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High-Resolution Physical Map of the *Sinorhizobium meliloti* 1021 pSyma Megaplasmid

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To facilitate sequencing of the *Sinorhizobium meliloti* 1021 pSyma megaplasmid, a high-resolution map was constructed by ordering 113 overlapping bacterial artificial chromosome clones with 192 markers. The 157 anonymous sequence tagged site markers (81,072 bases) reveal hypothetical functions encoded by the replicon.

The symbiotic soil bacterium *Sinorhizobium meliloti* forms nitrogen-fixing nodules on the roots of leguminous host plants and displays a complex genome consisting of a 3.7-Mb chromosome and two megaplasmids, pSyma (1.4 Mb) and pSymb (1.7 Mb) (8, 27, 41). Genes required for symbiosis are located on all three replicons (18, 20), but are more frequently found on the megaplasmids. Genes involved in nodulation and nitrogen fixation are located on pSyma (21, 22, 39), whereas those essential for extracellular polysaccharide synthesis and other symbiotic functions are located on pSymb (16, 44). These two genetic elements have both chromosome-like and plasmid-like features: both are 3 orders of magnitude larger than many cloning vectors and carry some copies of housekeeping genes, such as *groESL*, and genes associated with other metabolic functions (33). On the other hand, the megaplasmids can be mostly or entirely cured without affecting growth and reproduction (at least in permissive conditions) (13, 24; M. Hynes, personal communication). Moreover, the megaplasmids can be transferred to and maintained in at least one heterologous genus, *Agrobacterium* (25, 43). Maps for the chromosome and pSymb of strain 1021 exist (9, 10, 12, 20, 23), but concerning pSyma, only three markers outside the 250-kb region containing symbiotic genes have been identified (3). As part of the international effort to sequence the entire *S. meliloti* genome, we constructed a high-resolution physical map of the pSyma megaplasmid, using PCR-based screening and assembly of recombinant bacterial artificial chromosome (BAC) clones. In addition to providing a valuable tool for the total genome sequencing project, the data reported here provide new insights into the genetic information contained on pSyma.

A high-resolution map of the *S. meliloti* pSyma megaplasmid was constructed by using the same materials and methods that were successfully used for chromosome mapping (9) except that additional random sequences from a pSyma-enriched library were incorporated into the BAC screening (methods are described at http://cmgm.stanford.edu/~mbarnett/syma.htm). After screening 192 megaplasmid clones, we identified 88 pSyma clones. Additional clones from the total genomic BAC library were similarly screened to fill gaps in poorly represented regions of the pSyma contig. Thus, we assembled 113 BAC clones into a circular contig (Fig. 1A) encompassing the entire 1.4-Mb pSyma replicon, using a total of 192 markers, including 157 sequence tagged sites (STSs) (9) and 33 gene markers (Table 1) representing 14 individual genes, 15 operons (52 genes), and four insertion sequences available in the GenBank (7) and EMBL (42) databases. Assuming a random distribution of markers, average spacing was estimated at 7 kb, with a tiling path of 6.8 colinear BAC clones per marker. No region is represented by only one BAC, and we detected only five chimeric clones between pSyma and pSymb. All of the 108 other assembled BAC clones show an exact colinear distribution of markers, and pSyma is covered by a set of 19 BAC clones with minimal overlap. Assuming a map density of 7 kb, deletions or rearrangements on some clones should be smaller than 7 kb, if they do exist.

The relative positions of the nodulation and nitrogen fixation genes are consistent with previous mapping data (22, 23, 39), in particular the (i) presence of two *fixJ* loci flanking the *nod-nif* region (5, 35), (ii) organization of nodulation genes (39), (iii) orientation between *nos* and *fix* gene clusters (11), (iv) location of *syrA* (4), and (v) location of *groESLa* (33). All of these genes are clustered in a well-known symbiotic region. Based on the insert size of the clones covering this region, the total length was estimated to be between 250 and 300 kb, which is also in agreement with Renalier et al. (35). The remaining 1.1 Mb of the replicon does not contain any known symbiotic genes, except *syrB*. We also positioned several previously unmapped genes: *adhA*, *rhbF*, and a gene encoding a maturase. Concerning insertion sequences, we detected one copy of IS*Rm1* (46), at least five copies of the widespread IS*Rm2011-2* (40), five copies of IS*Rm3* (45), and one copy of IS*Rm5* (28), compared with five copies on the chromosome (9). We did not obtain PCR products with primers designed from IS*Rm2*, IS*Rm4*, IS*Rm6*, IS*Rm7*, IS*Rm8*, and IS*Rm9*.

Each STS was analyzed by BLASTX (1) comparison with the nonredundant protein database from the National Center for Biotechnology Information, and results are available at the website http://www-recomgen.univ-rennes1.fr/meliloti. STS match results were divided into four categories (Fig. 2A), and the most significant homologies were divided into functional groups according to Riley's classification for orthologous *Escherichia coli* genes (36, 37) (Fig. 2B).

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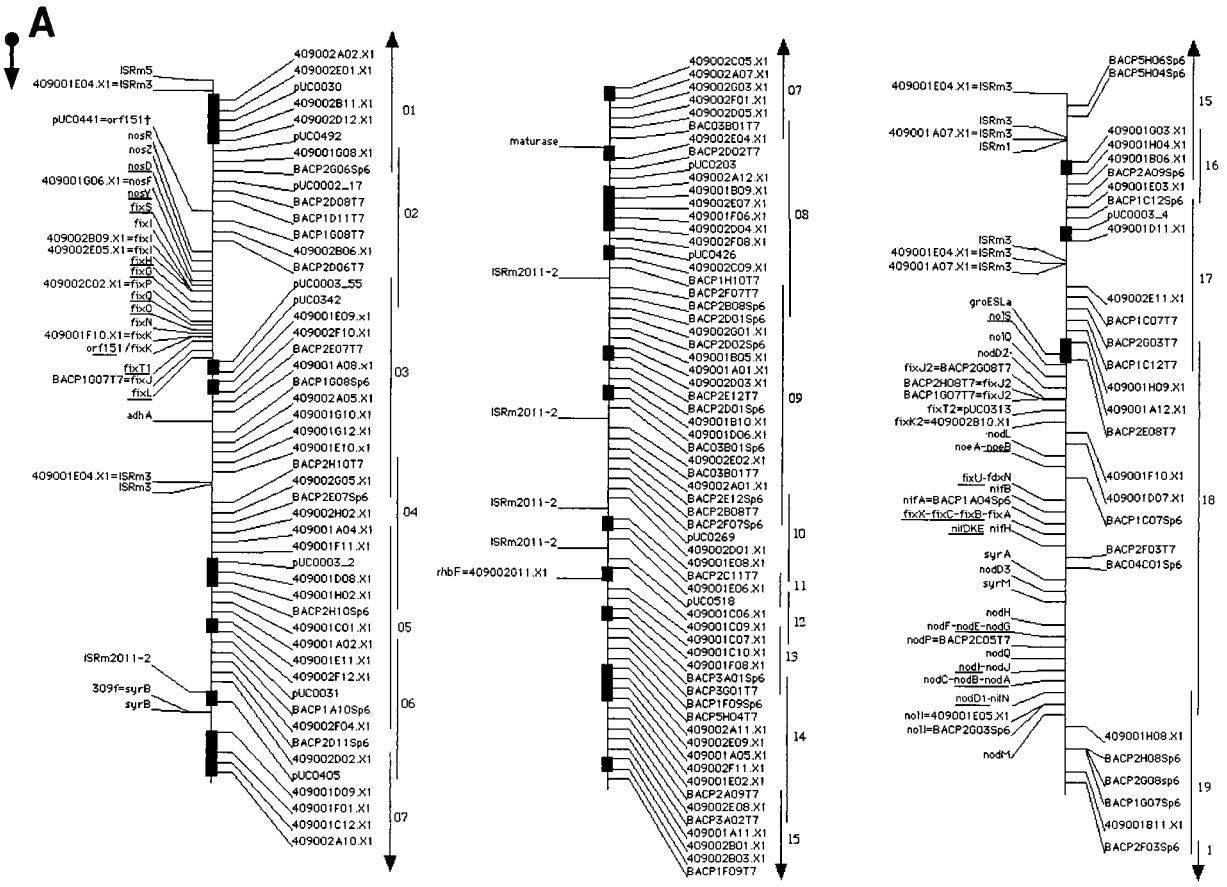
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nosZ
nosD
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fixS
fixI
409002B09.X1=fixI
409002E05.X1=fixI
fixH
fixG
409002C02.X1=fixP
fixQ
fixO
fixN
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orf151 /fixK
fixT1
BACP1G07T7=fixJ
fixL
adhA
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ISRm3
ISRm2011-2
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syrB
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02
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maturase
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ISRm2011-2
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groESLa
noIS
noI0
nodD2
fixJ2=BACP2G08T7
BACP2H08T7=fixJ2
BACP1G07T7=fixJ2
fixT2=pUC0313
fixK2=409002B10.X1
nodL
noeA-noeB
fixU-fdxN
nifB
nifA=BACP1A04Sp6
fixX-fixC-fixB-fixA
nifDKE nifH
syrA
nodD3
syrM1
nodH
nodF-nodE-nodG
nodP=BACP2C05T7
nodQ
nodI-nodJ
nodC-nodB-nodA
nodD1-nifN
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noII=BACP2G03Sp6
nodM
BACP2C07T7
BACP2G03T7
BACP1C12T7
409001H09.X1
409001A12.X1
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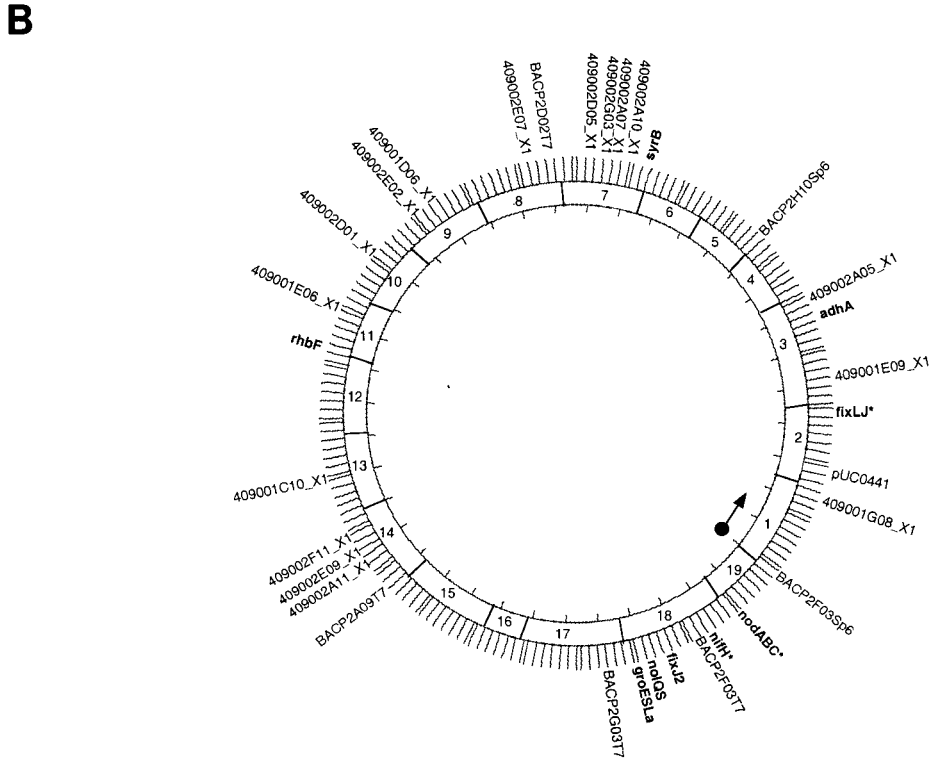
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nifH*
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fixJ2
noIGS
groESLa
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BACP2A09T7
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rhbF
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409002D01_X1
409001D06_X1
409002E02_X1
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TABLE 1. Previously identified *S. meliloti* genes mapped on the pSyma megaplasmid

Gene(s)	Encoded function or product
adhA	Alcohol dehydrogenase
fixABCX	Putative electron transport chain to nitrogenase
fixGHIS	Putative cation transport complex
fixJ2T2-fixK2	Transcriptional activators
fixKorf151	Transcriptional activator
fixLJT1	Hemoprotein kinase; transcriptional activator
fixNOQP	Putative bacteroid oxidase
groESLa	Chaperonin
nifABfdxNfixU	Nitrogen fixation regulatory protein; ferredoxin-like protein
nifHDKE	Nitrogenase reductase
nifN	FeMo-cofactor biosynthesis
nodABCIJ	Acyltransferase; N acetylase; chitin synthase; transporter of *nod* factors
nodD1	*nod* gene activator
nodD2	*nod* gene activator
nodD3	*nod* gene activator
nodFE	Acyl carrier protein; β-ketoacyl synthase
nodG	Putative dehydrogenase
nodH	Sulfotransferase
nodLnoeAB	*O*-Acetyltransferase
nodMnolFGHInodN	Glucosamine synthase; transport
nodPQ	ATP sulfurylase APS kinase
nolQS	Unknown function; similar to a thiamine biosynthetic enzyme
nosRZDFY	Nitrous oxide reduction proteins
maturase	Reverse transcriptase/maturase
rhbF	Siderophore biosynthesis in rhizobactin regulon
syrA	Increases exopolysaccharide abundance
syrB	Negatively affects *syrM* expression
syrM	*nod* gene activator

This distribution shows many STS markers containing genes involved in the metabolism of small molecules, such as (i) 4-deoxy-L-threo-5-hexosulose-uronate-ketol-isomerase and succinate-semialdehyde dehydrogenase (encoded by *gabD*), which is involved in carbohydrate (C_4-to-C_6) degradation (32), and (ii) serine hydroxymethyltransferase, which is the key enzyme of C_1 and C_2 compound assimilation and is necessary for the formation of effective nodules in *Bradyrhizobium japonicum* (38). The next largest group of STSs is made up of those possibly involved in cellular processes (chemotaxis and transport); no matches with cell division proteins or general housekeeping genes were detected other than those for the previously reported *groESLa* (33). We also found matches to genes involved in nitrogen metabolism: the periplasmic nitrate reductase precursor of *Paracoccus* and *Pseudomonas*, the NifX-like protein of *Rhizobium* sp. strain NGR234, an arginine deiminase of *Rhizobium etli* (15), and the NifL nitrogen fixation regulator of *Klebsiella* and other bacteria (31), previously unknown in *S. meliloti*. Also, there are several less stringent intriguing matches: an STS identical to stage IV sporulation protein FB of *Bacillus subtilis*, required for spore formation (14), and a marker identical to protein AttB of the plant pathogen *Agrobacterium tumefaciens*, required for the attachment of bacteria to plant cells (30). One STS is similar to VirB4 from *Agrobacterium* and TraB from *E. coli*, both of which are required for DNA transfer and may represent part of a region involved in conjugative transfer of the pSyma replicon. We also detected one sequence similar to the adducin-like protein AddA of the obligate intracellular parasite *Rickettsia prowazekii* and to alpha-adducin, which promotes the assembly of the spectrin-actin network in eukaryotic cells (2, 26). In addition, some STSs have matches to transcriptional regulators from the LysR family of transcriptional regulators, the AraC family activators, the GntR family regulators, the *trp* repressor, and a repressor of the TetR-AcrR family. We did not find any STSs with matches to regulators of two-component systems.

The most relevant comparison for the pSyma sequence will be with that of the closely related *Rhizobium* sp. strain NGR234, which has a complex genome (17), including a symbiosis plasmid of 536 kb, for which the complete nucleotide sequence has been established (19). Given that the pSyma megaplasmid of *S. meliloti* is almost three times the size of the pSym megaplasmid of NGR234, it will be interesting to determine how related they are. In this regard, we noted that seven of the *S. meliloti* pSyma STS markers had a match with the pSym of NGR234, while 150 of the *S. meliloti* pSyma markers did not (for $E \leq 1e^{-4}$).

The elucidation of the *S. meliloti* total genome sequence will aid greatly our understanding of the ancestry and behavior of the pSyma replicon as well as provide insight into the genomic

FIG. 1. (A) High-resolution map of the pSyma megaplasmid of *S. meliloti* 1021. The map is presented in three linear and contiguous parts of approximately 500 kb for convenience. Identified *S. meliloti* genes (genetic database or BLASTX results) are indicated on the left side while anonymous STS markers are located on the right side. The positions of underlined genes were deduced from mapped genes in the operon; i.e., no PCR primers were designed. Some genes are listed more than once because several sets of primers were used. Black rectangles indicate pairwise invertable markers. ‡, partial similarity. The minimum set of BAC clones covering the replicon is also presented. (B) Simplified map oriented according to the Honeycutt et al. map (23) showing STS markers mentioned in the text, selected genes, and the minimum set of BAC clones. Lengths of BAC inserts are shown relative to the sizes determined by field inversion gel electrophoresis. Genes previously mapped by Honeycutt et al. are marked with asterisks. Corresponding BAC insert sizes are as follows: BAC01, 110 kb; BAC02, 75 kb; BAC03, 110 kb; BAC04, 75 kb; BAC05, 55 kb; BAC06, 85 kb; BAC07, 120 kb; BAC08, 65 kb; BAC09, 80 kb; BAC10, 75 kb; BAC11, 60 kb; BAC12, 110 kb; BAC13, 100 kb; BAC14, 140 kb; BAC15, 80 kb; BAC16, 25 kb; BAC17, 100 kb; BAC18, 80 kb; and BAC19, 60 kb.

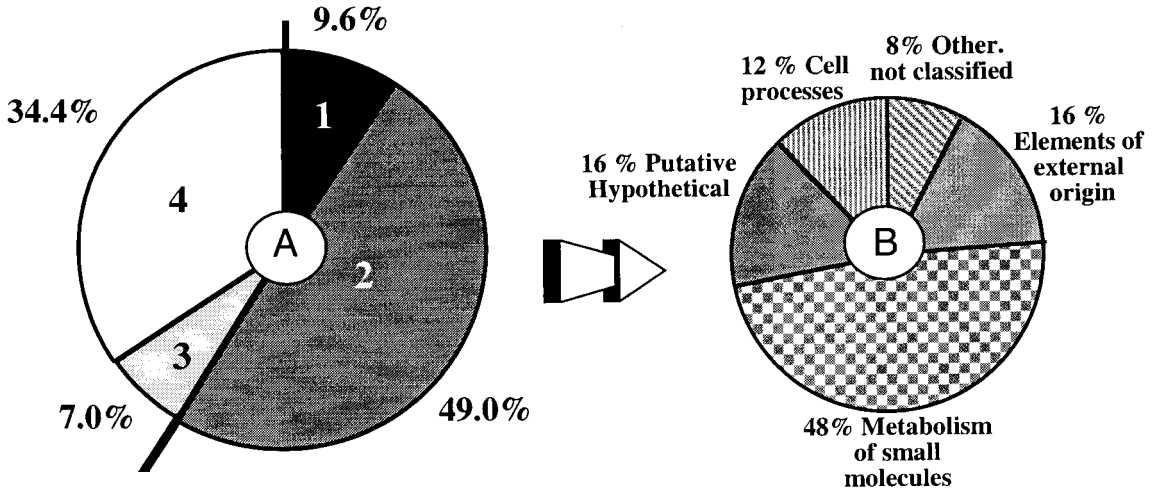

FIG. 2. (A) Distribution of BLASTX results among four categories of significance. 1, strong similarity with *S. meliloti* proteins ($E \leq 1e^{-6}$; identity, ≥85%); 2, strong similarity with sequences available in the databases ($E \leq 1e^{-6}$); 3, local or weaker similarity with sequences available in the databases ($1e^{-2} \leq E \leq 1e^{-6}$); 4, no similarity with sequences available in the databases. (B) Classification of the most significant matches ($E \leq 1e^{-6}$) using Riley's classification (34, 35).

plasticity, the presence of multicopy genes, and the relative involvement of each replicon, both in symbiotic and free-living bacteria.

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