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A portal for rhizobial genomes: *Rhizo*GATE integrates a *S. meliloti* genome annotation update with postgenome data

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Abstract

Sinorhizobium meliloti is a symbiotic soil bacterium of the alphaproteobacterial subdivision. Like other rhizobia, *S. meliloti* induces nitrogen-fixing root nodules on leguminous plants. This is an ecologically and economically important interaction, because plants engaged in symbiosis with rhizobia can grow without exogenous nitrogen fertilizers. The *S. meliloti-Medicago truncatula* (barrel medic) association is an important symbiosis model. The *S. meliloti* genome was published in 2001, and the *Medicago truncatula* genome currently is being sequenced. Many new resources and data have been made available since the original *S. meliloti* genome annotation and an update was needed. In June 2008, we submitted our annotation update to the EMBL and NCBI databases. Here we describe this new annotation and a new web-based portal *Rhizo*GATE. About 1000 annotation updates were made; these included assigning functions to 313 putative proteins, assigning EC numbers to 431 proteins, and identifying 86 new putative genes. *Rhizo*GATE incorporates the new annotion with the *S. meliloti* GenDB project, a platform that allows annotation updates in real time. Locations of transposon insertions, plasmid integrations, and array probe sequences are available in the GenDB project. *Rhizo*GATE employs the EMMA platform for management and analysis of transcriptome data and the IGetDB data warehouse to integrate a variety of heterogeneous external data sources.

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Keywords

Rhizobiales; α-proteobacteria; symbiotic nitrogen fixation; *Medicago*; symbiosis

Introduction

Rhizobia are a diverse group of bacteria that engage in symbioses with the roots of leguminous host plants. Once established as symbionts, these bacteria reduce or "fix" dinitrogen to a form the plant can use for growth (reviewed in Prell and Poole, 2006; Jones et al., 2007). With their potential to fix up to 300 kg of molecular nitrogen/ha cultivated land, symbiotic bacteria contribute significantly to an ecologically-sound and cost-efficient nitrogen supply. Most rhizobia (order Rhizobiales) belong to families within the α-proteobacteria subdivision (Garrity et al., 2002), although symbiotic nitrogen-fixing β-proteobacteria (β-rhizobia) have also been reported (Chen et al., 2003; Rasolomampianina et al., 2005). The Rhizobiales also include the plant pathogen *Agrobacterium* and the mammalian pathogens *Bartonella* and *Brucella*. Almost 10 years ago, the first rhizobial genomes were sequenced: *Mesorhizobium loti* MAFF303099 (Phylobacteriaceae) (Kaneko et al., 2000) and *Sinorhizobium meliloti* 1021 (Rhizobiaceae) (Barnett et al., 2001; Capela et al., 2001; Finan et al. 2001; Galibert et al., 2001). Since then, more than 20 complete genome sequences from the Rhizobiales have been released. These sequences are a valuable resource that extends our knowledge of the genetic basis of the rhizobial lifestyle (MacLean et al., 2007).

The annotated *S. meliloti* 1021 genome sequence was submitted to the EMBL/GenBank database in 2001 (chromosome, AL591688; pSymA, NC_003037; pSymB, AL591985). At that time, nearly half of the predicted genes lacked a functional assignment. Hence, we wanted to exploit the wealth of newly available data to update the *S. meliloti* 1021 genome annotation. In addition, in the *S. meliloti* postgenome era, a broad range of strategies was developed to use genomic data to obtain functional information. These comprise transcriptome and proteome studies, mutagenesis, and gene fusion techniques. The number of studies applying high throughput methods is continually increasing. We integrated data from these diverse resources, and a web-based genome browser, into a portal that facilitates data management and access to the above mentioned information. Here we describe the annotation update and introduce the *Rhizo*GATE portal. These data resources are publicly available from one access site.

The *S. meliloti* 1021 annotation update

Rhizobia often possess large, multipartite genomes. The *S. meliloti* genome contains a circular chromosome (3.65 Mb) and two megaplasmids, pSymA (1.35 Mb) and pSymB (1.68 Mb) (Galibert et al., 2001). The original annotation defined 6204 protein-encoding genes: 3341 on the chromosome (Capela et al., 2001), 1293 on pSymA (Barnett et al., 2001), and 1570 on pSymB (Finan et al., 2001). There was no experimentally proven function for a vast majority of the predicted genes and 40 % could not be placed in a functional category. Moreover, 8 % were orphan genes, defined as those not found in any other sequenced genome.

There are numerous changes in this annotation update (summarized in Fig. 1). The update incorporates pertinent information published from 2001 to 2008. Improved prediction tools and the availability of additional rhizobial genomes helped to identify 86 new putative genes, and adjust the start positions of 360 coding regions. There were 66 previously predicted orphan genes removed from the annotation. These were removed either because a new, more plausible candidate, was encoded on the opposite strand, or because of short length, no database match and poor *S. meliloti* codon usage patterns. It was previously suggested that short genes may be overpredicted in GC-rich genomes because stop triplets are less frequent (Skovgaard et al.,

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2001;Fukuchi and Nichikawa, 2004). This updated annotation is more conservative with respect to short ORFs. About 1000 updates added new information to the annotation of gene products. Putative functions were assigned to 313 putative proteins formerly classified as hypothetical or conserved hypothetical. As a result, more than 71 % of genes now have a predicted function. The Enzyme classification (EC) numbers were changed for 160 genes and were newly assigned to 431 genes. Altogether, there have been changes in annotation in about a quarter of the genes. This number does not include minor changes such as spelling or grammar. We assigned COG (Clusters of Orthologous Groups) (Tatusov et al., 2000) and GO (Gene Ontology) (Ashburner et al., 2000) (http://www.geneontology.org/) classification terms to facilitate queries and genome comparisons by functional class and standard descriptors. Table 1 summarizes the update of the general features of the *S. meliloti* strain 1021 genome. The annotation update was released in the EMBL/GenBank/DDBJ database.

The *Rhizo*GATE portal

The *Rhizo*GATE portal (www.rhizogate.de) was constructed to integrate the *S. meliloti* genome sequence, postgenome data sets, and bioinformatics tools (Fig. 2). Organization of tools and data sets in a single portal allows easy access and exploitation of the wealth of information available for the *S. meliloti* genome. The IGetDB data repository is an integral part of this portal and allows complex queries and data mining.

The S. meliloti GenDB genome browser and annotation platform

The core of the *Rhizo*GATE portal is the *S. meliloti* GenDB project. GenDB is a genome annotation system for prokaryotic genomes (Meyer et al., 2003) (http://www.cebitec.unibielefeld.de/groups/brf/software/gendb_info/) that offers automatic identification, classification and annotation of genes using a variety of software tools. Its user interface allows expert annotation by geographically dispersed teams. Our consortium of research groups used this platform to update the 2001 *S. meliloti* genome annotation. The most important feature of the *S. meliloti* GenDB project is that it allows for continual updates, while retaining a complete annotation history. Moreover, a recent Metanor (Goesmann et al., 2005) automatic annotation is offered as an additional information source. Metanor uses a combination of standard tools like BLAST, HMMer and InterPro to assign a gene name, gene product, description, functional category, GO numbers and other attributes to each CDS. It is feasible to keep pace with the increasing amount of information and make this knowledge available to the community in real-time. In addition to the annotation changes described above, the *S. meliloti* GenDB project was enriched with information on novel genes, available mutant and gene fusion libraries, experimentally proven operons, and microarray probes (Table 2) (Fig. 3).

Recently, Mao et al. (2008) identified 20 new transcripts in a pilot study applying ultrafast transcript sequencing to *S. meliloti*. Although this study generated just 1854 non-ribosomal RNA sequences, it demonstrated that the most recent sequencing technologies will greatly facilitate gene discovery and improve genome annotation. Four of these new transcripts were independently predicted in our annotation update submitted to EMBL/GenBank/DDBJ. Since the ultrafast sequencing approach was published after submission of the update to the databases the additional 16 new transcripts could not be considered. However, these genes as well as non-coding RNA genes predicted by the Rfam database (http://www.sanger.ac.uk/Software/Rfam/) were included into the GenDB project.

Microarrays based on PCR fragments and oligonucleotide probes have been published for the *S. meliloti* genome (Rüberg et al., 2003; Krol and Becker, 2004). Positions and sequences of the probes represented on these microarrays are also denoted.

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Pobigaylo et al. (2006) made a signature-tagged (STM) mini-Tn*5* mutant library in *S. meliloti* 2011, a wild type strain closely related to the reference strain *S. meliloti* 1021. Both are spontaneous streptomycin resistant derivatives of *S. meliloti* SU47 (Meade and Signer, 1977; Casse et al., 1979). A set of 412 different mini-Tn*5* transposons, each carrying two individual short sequence tags, was used for this mutagenesis approach. Each individual mutant of a set can be distinguished from every other mutant based on the tags carried by its transposon. These signature-tagged mutants can be used in large-scale genetic screens. The transposon carries a promoterless *gusA* gene, therefore mutants can be used in expression studies. Insertion sites for 5089 mutants were mapped and are marked in the GenDB project; 44 % of predicted protein-coding genes contain at least one of these mapped transposons.

In addition, plasmid integration was employed to generate defined *S. meliloti* mutants. In this approach, internal fragments, 200 to 350 bp in length, were inserted into the mobilizable suicide vector pK19mob2ΩHMB (Luo et al., 2005). These plasmids can be transferred by conjugation from the broad host range mobilizing strain *E. coli* S17-1 (Simon et al., 1983) to *S. meliloti* strains. Plasmids targeting 3207 *S. meliloti* genes are available in *E. coli* S17-1. So far, 727 plasmids from this library have been integrated into the *S. meliloti* 1021 genome. The positions of these plasmids and mutants are denoted in the GenDB project. A study of plasmid integration mutants for 90 genes encoding LysR-family transcriptional regulators has identified new regulatory genes involved in symbiosis (Luo et al., 2005). Information about this comprehensive collection of defined mutants is easily accessible via the *Rhizo*GATE portal.

A high-throughput approach was used to determine co-transcribed genes in *S. meliloti* (Krol et al., unpublished). This approach combined polar plasmid integrations in the first gene of putative operons with transcriptional reporter gene fusions in downstream genes. These reporter gene fusions were derived from the mini-Tn*5* mutant library (Pobigaylo et al., 2006). Significant reduction of reporter gene expression caused by plasmid interruption of transcription indicates an operon structure. This study confirmed co-transcription from 75 operons comprising 225 genes. These data, along with published co-transcription data were included into the *S. melioti* GenDB project.

Recently, Cowie et al. (2006) generated a library of 6,298 plasmids carrying randomly generated *S. meliloti* DNA fragments fused to flanking pairs of reporter genes, *gfp+*, *lacZ* and *gusA*, *rfp*. These plasmids were integrated into the *S. meliloti* genome. Positional information and orientation of the fragments is given in the GenDB project.

In addition to the *S. meliloti* 1021 genome, published annotations were included for ten more Rhizobiales genomes (Table 3). These genomes are presented in one GenDB project, which facilitates searches on all genomes.

The EMMA platform for management and analysis of transcriptome data

We implemented the open source platform EMMA (Dondrup et al., 2003, 2008) (http://www.cebitec.uni-bielefeld.de/groups/brf/software/emma_info/) within *Rhizo*GATE to store and analyze transcriptome data from *S. meliloti*. EMMA provides access to gene expression data sets and processing tools including normalizations to reduce noise and systematic errors, statistical tests for the identification of significantly regulated genes, and clustering algorithms. It allows import of data from spotted microarrays, Affymetrix GeneChips, and NimbleGene DNA chips. This platform connects to the genomic sequences in GenDB so that expression data is linked to the most recent annotation. The *S. meliloti* EMMA project currently holds 277 hybridizations arranged in 22 experiments. These transcriptome data sets can be queried by using the IGetDB data warehouse (Table 4) or the EMMA web interface accessible via the *Rhizo*GATE portal.

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The IGetDB data warehouse

IGetDB (http://www.cebitec.uni-bielefeld.de/groups/brf/software/igetdb_info/) is an online platform for data mining that stores and integrates data from various sources. Currently, it holds annotation, mutant (Pobigaylo et al., 2006, 2008), gene fusion library (Cowie et al., 2006), and ORFeome data (Schroeder et al, 2005), as well as transcriptome and proteome data (Table 4). Annotation data and positions of mutants are derived automatically from the GenDB project. Published mutant phenotypes from high-throughput studies (Pobigaylo et al., 2006, 2008) and from the NodMutDB mutant data collection (Mao et al., 2005) as well as symbiotic phenotypes of about 250 mTn*5* or plasmid insertion mutants (Capela et al., unpublished data) are also accessible via the IGetDB interface. Furthermore, this interface links to expression data of the gene fusion library that was assayed in complex medium and minimal medium with either glucose or succinate as sole carbon source (Cowie et al., 2006). Also, *Rhizo*GATE provides a link to information about the Affymetrix SymbiosisChip, including gene and intergenic region sequences that were used for GeneChip probe set design. Published expression data applying this GeneChip were included in the data warehouse.

Implications and summary

For decades, *S. meliloti* has been the focus of numerous research groups from around the world. This research has led to many important discoveries, some of which are applicable to prokaryotes in general, making *S. meliloti* a model bacterium in its own right. For example, a long sought after step in cobalamin biosynthesis was first elucidated in *S. meliloti* (Campbell et al., 2006). Recently, substrates were experimentally determined for many members of the *S. meliloti* ABC transporter (Mauchline et al., 2006) and short-chain dehydrogenase/reductase (Jacob et al., 2008) families. Together with its model plant host, *Medicago truncatula*, *S. meliloti* is one of the most important symbiosis model systems.

To facilitate research on this important model organism, we established the *Rhizo*GATE portal as a single access site for the growing volume of *S. meliloti* genome and postgenome data and included additional Rhizobiales genomes. We intend to add further value to this platform by integrating additional publicly available transcriptome, proteome, metabolome, and mutant datasets into the IGetDB data repository, and implementing more analysis tools.

About one quarter of soil fixed nitrogen results from rhizobial symbioses (Madigan et al. 1997; Zahran, 1999; Werner and Newton, 2005). This amount is insufficient to sustain food production, so use of chemical nitrogen fertilizers is necessary. Unfortunately, manufacture of these fertilizers requires vast amounts of energy, mostly in the form of non-renewable fossil fuels. Therefore, gains in understanding rhizobial symbiosis have the potential to help improve nutrition, climate, and world economies. We hope that this portal will be one small step in aiding such research.

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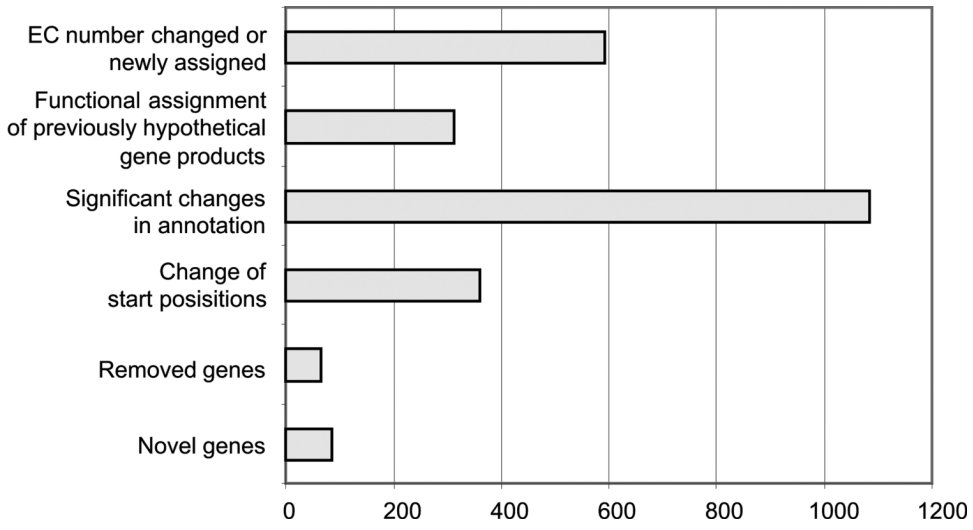


Fig. 1.
Comparison of the annotation update to the original annotation (Galibert et al., 2001).

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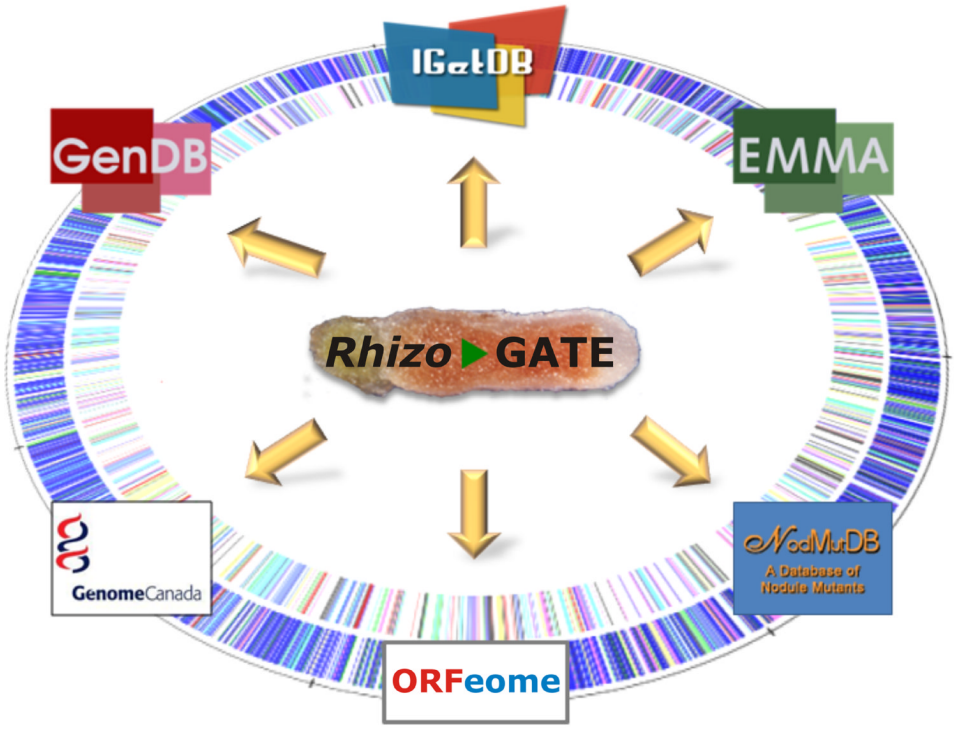


Fig. 2.

The *Rhizo*GATE portal (www.rhizogate.de) provides access to genome (GenDB, Bielefeld University) and transcriptome (EMMA, Bielefeld University) data, includes a data warehouse (IGetDB, Bielefeld University), and links to *S. meliloti* gene fusion (McMaster University, GenomeCanada) and ORFeome library data (Washington State University), and to the NodMutDB mutant database (Virginia Bioinformatics Institute).

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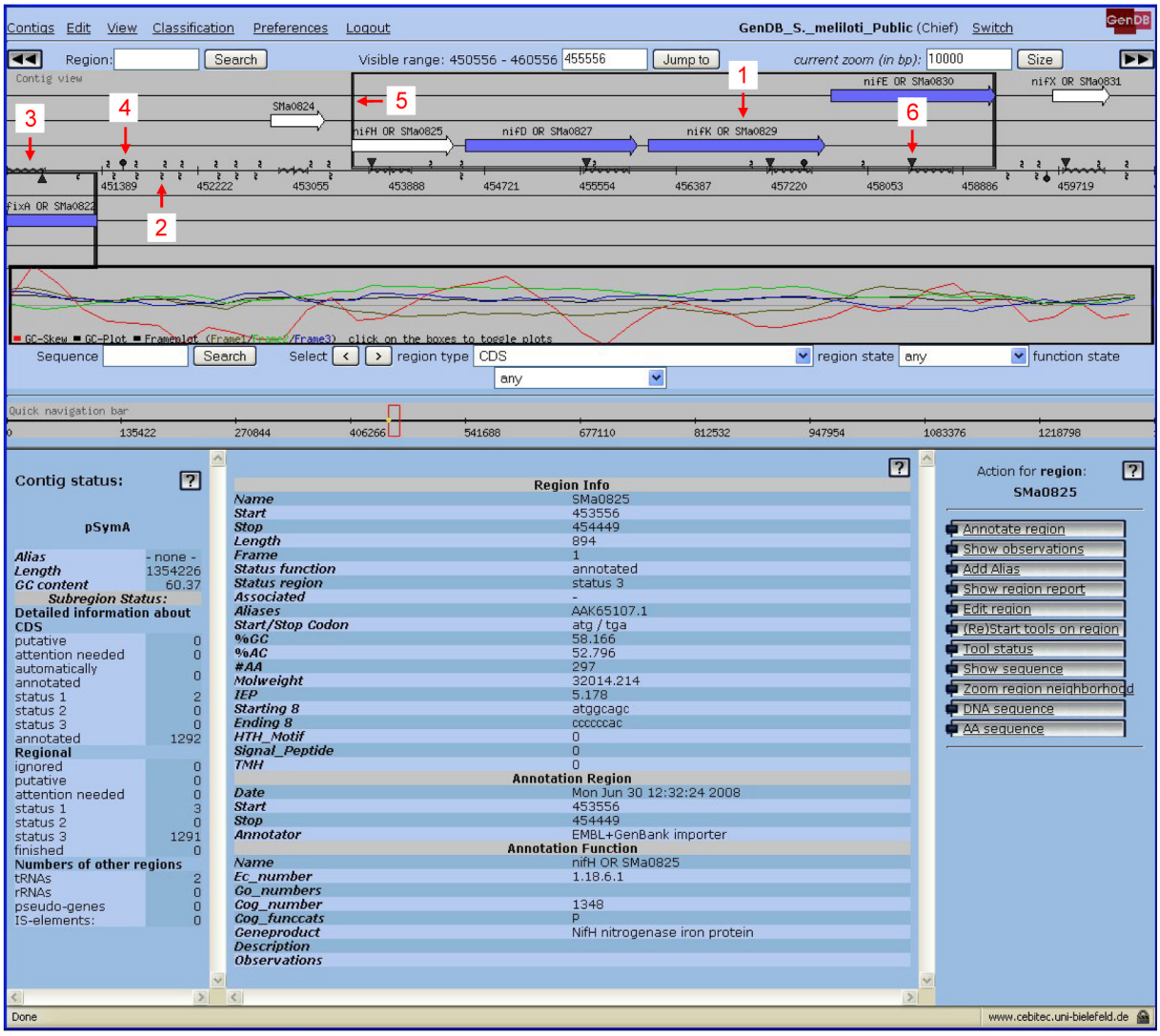


Fig. 3. Main contig view of the GenDB *Rhizo*GATE project

1, coding region; 2, microarray oligonucleotide probe; 3, microarray PCR fragment probe; 4, transposon insertion; 5, operon; 6, integrated plasmid

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Table 1

General features of the *S. meliloti* strain 1021 genome

	Chromosome 2001	update	pSymA 2001	update	pSymB 2001	update	Genome 2001	update
Length (bp)	3,654,135	3,654,135	1,354,226	1,354,226	1,683,333	1,683,333	6,691,694	6,691,694
G+C ratio	62.7 %	62.7 %	60.4 %	60.4 %	62.4 %	62.4 %	62.1 %	62.1 %
tRNAs	51	51	2	2	1	1	54	54
rRNA operons	3	3	0	0	0	0	3	3
Protein-coding genes	3341	3351	1293	1291	1570	1583	6204	6225
Genes with functional assignment	59 %	74.5 %	56.5 %	60.6 %	64.4 %	74.7 %	59.7 %	71.6 %

* % of total protein-coding genes

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Table 2

Data types accessible via the *S. meliloti* GenDB project

Data type	Reference
Genome sequence, coding regions, RNA genes, repeat motifs (insertion elements, transposons, RIME elements)	Galibert et al., 2001
mTn*5*-STM mutants	Pobigaylo et al., 2006
Plasmid integration mutants	Rüberg and Becker; Capela et al.; unpublished
Inserts of gene fusion plasmids	Cowie et al., 2006
Operons	Krol et al., unpublished
Sm6kPCR microarray probes	Rüberg et al., 2003
Sm6kOligo microarray probes	Krol and Becker, 2004
Sm14kOLI microarray probes	Becker, 2008
Observations (Blast matches, InterPro, Pfam, TMHMM, SignalP, helix-turn-helix, TIGRFAM, Priam)	

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Table 3

Rhizobiales genomes in the GenDB project

Organism	Accession numbers
Sinorhizobium meliloti 1021	AL591688, NC_003037, AL591985
Sinorhizobium medicae WSM419	NC_009636, NC_009620, NC_009621, NC_009622
Rhizobium etli CFN42	NC_007761, NC_007762, NC_007763, NC_007764, NC_007765, NC_007766, NC_004041
Rhizobium etli CIAT 652	NC_010994, NC_010998, NC_010996, NC_010997
Rhizobium leguminosarum bv. viciae 3841	NC_008380, NC_008381, NC_008384, NC_008378, NC_008382, NC_008383, NC_008379
Rhizobium sp. NGR234	NC_000914
Mesorhizobium loti MAFF303099	NC_002678, NC_002679, NC_002682
Mesorhizobium sp. BNC1	NC_008254, NC_008242, NC_008243, NC_008244
Bradyrhizobium japonicum USDA 110	NC_004463
Bradyrhizobium sp. BTAi1	NC_009485, NC_009475
Bradyrhizobium sp. ORS278	NC_009445

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Table 4

Data sources accessible via the IGetDB repository

Data type	Reference
GenDB region information: e.g. coding regions, mutants, microrray probes, operons Transcriptome data	Galibert et al., 2001; Rüberg et al., 2003; Krol and Becker, 2004; Pobigaylo et al., 2006; Becker, 2008 Barnett et al., 2004; Becker et al., 2004; Chao et al., 2004, 2005; Capela et al., 2005 ; Hoang et al., 2004; Krol and Becker, 2004; Bobik et al., 2006; Capela et al., 2006; Dominguez-Ferreras et al., 2006; Gibson, 2007; Sauviac et al., 2007; Wells et al., 2007 ; Rossbach et al., 2008 Djordjevic et al., 2003
Proteome data	Djordjevic et al., 2003
Gene fusion library: expression information	Cowie et al. 2006
ORFeome: clone information	Schroeder et al., 2005
mTn*5*-STM mutants: phenotypes	Pobigaylo et al., 2006; Pobigaylo et al. 2008 ; Capela et al. unpublished
Plasmid integration mutants: phenotypes	Luo et al. 2005 ; Capela et al., unpublished

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