS recommendations [17,18] |
|---|---|---|
| **MIGS ID** | **Property** | **Term** | **Evidence code** |
| Current classification | Domain | Bacteria | TAS [18] |
| | Phylum | Proteobacteria | TAS [19] |
| | Class | Alphaproteobacteria | TAS [20,21] |
| | Order | Rhizobiales | TAS [21,22] |
| | Family | Methylobacteriaceae | TAS [21,23] |
| | Genus | Methylobacterium | TAS [24-26] |
| | Species | Methylobacterium sp. | TAS [10] |
| | Strain | WSM2598 | TAS [10] |
| Gram stain | Negative | IDA |
| Cell shape | Rod | IDA |
| Motility | Motile | IDA |
| Sporulation | Non-sporulating | NAS |
| Temperature range | Mesophile | IDA |
| Optimum temperature | 28°C | NAS |
| Salinity | Non-halophile | NAS |
| MIGS-22 | Oxygen requirement | Aerobic | IDA |
| MIGS-6 | Carbon source | Formate, succinate & glutamate | TAS [14] |
| | Energy source | Chemoorganotroph | TAS [14] |
| MIGS-15 | Habitat | Soil, root nodule on host | TAS [10] |
| MIGS-14 | Biotic relationship | Free living, symbiotic | TAS [10] |
| MIGS-4 | Pathogenicity | Non-pathogenic | NAS |
| Biosafety level | 1 | TAS [27] |
| Isolation | Root nodule of *Listia bainesii* | TAS [10] |
| MIGS-4 | Geographic location | Estcourt Research Station, South Africa | TAS [10] |
| MIGS-5 | Sample collection date | May 27, 2002 | TAS [10] |
| MIGS-4.1 | Latitude | ~29.9125 | TAS [10] |
| MIGS-4.2 | Longitude | 29.16667 | TAS [10] |
| MIGS-4.3 | Depth | Not reported | NAS |
| MIGS-4.4 | Altitude | 1,200 m | IDA |

Evidence codes – IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [31].
A pigmented *Methylobacterium* strain, WSM2598, isolated from a root nodule of *L. bainesii* cv “Miles” in South Africa in 2002, was found to be a highly effective nitrogen fixing microsymbiont of both *L. bainesii* and *Listia heterophylla* (previously *Lotononis listii*) [10]. Here we present a set of preliminary classification and general features for *Methylobacterium* sp. strain WSM2598, together with the description of the genome sequence and annotation.

**Figure 2** Phylogenetic tree showing the relationships of *Methylobacterium* sp. WSM2598 (shown in blue print) with some of the root nodule bacteria in the order *Rhizobiales* based on aligned sequences of the 16S rRNA gene (1,340 bp internal region). All sites were informative and there were no gap-containing sites. Phylogenetic analyses were performed using MEGA, version 5 [28]. The tree was built using the maximum likelihood method with the General Time Reversible model. Bootstrap analysis [29] with 500 replicates was performed to assess the support of the clusters. Type strains are indicated with a superscript T. Brackets after the strain name contain an accession number. Strains with a genome sequencing project registered in GOLD [30] are in bold print and the GOLD ID is mentioned after the accession number. Published genomes are designated with an asterisk.

**Organism information**

*Methylobacterium* sp. strain WSM2598 is a motile, non-sporulating, non-encapsulated, Gram-negative rod with one to several flagella. It is a member of the family *Methylobacteriaceae* in the class *Alphaproteobacteria*. The rod-shaped form varies in size with dimensions of approximately 0.5 μm in width and 1.0-1.5 μm in length (Figure 1 Left and 1 Center). WSM2598 is medium to slow growing, forming
0.5–1.5 mm diameter colonies within 6–7 days at 28°C. WSM2598 is pigmented, an unusual property for rhizobia. When grown on half strength Lupin Agar (½LA) [10], WSM2598 forms dark pink pigmented, opaque, slightly domed colonies with smooth margins (Figure 1 Right).

WSM2598 alkalinizes ½LA containing universal indicator (BDH Laboratory Supplies). WSM2598 cultured in minimal medium [16] is unable to utilize arabinose, galactose, glucose, mannitol, methanol, methylamine or formaldehyde as sole carbon sources, but grows poorly on formate and well on succinate and glutamate [14]. Minimum Information about the Genome Sequence (MIGS) is provided in Table 1 and Additional file 1: Table S1.

Table 2 Compatibility of Methylobacterium sp. WSM2598 with 11 host legume genotypes for nodulation (Nod) and N₂-Fixation (Fix)

| Species name | Nod  | Fix  | Reference |
|--------------|------|------|-----------|
| Listia angolensis (Welw. ex Bak.) B.-E. van Wyk & Boatwr. | +(w) | - | [7,10] |
| Listia bainesii (Bak.) B.-E. van Wyk & Boatwr. | + | + | [7,10] |
| Listia heterophylla E. Mey. | + | + | [7,10] |
| Listia marlothii (Engl.) B.-E. van Wyk & Boatwr. | + | + | [7,10] |
| Listia solutudinis (Dümmer) B.-E. van Wyk & Boatwr. | + | + | [10] |
| Listia subulata (B.-E. van Wyk) B.-E. van Wyk & Boatwr. | + | + | [10] |
| Leobordea lanata (Thunb.) B.-E. van Wyk & Boatwr. (=Lotononis bolusii) | +(w) | - | [7] |
| Leobordea langiflora (H. Bolus) B.-E. van Wyk & Boatwr. | +(w) | - | [7] |
| Leobordea stipulosa (Bak. f.) B.-E. van Wyk & Boatwr. | +(w) | - | [7] |
| Macroptilium atropurpureum (DC.) Urb. cv. Siratro | +(w) | - | [10] |

(w) indicates nodules present were white.

Figure 2 shows the phylogenetic neighborhood of Methylobacterium sp. WSM2598 in a 16S rRNA sequence based tree. The 16S rDNA sequence of WSM2598 has 99% (1,358/1,364 bp) and 98% (1,334/1,365 bp) sequence identity to the 16S rRNA of the fully sequenced strains Methylobacterium sp. 4–46 (Gc00857) and M. nodulans ORS2060 (Gc00935), respectively.

Symbiotaxonomy
Methylobacterium sp. WSM2598 forms nodules on (Nod+), and fixes N₂ (Fix+), with southern African species of Listia. On Listia angolensis, some species of the crotalarioid genus Leobordea and the promiscuous legume Macroptilium atropurpureum, WSM2598 forms white, ineffective (Fix-)

Table 3 Genome sequencing project information for Methylobacterium sp. WSM2598

| MIGS ID | Property | Term |
|---------|----------|------|
| MIGS-31 | Finishing quality | Improved high quality draft |
| MIGS-28 | Libraries used | Illumina GAII standard PE and CLIP PE libraries |
| MIGS-29 | Sequencing platforms | Illumina GAII technology |
| MIGS-312 | Sequencing coverage | 685x Illumina |
| MIGS-30 | Assemblers | Velvet, version 1.0.05; Allpaths r99750 |
| MIGS-32 | Gene calling method | Prodigal 1.4 |
| GenBank | | ARAA0000000.1 |
| GenBank release date | | August 28, 2013 |
| GOLD ID | | G08887 |
| NCBI project ID | | 88639 |
| Database: IMG | | 2517572068 |
| Project relevance | | Symbiotic N₂ fixation, agriculture |

Table 4 Genome statistics for Methylobacterium sp. WSM2598

| Attribute | Value | % of total |
|-----------|-------|-----------|
| Genome size (bp) | 7,669,765 | 100.00 |
| DNA coding region (bp) | 6,286,667 | 81.97 |
| DNA G+C content (bp) | 5,458,294 | 71.17 |
| Number of scaffolds | 5 | |
| Number of contigs | 83 | |
| Total genes | 7,349 | 100.00 |
| RNA genes | 18 | 0.24 |
| rRNA operons | 6 | 0.08 |
| Protein-coding genes | 7,236 | 98.46 |
| Genes with function prediction | 5,234 | 71.22 |
| Genes assigned to COGs | 5,025 | 68.38 |
| Genes assigned Pfam domains | 5,314 | 72.31 |
| Genes with signal peptides | 736 | 10.01 |
| Genes with transmembrane helices | 1,492 | 20.30 |
| CRISPR repeats | 3 | |
Figure 3 (See legend on next page.)
nodules. It does not form nodules on other tested legumes [7], [Table 2].

**Genome sequencing and annotation information**

**Genome project history**

This organism was selected for sequencing on the basis of its environmental and agricultural relevance to issues in global carbon cycling, alternative energy production, and biogeochemical importance, and is part of the Community Sequencing Program at the U.S. Department of Energy, Joint Genome Institute (JGI) for projects of relevance to agency missions. The genome project is deposited in the Genomes OnLine Database [30] and an improved-high-quality-draft genome sequence in IMG. Sequencing, finishing and annotation were performed by the JGI. A summary of the project information is shown in Table 3.

**Growth conditions and DNA isolation**

*Methylobacterium* sp. WSM2598 was grown to mid-logarithmic phase in TY rich media on a gyratory shaker at 28°C [32]. DNA was isolated from 60 mL of cells using a CTAB (Cetyl trimethyl ammonium bromide) bacterial genomic DNA isolation method [33].

**Genome sequencing and assembly**

The draft genome of *Methylobacterium* sp. WSM2598 was generated at the DOE Joint Genome Institute (JGI) using Illumina technology [34,35]. For this genome, we constructed and sequenced an Illumina short-insert paired-end library with an average insert size of 270 bp which generated 19,048,548 reads and an Illumina long-insert paired-end library with an average insert size of 6354.14 +/− 3100.07 bp which generated 18,876,864 reads totaling 5,689 Mbp of Illumina data. (unpublished, Feng Chen). All general aspects of library construction and sequencing performed at the JGI can be found at the JGI website. The initial draft assembly contained 141 contigs in 41 scaffold(s). The initial draft data was assembled with Allpaths, version 39750, and the consensus was computationally shredded into 1 Kbp overlapping fake reads. The Illumina draft data was also assembled with Velvet, version 1.1.05 [36] and the consensus sequences were computationally shredded into 1.5 Kbp overlapping fake reads (shreds). The Illumina draft data was assembled again with Velvet using the shreds from the first Velvet assembly to guide the next assembly. The consensus from the second VELVET assembly was shredded into 1.5 Kbp overlapping fake reads. The fake reads from the Allpaths assembly and both Velvet assemblies and a subset of the Illumina CLIP paired-end reads were assembled using parallel phrap, version 4.24 (High Performance Software, LLC). Possible mis-assemblies were corrected with manual editing in Consed [37-39]. Gap closure was accomplished using repeat resolution software (Wei Gu, unpublished), and sequencing of bridging PCR fragments with Sanger and/or PacBio (unpublished, Cliff Ardley et al. Standards in Genomic Sciences 2014, 9:5 http://www.standardsingenomics.com/content/9/1/5

| Table 5 Number of protein coding genes of *Methylobacterium* sp. WSM2598 associated with the general COG functional categories |
|---|---|---|---|
| Code | Value | % age | COG category |
| J | 176 | 3.15 | Translation, ribosomal structure and biogenesis |
| A | 3 | 0.05 | RNA processing and modification |
| K | 398 | 7.13 | Transcription |
| L | 384 | 6.88 | Replication, recombination and repair |
| B | 5 | 0.09 | Chromatin structure and dynamics |
| D | 44 | 0.79 | Cell cycle control, mitosis and meiosis |
| Y | 0 | 0.00 | Nuclear structure |
| V | 78 | 1.40 | Defense mechanisms |
| T | 422 | 7.56 | Signal transduction mechanisms |
| M | 306 | 5.48 | Cell wall/membrane biogenesis |
| N | 139 | 2.49 | Cell motility |
| Z | 2 | 0.04 | Cytoskeleton |
| W | 0 | 0.00 | Extracellular structures |
| U | 96 | 1.72 | Intracellular trafficking and secretion |
| O | 155 | 2.78 | Posttranslational modification, protein turnover, chaperones |
| C | 399 | 7.15 | Energy production conversion |
| G | 307 | 5.50 | Carbohydrate transport and metabolism |
| E | 526 | 9.42 | Amino acid transport metabolism |
| F | 80 | 1.43 | Nucleotide transport and metabolism |
| H | 208 | 3.73 | Coenzyme transport and metabolism |
| I | 234 | 4.19 | Lipid transport and metabolism |
| P | 285 | 5.11 | Inorganic ion transport and metabolism |
| Q | 174 | 3.12 | Secondary metabolite biosynthesis, transport and catabolism |
| R | 640 | 11.47 | General function prediction only |
| S | 520 | 9.32 | Function unknown |
| - | 2,324 | 31.62 | Not in COGS |
Han) technologies. One round of manual/wet lab finishing was also completed. 17 PCR PacBio consensus sequences were completed to close gaps and to raise the quality of the final sequence. The total (“estimated size” for the unfinished) size of the genome is 8.3 Mb and the final assembly is based on 5.689 Mbp of Illumina draft data, which provides an average 685× coverage of the genome.

**Genome annotation**

Genes were identified using Prodigal [40] as part of the DOE-JGI Annotation pipeline [41], followed by a round of manual curation using the JGI GenePRIMP pipeline [42]. Within the Integrated Microbial Genomes (IMG-ER) system [43], predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGRfam, Pfam, PRIAM, KEGG, COG, and InterPro databases. These data sources were combined to assert a product description for each predicted protein. Non-coding genes and miscellaneous features were predicted using tRNAscan-SE [44], RNAMer [45], Rfam [46], TMHMM [47], and SignalP [48]. Additional gene prediction analyses and functional annotation were performed within IMG.

**Genome properties**

The genome is 7,669,765 nucleotides with 71.17% GC content (Table 4) and comprised of 5 scaffolds (Figure 3) of 83 contigs. From a total of 7,349 genes, 7,236 were protein encoding and 18 RNA only encoding genes. The majority of genes (71.22%) were assigned a putative function whilst the remaining genes were annotated as hypothetical. The distribution of genes into COGs functional categories is presented in Table 5.

**Conclusion**

WSM2598 was sequenced as part of the DOE Joint Genome Institute GBEA-RNB project. In common with other sequenced rhizobial strains, WSM2598 has a comparatively large genome of around 7.69 Mbp, with a high proportion of genes assigned to the COG functional categories associated with transcription control and signal transduction (14.69%), transport and metabolism (29.38%) and secondary metabolite biosynthesis (3.12%). These features are characteristic of soil bacteria, which inhabit oligotrophic environments with typically diverse but scarce nutrient sources. Rhizobial methylobacteria are unusual, however, in that they form symbiotic associations exclusively with African crotalarioid legume hosts, several species of which are well-adapted to arid climates and acid, infertile soils and are therefore potentially useful pasture plants in marginal agricultural systems. The molecular basis for this symbiotic specificity has yet to be determined. As WSM2598 is highly effective for N₂-fixation on several of these hosts, its sequenced genome is a valuable resource for gaining an understanding of symbiotic specificity and N₂-fixation in a currently understudied group of legumes and rhizobia.

**Additional file**

Additional file 1: Table S1. Associated MG5 record.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

JA, JH and RY supplied the strain and background information for this project and contributed to the assembly of the manuscript with WR, TR supplied DNA to JGI and performed all imaging, WR coordinated the project and all other authors were involved in either sequencing the genome and/ or editing the paper. All authors read and approved the final manuscript.

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