A locus necessary for the transport and catabolism of erythritol in *Sinorhizobium meliloti*

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In this work we have genetically defined an erythritol utilization locus in *Sinorhizobium meliloti*. A cosmid containing the locus was isolated by complementation of a transposon mutant and was subsequently mutagenized using Tn5::B20. The locus was found to consist of five transcriptional units, each of which was necessary for the utilization of erythritol. Genetic complementation experiments using genes putatively annotated as erythritol catabolic genes clearly showed that, of the 17 genes at this locus, six genes are not necessary for the utilization of erythritol as a sole carbon source. The remaining genes encode EryA, EryB, EryC and TpiB as well as an uncharacterized ABC-type transporter. Transport experiments using labelled erythritol showed that components of the ABC transporter are necessary for the uptake of erythritol. The locus also contains two regulators: EryD, a SorC class regulator, and SMc01615, a DeoR class regulator. Quantitative RT-PCR experiments showed that each of these regulators negatively regulates its own transcription. In addition, induction of the erythritol locus was dependent upon EryD and a product of erythritol catabolism. Further characterization of polar mutations revealed that in addition to erythritol, the locus contains determinants for adonitol and L-arabitol utilization. The context of the mutations suggests that the locus is important for both the transport and catabolism of adonitol and L-arabitol.

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INTRODUCTION

Sinorhizobium meliloti is a Gram-negative soil microbe that can exist either as a free-living organism in the soil or in a symbiotic relationship with legume plants. *S. meliloti* can interact with the legume alfalfa to form root nodules, and within these nodules it can reduce nitrogen gas and provide this fixed nitrogen to the plant (Spaink, 2000). Prior to the colonization of the root and the initiation of infection threads that lead to the release of the bacteria into the root nodule, the bacteria must be able to survive in the rhizosphere. It has been shown that the ability to use organic compounds can strongly influence competition in the rhizosphere (Triplett & Sadowsky, 1992). In *Rhizobium leguminosarum*, the inability to utilize erythritol has been shown to affect a strain's ability to effectively compete for nodule occupancy (Yost *et al.*, 2006).

The ability to utilize erythritol as a sole carbon source is not universal among the *Rhizobiaceae* (Jordan, 1984; Stowers, 1985). Evidence has been presented that suggests the erythritol catabolic locus may have been horizontally transferred from *Brucella* to *R. leguminosarum* (Yost *et al.*, 2006). In addition to the *eryABCD* locus that had been

previously shown to be necessary for erythritol catabolism (Sangari *et al.*, 2000), it was shown that two sets of genes flanking *eryABCD* of *R. leguminosarum* were also necessary for erythritol catabolism. These are *eryEFG*, encoding three ABC transporter genes, and an operon consisting of a DeoR type regulator, a triose phosphate isomerase and a putative ribulose phosphate isomerase (Yost *et al.*, 2006).

The inability to utilize erythritol by *Brucella abortus* strain S19 has been correlated with attenuated virulence (Meyer, 1966, 1967). This association prompted the elucidation of a biochemical pathway for its catabolism (Sperry & Robertson, 1975a, b). Subsequently, the genes for the first three steps of this biochemical pathway were characterized (*eryA*, *eryB* and *eryC*) and found to appear in an operon with a negative regulator *eryD* (Sangari *et al.*, 1998, 2000). More recent work, using proteomic as well as comparative genomic approaches, has reinforced the role erythritol transport and catabolism may play in virulence (Burkhardt *et al.*, 2005).

In *S. meliloti*, screening for mutants unable to use rhamnose as a sole carbon source identified two regions: the genes homologous to the *R. leguminosarum* genes necessary for rhamnose catabolism, *rhaDI* and *rhaRSTPQMK* (Richardson *et al.*, 2004; Richardson & Oresnik, 2007; Richardson *et al.*, 2008), and a gene encoding a triose-phosphate isomerase (*tpiA*) (Poysti &

Abbreviation: VMM, Vincent's minimal medium.

A supplementary table, listing PCR primers used, is available with the online version of this paper.

Oresnik, 2007). The *S. meliloti* genome contains another gene, *tpiB*, that also encodes triose-phosphate isomerase activity (Poysti & Oresnik, 2007). Although both genes encode functional triose-phosphate isomerases, they appear to play distinct biochemical roles in the organism. *TpiA* is necessary for glycerol catabolism and has been hypothesized to play a role in central metabolism, whereas *TpiB* is necessary for the catabolism of erythritol (Poysti & Oresnik, 2007). It is of note that the overexpression of the wild-type *tpiB* can complement *tpiA*-associated mutant phenotypes whereas it has been shown that in *S. meliloti* a *tpiB* mutation was unable to be genetically complemented by *tpiA* (Poysti & Oresnik, 2007). Since only a *tpiA/B* double mutant, and not a *tpiA* or *tpiB* single mutant, is impaired for autotrophic and gluconeogenic growth, *tpiB* is expressed at low levels in the absence of erythritol (Pickering & Oresnik, 2008; Poysti & Oresnik, 2007).

Our interest in erythritol stems from our initial studies with *tpiB* (Poysti & Oresnik, 2007). Additionally, as noted by Yost *et al.* (2006), the genetic content and arrangement of the putative erythritol locus adjacent to *tpiB* is drastically different from that previously described in *R. leguminosarum* and *B. abortus* (Galibert *et al.*, 2001; Halling *et al.*, 2005; Young *et al.*, 2006). Therefore, the goal of this work was to genetically characterize the locus and to determine what components are necessary for the transport and catabolism of erythritol.

METHODS

Bacterial strains, plasmids and media. Bacterial strains and plasmids used and generated in this work are listed in Table 1. *S. meliloti* strains were grown routinely at 30 °C on complex Luria-Bertani medium (Sambrook *et al.*, 1989) or on defined Vincent's minimal medium (VMM) (Vincent, 1970). Carbon sources were filter-sterilized and added to VMM to a final concentration of 15 mM. When required, *S. meliloti* and *Escherichia coli* strains were grown with the following antibiotics (concentrations in µg ml⁻¹): chloramphenicol (Cm) 20; gentamicin (Gm) 20 or 60; kanamycin (Km) 20; neomycin (Nm) 200; rifampicin (Rf) 50; streptomycin (Sm) 200; tetracycline (Tc) 5. All antibiotics were filter-sterilized before use.

DNA manipulations and constructions. Standard techniques were used for plasmid isolation, restriction enzyme digests, ligations, transformations and agarose gel electrophoresis (Sambrook *et al.*, 1989). Oligonucleotide primers used for PCR amplification are listed in Supplementary Table S1, available with the online version of this paper.

To create an *eryD* mutation, pBG1 was first constructed by amplification of a 400 bp internal fragment of *eryD* by PCR using Rm1021 genomic DNA as a template and primers 1 and 2. The amplicon was restricted and ligated into the suicide vector pKNOCK-Gm (Alexeyev, 1999) using *Bam*HI and *Cl*AI. pBG1 was transformed into competent *E. coli* S17-1 and selected for using Gm (20 µg ml⁻¹). Single crossover *eryD* mutants, SRmA855 and SRmA856, were isolated by conjugating pBG1 into Rm1021 and SRmA449, respectively, and selecting for Sm^R and Gm^R transconjugants as previously described (Poysti & Oresnik, 2007; Richardson *et al.*, 2004). Colonies were screened for phenotype and mutations were verified by sequencing.

To construct a strain carrying a mutation in *SMc01615*, the gene upstream of *tpiB* that putatively encodes a DeoR-type regulator, a 1053 bp product was amplified from Rm1021 genomic DNA by PCR with primers 9 and 10, digested with *Ap*AI and *Sst*II, and cloned into pBluescript II SK to yield pBP149. pBP149 was digested with *Sma*I into which a *Sma*I-digested *nptII* cassette from pMM22 was ligated to generate pBP151. pBP151 was subsequently recloned into pJQ200SK to produce pBP173. pBP173 was mobilized into Rm1021 with MT616 and selected for double recombinants. Colonies were screened onto selective media and sequenced to verify the insertion of *nptII* in *SMc01615*. One such mutant was designated SRmA966.

Two different methods were used to construct constitutively expressed erythritol genes. ORFs of *eryA*, *eryB* and *eryD* were amplified by PCR using primers 5 and 6, 7 and 8, and 3 and 4, respectively, and cloned into the broad-host-range vector, pRK7813. The 5' primers 5, 7 and 3 were all designed to have a ribosome-binding site approximately 8 bp upstream of the annotated ATG to allow constitutive expression. pBG2 was constructed by using *Pst*II and *Bam*HI restriction sites that were added to the primers to digest and ligate the 960 bp *eryD* ORF into pRK7813 such that it would be expressed from the *lacZ* promoter. pBG3 and pHY112 were constructed similarly using the *eryA* ORF with *Hind*III and *Eco*RI restriction sites and the *eryB* ORF with *Pst*II and *Bam*HI restriction sites, respectively. Additionally, the ORFs for *eryA*, *eryC* and *eryD* were recombined into pCO37 from the *S. meliloti* ORFeome using the methods described previously (House *et al.*, 2004; Jacob *et al.*, 2008; Schroeder *et al.*, 2005).

Genetic manipulations. Conjugations between *E. coli* strains and *S. meliloti* were performed as described previously using the mobilizing strain MT616 (Finan *et al.*, 1985). Transposon mutagenesis of Rm1021 using pRK602 was carried out as described previously (Finan *et al.*, 1985). Mutants were routinely single-colony purified three times and subsequently transduced into Rm1021 with phage ΦM12 to ensure that the mutation was 100% genetically linked to the transposon (Finan *et al.*, 1985). The erythritol-complementing cosmid pEL6 was isolated by conjugating a cosmid bank (Wang *et al.*, 2006) of *S. meliloti* into SRmA723 and plating on defined medium using erythritol as a sole carbon source to select for complementation. The cosmid pEL6 was then mutagenized by mating the cosmid into the Tn5::B20 carrying EcA101 (Clark *et al.*, 2001) and selecting for co-transfer of the cosmid and a Tn5::B20 marker when mated out of EcA101 and into DH5α yielding pHC1-pHC12 essentially as described previously (Poysti *et al.*, 2007). The position of Tn5::B20 insertions was verified using arbitrary PCR as described previously (Miller-Williams *et al.*, 2006; Poysti *et al.*, 2007). Insertions confirmed to be in the erythritol locus were recombined into Rm1021 as previously described (Glazebrook & Walker, 1991). The inserts were confirmed using arbitrary PCR and were transduced into Rm1021 yielding SRmA778-SRmA787.

β-Galactosidase assays. Initial expression studies were carried out using *lacZ* fusions that were isolated in pEL6 (Table 1). The assays were essentially carried out as described previously (Clark *et al.*, 2001; Oresnik *et al.*, 1998). These data, although not presented, were corroborated by quantitative RT-PCR data (Table 4).

RNA isolation and cDNA synthesis. Bacterial cultures were grown to an OD₆₀₀ of approximately 0.8 in VMM containing either 15 mM erythritol and 15 mM glycerol or 15 mM glycerol alone. Cells were harvested by centrifugation and resuspended in TE buffer with lysozyme (0.4 mg ml⁻¹). RNA was then isolated using the Qiagen RNA isolation technique described previously (Barnett *et al.*, 2004). All RNA samples were treated twice with Qiagen on-column RNase-free DNase kit during the DNase step to remove DNA contamination.

The RNA was analysed spectrophotometrically and the absorbance ratio of the sample at 260 nm and 280 nm was compared to ensure

Table 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or phenotype	Reference or source
<i>S. meliloti</i>		
Rm1021	SU47 <i>str-21</i> , Sm ^R	Meade <i>et al.</i> (1982)
SRmA428	Rm1021 <i>SMc01627::Tn5</i> , Nm ^R	This work
SRmA449	Δ <i>tpiA</i>	Poysti & Oresnik (2007)
SRmA465	ϕ (SRmA428) $\rightarrow$ Rm1021, Nm ^R	This work
SRmA584	Rm1021 <i>tpiB2</i> , Nm ^r	Poysti & Oresnik (2007)
SRmA723	Rm1021 <i>SMc01627::Tn5-233</i> , Gm ^R Sp ^R	This work
SRmA778	Rm1021 <i>ery-1::Tn5::B20</i> , Nm ^R	This work
SRmA779	Rm1021 <i>ery-2::Tn5::B20</i> , Nm ^R	This work
SRmA780	Rm1021 <i>ery-3::Tn5::B20</i> , Nm ^R	This work
SRmA781	Rm1021 <i>ery-5::Tn5::B20</i> , Nm ^R	This work
SRmA782	Rm1021 <i>eryA6::Tn5::B20</i> , Nm ^R	This work
SRmA783	Rm1021 <i>ery-7::Tn5::B20</i> , Nm ^R	This work
SRmA784	Rm1021 <i>eryB9::Tn5::B20</i> , Nm ^R	This work
SRmA785	Rm1021 <i>ery-10::Tn5::B20</i> , Nm ^R	This work
SRmA787	Rm1021 <i>eryC12::Tn5::B20</i> , Nm ^R	This work
SRmA885	Rm1021 <i>eryD13::pKNOCK-Gm</i> , Gm ^R	This work
SRmA966	Rm1021 <i>ery-14::nptII</i> , Nm ^R	This work
<i>E. coli</i>		
DH5 α	λ^- Φ 80dlacZ M15 (<i>lacZYA-argF</i>)U169 <i>recA1endA1 hsdR17(r_K⁻ m_K⁻) supE44 thi-1 gyrA relA1</i>	Hanahan (1983)
DH5 α Rif	Rifampicin-resistant DH5 α	House <i>et al.</i> (2004)
MM294A	<i>pro-82-thi-1 hsdR17 supE44</i>	Finan <i>et al.</i> (1986)
MT607	MM294A <i>recA56</i>	Finan <i>et al.</i> (1986)
MT616	MT607 (pRK600)	Finan <i>et al.</i> (1986)
S17-1	<i>recA</i> derivative of MM294A with integrated RP4-2 (<i>Tc::Mu::Km::Tn7</i>)	Simon <i>et al.</i> (1983)
EcA101	MT607 Ω Tn5::B20, Kan ^R	Clark <i>et al.</i> (2001)
Plasmids		
pBG1	pKNOCK-Gm + 400 bp internal <i>eryD</i> fragment (<i>Bam</i> HI/ <i>Cl</i> AI), Gm ^R	This work
pBG2	pRK7813 + <i>eryD</i> (<i>Pst</i> I, <i>Bam</i> HI), Tc ^R	This work
pBG3	pRK7813/ <i>eryA</i> , (<i>Eco</i> RI, <i>Hind</i> III), Tc ^R	This work
pBG13	pCO37/ <i>eryA</i> , Tc ^R	This work
pBG14	pCO37/ <i>eryC</i> , Tc ^R	This work
pBG18	pCO37/ <i>eryD</i> , Tc ^R	This work
pBP149	pBS/ <i>SMc01615</i> , Ap ^R	This work
pBP151	pBS/ <i>SMc01615::nptII</i> , Ap ^R	This work
pBP173	pJQ200/ <i>SMc01615::nptII</i> , Gm ^R	This work
pBluescript II SK	Cloning vector, Ap ^R	Stratagene
pCO37	pRK7813 containing <i>attB</i> sites, Gateway compatible destination vector	Jacob <i>et al.</i> (2008)
pEL6	CX1 erythritol-complementing cosmid, Tc ^R	Wang <i>et al.</i> (2006)
PHY112	pRK7813 / <i>eryB</i> , Tc ^R	This work
pHC1	pEL6 <i>ery-1::Tn5::B20</i> , Nm ^R Tc ^R	This work
pHC2	pEL6 <i>ery-2::Tn5::B20</i> , Nm ^R Tc ^R	This work
pHC3	pEL6 <i>ery-3::Tn5::B20</i> , Nm ^R Tc ^R	This work
pHC5	pEL6 <i>ery-5::Tn5::B20</i> , Nm ^R Tc ^R	This work
pHC6	pEL6 <i>eryA6::Tn5::B20</i> , Nm ^R Tc ^R	This work
pHC7	pEL6 <i>ery-7::Tn5::B20</i> , Nm ^R Tc ^R	This work
pHC9	pEL6 <i>eryB9::Tn5::B20</i> , Nm ^R Tc ^R	This work
pHC10	pEL6 <i>ery-10::Tn5::B20</i> , Nm ^R Tc ^R	This work
pHC12	pEL6 <i>eryC12::Tn5::B20</i> , Nm ^R Tc ^R	This work
pKNOCK-Gm	Suicide vector, Gm ^R	Alexeyev (1999)
pNP163	<i>tpiB</i> -complementing cosmid	Poysti & Oresnik (2007)
pPH1JI	IncP plasmid, Gm ^R	Beringer <i>et al.</i> (1978)
pRK600	pRK2013 <i>nptI::Tn9</i> , Cm ^R	Finan <i>et al.</i> (1986)
pRK602	pRK600 Ω Tn5, Cm ^R Nm ^R	Finan <i>et al.</i> (1985)
pRK7813	Broad-host-range vector, Tc ^R	Jones & Guttererson (1987)
pJQ200SK	Gene replacement suicide vector, Gm ^R	Quandt & Hynes (1993)
pMM22	Kan ^r fragment cloned as <i>Sma</i> I in pBlueScript	Pickering & Oresnik (2008)

the purity of the RNA sample. The concentration of RNA was determined using the absorption coefficient for RNA [$0.027 (\mu\text{g ml}^{-1}) \text{ cm}^{-1}$]. First strand cDNA synthesis was performed using 1 μg RNA as suggested by the supplier (Invitrogen). The quality of the cDNA synthesis was checked by electrophoresis and quantified spectrophotometrically.

Junction PCR. To determine the extent of transcripts, PCRs across putative junctions in the erythritol locus were performed using the cDNA from either induced cells grown on erythritol/glycerol or non-induced cells grown on glycerol. An identical PCR using the RNA samples that were used for the cDNA synthesis as a template was carried out to control for possible genomic DNA contamination. A similar reaction was also performed using genomic template as a positive primer control. PCRs across three gene junctions were performed using primers 21 and 22, 23 and 24, and 25 and 26 (Supplementary Table S1). PCR conditions were as follows: 95 °C for 60 s, 30 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 °C for 90 s, and 72 °C for 300 s.

Quantitative RT-PCR (qRT-PCR). Approximately 100 ng cDNA sample was used as a template for qRT-PCR. Reactions were performed using the SYBR green RT-PCR kit from Invitrogen as recommended by the manufacturer. Primers 11 and 12 were used to analyse *SMc01628* transcription; 13 and 14 were used to analyse *eryD* transcription; 15 and 16 were used to analyse *eryA* transcription; 17 and 18 to analyse *eryC* transcription and finally 19 and 20 to analyse *SMc01615* transcription. Primers were designed upstream of all polar insertion mutations in strains in this work. The qRT-PCR was performed using a Cepheid Smart Cycler with the following program: stage 1, 95 °C for 120 s, once; stage 2, 95 °C for 15 s followed by 60 °C for 30 s, repeated 40 times; stage 3, melting curve analysis of PCR products.

Transport assay. Transport assays were carried out essentially as previously described (Poysti *et al.*, 2007). Labelled D-[1- ^{14}C] erythritol (370 MBq mmol^{-1}) was purchased from American Radiolabeled Chemicals. Transport assays were initiated by the addition of [^{14}C] erythritol to a final concentration of 2 μM to the samples and aliquots of 0.25 or 0.5 ml were withdrawn at appropriate time points and rapidly filtered through a Millipore 0.45 μm Hv filter on a Millipore sampling manifold. Samples were taken every 15 or 20 s and continued for up to 2 min. The amount of radioactivity retained by the cells was quantified by a liquid scintillation counter (Beckman LS6500).

Competition assay. Alfalfa plants were grown as previously described except that Leonard jar assemblies were made from Magenta jars (Sigma) (Oresnik *et al.*, 1994). Competition for nodule occupancy assays were carried out as described previously (Oresnik *et al.*, 1999).

RESULTS

Isolation of erythritol transport and catabolic mutants

Comparison of the putative erythritol utilization locus of *S. meliloti* with that of *R. leguminosarum* shows that the organization of the catabolic genes is very different (Yost *et al.*, 2006). In addition, the ABC-type transporter genes that are found downstream from *eryD* in *S. meliloti* (*SMc01628*–*SMc01624*) do not appear to be orthologues of *eryEFG* in *R. leguminosarum*, suggesting that components involved in erythritol transport may not always be found in close proximity to the putative erythritol catabolic genes

eryA, *eryB* and *eryC*. In an attempt to isolate genes necessary for the transport of erythritol, Tn5 mutagenesis of the wild-type was carried out.

Approximately 3000 transposon mutants were initially screened for their inability to grow on erythritol as a sole carbon source. The single mutant that was isolated was purified and designated SRmA428 (Table 1). The transposon was subsequently transduced into Rm1021 and the transductants were retested for phenotype. The transduced strain was designated SRmA465. The transposon was found to be within *SMc01627*, which is predicted to encode a permease that appears to be part of an ABC-type transporter located between the putative negative regulator *eryD* and the gene encoding the putative erythritol kinase, *eryA*.

Based on the close proximity of the putative start and stop codons found immediately upstream and downstream of each of the genes annotated as being necessary for erythritol catabolism, it was initially assumed that all the genes from *eryD* to *eryC* constituted a single operon (Fig. 1). Combined with the ABC gene transporter gene locus, this suggests close proximity of all the erythritol utilization genes. Attempts to complement SRmA465 with the complementing cosmid for *tpiB*, pNP163 (Poysti & Oresnik, 2007), were unsuccessful, suggesting that pNP163 did not contain the entire locus necessary for erythritol catabolism (data not shown). To obtain a complementing cosmid for the erythritol catabolic locus, a cosmid bank was mated en masse into SRmA465 (Wang *et al.*, 2006) and the *S. meliloti* transconjugants that had regained their ability to use erythritol as a sole carbon source were isolated. One such isolate, that had the ability to complement the *SMc01627* mutation in SRmA465 as well as the *tpiB2* allele in SRmA584, was retained and designated pEL6 (Table 1).

To generate additional mutations in the erythritol locus, a saturation mutagenesis of pEL6 was performed using Tn5::B20 to generate representative insertions across pEL6. Inserts were identified by generating a PCR fragment from the IS50 element on the Tn5::B20 using an arbitrary PCR protocol, and sequencing the products using a primer designed to match the IS50 element (Poysti *et al.*, 2007). The plasmids carrying insertions within the erythritol locus were designated pHc112 (Table 1, Fig. 1). Each of these insertions was marker exchanged into the chromosome of Rm1021 to yield the corresponding genomic mutation as described in Methods. These mutations were designated SRmA778–787 (Table 1). Each of these strains was unable to use erythritol as a sole carbon source. Also, each strain containing an insertion in a gene downstream of *eryA* was unable to grow when erythritol was present with a secondary carbon source such as glycerol. Similar toxic effects have been previously noted for erythritol as well as other sugars in both *R. leguminosarum* and *E. coli* (Adhya & Shapiro, 1969; Power, 1967; Richardson *et al.*, 2004; Sperry & Robertson, 1975a; Yost *et al.*, 2006).

Although a good distribution of transposon insertions across the transport and catabolic genes of the locus were

Fig. 1. Predicted ORFs and isolated mutant alleles in the erythritol locus of *S. meliloti*. Vertical lines with flags represent the location and orientation of Tn5::B20 insertion alleles. Vertical lines without flags represent other insertion mutations. Arrows above the diagram represent transcriptional units as defined by this work. Lines below the diagram represent regulation as defined by this work while plus signs (+) are positioned at the beginning of other inducible transcripts (see text for details).

isolated, insertions in the two putative regulators were not (Fig. 1). Therefore single crossover mutations in *eryD* and *SMc01615* were constructed giving rise to SRmA885 and SRmA966 respectively. Neither strain was able to use erythritol as a sole carbon source. In addition it was found that SRmA966 was unable to grow on glycerol in the presence of erythritol. Since *eryD* and *SMc01615* are annotated as negative regulators, these phenotypes suggested polarity on downstream genes and were consistent with our hypothesis that the entire locus was transcribed as a single unit.

Erythritol utilization genes are in different complementation groups

To address the hypothesis that the erythritol locus in *S. meliloti* was a single operon, plasmids containing inserts within genes that were presumed to be necessary for erythritol catabolism were conjugated into each of SRmA778–SRmA787 to test for complementation. In addition, these plasmids were also conjugated into SRmA885 (*eryD*), SRmA966 (*SMc01615*) and SRmA584 (*tpiB*). The resulting transconjugants were screened for their ability to utilize erythritol as a sole carbon source. The results show that contrary to our initial assumptions, the erythritol locus in *S. meliloti* does not consist of a single

transcript from *eryD* to *eryC* (Table 2). The locus appears to consist of at least five transcripts: a transcript containing *eryD*, a transcript encoding the components for one or more ABC transporters (*SMc01628–SMc01624*), a transcript that spans *eryA* through *eryB*, a transcript containing *eryC* and a transcript containing the genes *SMc01615*, *tpiB* and *rpiB*. Interestingly, since *eryD* appears to be transcribed independently, the inability of SRmA885 to use erythritol as a sole carbon source cannot be explained by polar effects on the ABC transporter. Similarly, SRmA428 (*SMc01627*) has no polar effects on the downstream erythritol catabolic genes.

***eryA* and *eryB* are contained in a single transcript**

Although the complementation data are consistent with *eryA* and *eryB* being in a single transcript (Table 2), the data could be viewed as ambiguous with respect to *eryA* and *eryB* (Table 2). We note that SRmA782, containing *eryA*, as well as SRmA784, containing *eryB*, often grew poorly when carrying pHC6 or pHC9. We reasoned that if *eryA*, *SMc01622*, *fucA1* and *eryB* constituted a single transcript, they should all be present on a single mRNA. To test this, total RNA from erythritol-grown Rm1021 was isolated and used to create cDNA. Primers to the junctions of each of these genes were designed. In each case, we were able to generate a PCR product from the cDNA suggesting

Table 2. Erythritol catabolic genes are in separate complementation groups

Growth is as follows: +, wild-type; +/-, marginal growth; -, no growth.

Strain	Genotype	Plasmid						
		none	pHC10	pHC3 (1628)	pHC6 (1627)	pHC9 (<i>eryA</i>)	pHC12 (<i>eryB</i>)	pEL6 (<i>eryC</i>) Wild-type
Rm1021	Wild-type	+	+	+	+	+	+	+
SRmA855	<i>eryD</i>	-	+	+	+	+	+	+
SRmA785	<i>SMc01628</i>	-	-	-	+	+	+	+
SRmA780	<i>SMc01627</i>	-	-	-	+	+	+	+
SRmA782	<i>eryA</i>	-	+	+	-	+/-	+	+
SRmA784	<i>eryB</i>	-	+	+	+/-	-	+	+
SRmA787	<i>eryC</i>	-	+	+	+	+	-	+
SRmA966	<i>SMc01615</i>	-	+	+	+	+	+	+
SRmA584	<i>tpiB</i>	-	+	+	+	+	+	+

that these genes were indeed within a single transcript (Fig. 2). Primers (27 and 28) designed to amplify the junction between *eryB* and *SMc01619* did not generate a PCR product from cDNA suggesting that *eryB* was the final gene in this transcript (data not shown).

The erythritol locus does not contain uncharacterized genes involved in erythritol catabolism

The metabolic processes necessary for the breakdown of erythritol in *B. abortus* have been shown to consist of five steps (Sperry & Robertson, 1975b). Of the five reactions, the genes for only three of these, *eryA*, *eryB* and *eryC* have been discovered (Sangari *et al.*, 2000). The two transcripts that contain *eryA*, *eryB* and *eryC* in *S. meliloti* include five other genes (Fig. 1). These genes encode a putative oxidoreductase (*SMc01622*), a putative aldolase (*FucA1*), a putative class II aldolase/adducin protein (*SMc01619*), a putative sugar kinase (*SMc01618*) and a putative hydrolase (*SMc01617*). To determine if any of these genes encoded proteins that play a role in the catabolism of erythritol, *eryA*, *eryB* and *eryC* were introduced and overexpressed in each of the mutants that we had previously isolated (Table 3).

When *eryB* was overexpressed in SRmA781, SRmA779 and SRmA784, carrying mutations in *SMc01622*, *fucA1* and *eryB*, respectively, each strain was complemented for the inability to utilize erythritol as a sole carbon source (Table 3). SRmA782, carrying an *eryA* mutation, was not complemented by the introduction of *eryB*. Taken together, these data strongly suggest that only *eryA* and *eryB* are necessary for erythritol breakdown and that the insertions in SRmA781 and SRmA779 have polar effects on *eryB*. Similarly, it was found that SRmA783 and SRmA778 were complemented by the introduction of *eryC* on a plasmid suggesting that proteins encoded by *SMc01619*, *SMc01618* and *SMc01617* do not play a role in erythritol catabolism (Table 3). These data

suggest that the transcript that contains *eryC* also contains *SMc01617–SMc01619* (Tables 2 and 3).

Introduction of a plasmid containing *eryA* into SRmA782 did not complement the ability to utilize erythritol (Table 3). Although this is not surprising since all the data presented support the hypothesis that an *eryA* mutation has a polar effect on *eryB* (Tables 2 and 3, Fig. 2), it is worth noting that when *eryA* was expressed in SRmA782, the strain was unable to grow on a medium that contained erythritol with a second carbon source that could support growth (Table 3). This correlates with previous suggestions that toxic effects are the result of the build-up of a phosphorylated intermediate (Adhya & Shapiro, 1969; Power, 1967; Richardson *et al.*, 2004; Sperry & Robertson, 1975a; Yost *et al.*, 2006).

Components of the ABC-type transporter defined by *SMc01628–SMc01624* are necessary for transport of erythritol

BLAST analysis using Hyp-EryEFG from *R. leguminosarum* VF39SM against the *S. meliloti* Rm1021 database identified SMb20349–SMb20352 as having the highest similarity at the amino acid level (about 67 % for EryEFG, about 47 % for Hyp). Data from our transposon mutagenesis experiments show that inserts that affect the *SMc01628–SMc01624* lead to an inability to use erythritol as a sole carbon source (Table 2). Since duplication of function is not uncommon in *Rhizobium* (Oresnik *et al.*, 1998; Renalier *et al.*, 1987; Schlüter *et al.*, 1997; Schwedock & Long, 1992), we wanted to determine if erythritol transport was inducible in *S. meliloti* and if the transporter genes found between *eryA* and *eryD* were indeed necessary for erythritol transport.

Rm1021 grown in defined medium containing glucose or glycerol was not capable of taking up labelled erythritol at levels above those observed in dead-cell controls (data not shown). In contrast, Rm1021 grown in medium containing

Fig. 2. (a) Schematic diagram showing the arrangement of genes between *eryA* and *SMc01619*. Arrows represent approximate positions of primers that were used for junction PCR analysis. (b) Junction PCR of erythritol-induced cDNA. The primers used span the junction between the two genes, as labelled. Lanes 1, 4 and 7 show the PCR product using wild-type genomic DNA as template. Lanes 2, 5 and 8 show PCR product using isolated RNA from erythritol-induced wild-type cells. Lanes 3, 6 and 9 show PCR product using cDNA synthesized from isolated RNA from erythritol-induced wild-type cells.

Table 3. Complementation of mutations in erythritol catabolic genes

Growth is as follows: +, wild-type; +/-, marginal growth; -, no growth. Ery/glyc, media contained both 15 mM erythritol and 15 mM glycerol.

Strain	Genotype	Plasmid											
		None			pBG3 (<i>eryA</i> ⁺)			pBG14 (<i>eryB</i> ⁺)			pHY112 (<i>eryC</i> ⁺)		
		Ery	Ery/glyc	Glyc	Ery	Ery/glyc	Glyc	Ery	Ery/glyc	Glyc	Ery	Ery/glyc	Glyc
Rm1021	Wild-type	+	+	+	+	+	+	+	+	+	+	+	+
SRmA782	<i>eryA</i>	-	+	+	-	-	+	-	+	+	-	+	+
SRmA781	<i>SMc01622</i>	-	-	+	-	-	+	+	+	+	-	-	+
SRmA779	<i>fucA1</i>	-	-	+	-	-	+	+	+	+	-	-	+
SRmA784	<i>eryB</i>	-	-	+	-	-	+	+	+	+	-	-	+
SRmA783	<i>SMc01619</i>	-	-	+	-	-	+	-	-	+	+	+	+
SRmA778	<i>SMc01618</i>	-	-	+	-	-	+	-	-	+	+	+	+
SRmA787	<i>eryC</i>	-	-	+	-	-	+	-	-	+	+	+	+

either erythritol or erythritol and glycerol displayed robust transport rates (Fig. 3). To determine if the ABC transporter genes between *eryA* and *eryD* were necessary, SRmA465, carrying a Tn5 insertion in *SMc01627*, was grown in medium containing erythritol and glycerol. The data demonstrate that SRmA465 was unable to transport labelled erythritol into the cell suggesting that this gene(s) encodes a protein(s) used in an ABC transporter involved in the transport of erythritol (Fig. 3).

EryD and SMc01615 negatively regulate their own transcripts and affect the expression of other *ery* transcripts

We had previously shown that *tpiB* could be induced by low concentrations of erythritol, thus allowing it to complement a *tpiA* mutation in Rm1021 (Poysti & Oresnik, 2007). Since *tpiB* is in an operon that includes a DeoR-type negative regulator, encoded by *SMc01615*, it was postulated that this regulator would regulate *tpiB* expression and that second-site mutations that allowed phenotypic suppression of *tpiA* would be isolated readily (Poysti & Oresnik, 2007). Although more than 10¹⁰ cells were plated, this class of second-site mutations was never isolated, which led to the suggestion that *tpiB* may not be solely under the regulation of SMc01615 (Poysti & Oresnik, 2007). To address the regulation of *tpiB* and the other components of the erythritol catabolic genes, RT-PCR experiments were carried out.

Consistent with preliminary β -galactosidase assays using genetic fusions (data not shown), it was found that, in the presence of erythritol, components of the erythritol catabolic pathway were induced 5–10-fold when compared with transcript levels found in glycerol-grown cells (Table 4). Similar analysis with the *eryD* mutant, SRmA885, showed greater than 10-fold increased expression of *eryD* in glycerol conditions compared with wild-type levels demonstrating that, consistent with the annotation, EryD is a negative regulator and that it

regulates the expression of its own transcript (Table 4). Intriguingly, every other transcript in the erythritol locus was expressed at, or below, background levels when SRmA885 (*eryD*) was grown under inducing conditions; a phenotype more commonly associated with a positive regulator (Table 4). The inability of SRmA885 to induce erythritol transport and catabolic genes is consistent with its inability to grow using erythritol as a sole carbon source (Table 2).

To address the role that SMc01615 plays in the regulation of erythritol catabolic genes, a strain (SRmA966) containing a mutation in this gene was constructed. Since this strain is unable to grow in the presence of erythritol, only the effect of the mutation under non-inducing conditions could be analysed. The results clearly show that lack of *SMc01615* resulted in a greater than 10-fold induction of its own transcript under non-inducing conditions. We note

Fig. 3. Erythritol transport assays of *S. meliloti* wild-type (Rm1021, ●) and the erythritol transport mutant SRmA465 (*SMc01627*, ■) induced with defined medium containing erythritol and glycerol. Where not shown error bars are smaller than the symbol. Error bars represent SD ($n=3$).

Table 4. Regulation of gene expression by *eryD* and *SMc01615* analysed by qRT-PCR

Data are expressed as $2^{\Delta Ct}$ and represent fold expression over uninduced Rm1021 grown in glycerol. The experiment also included *SMc00128* as an internal control (Krol & Becker, 2004). The table shows representative data from a single experiment. The experiment was repeated three times, showing consistent results. Ery/glyc, media contained both 15 mM erythritol and 15 mM glycerol.

Strain	Relevant genotype	Carbon source	<i>eryD</i>	<i>SMc01628</i>	<i>eryA</i>	<i>eryC</i>	<i>SMc01615</i>
Rm1021	Wild-type	Glyc	1	1	1	1	1
Rm1021	Wild-type	Ery/glyc	11.0	19.4	16.9	11.9	6.5
SRmA885	<i>eryD</i>	Glyc	14.7	0.8	0.1	0.9	0.3
SRmA885	<i>eryD</i>	Ery/glyc	18.6	0.3	0.1	1.1	0.6
SRmA966	<i>SMc01615</i>	Glyc	5.5	0.7	0.6	1.4	14.6
SRmA782	<i>eryA</i>	Glyc	0.3	0.3	1.1	1.1	0.3
SRmA782	<i>eryA</i>	Ery/glyc	1.47	0.8	0.4	0.7	0.9

that in SRmA966 we observed an increased level of transcription of *eryD* suggesting that *SMc01615* may also play a role in regulating *eryD* (Table 4).

The inability to catabolize erythritol affects gene expression

It is unclear if the regulators of *ery* catabolic genes respond to either erythritol and/or an erythritol metabolite as an effector molecule (Sangari *et al.*, 2000; Yost *et al.*, 2006). Since the organization of the operons containing the *ery* catabolic and transport genes is so different from that of either *B. abortus* or *R. leguminosarum*, it was thought that investigating the effect of erythritol catabolic mutations on the regulation of these genes may provide some insight. Analysis of transcription in SRmA782 (*eryA*), revealed that only background levels of transcription occurred across the entire erythritol region when it was grown on media containing erythritol (Table 4). This suggests that in addition to its own induction, a functional EryA is essential for the induction of the entire erythritol locus including *tpiB* and *eryD*. In addition, it suggests that the regulators of the erythritol locus probably respond to an intermediate of erythritol catabolism.

The erythritol locus contains determinants for adonitol and arabitol utilization

The transcripts that contain the genes necessary for erythritol catabolism (*eryA*, *eryB*, *eryC* and *tpiB*) also contain six other genes that are annotated as being involved in small-molecule metabolism. Moreover, the genes that encode the components of the ABC transporter contain two ABC proteins and two permease components, formally making it possible that this may represent more than one transport system. To attempt to define what role the other genes in this cluster may play, representative insertion mutants from each of the transcripts (except *eryD*) were analysed for carbon utilization phenotypes using Biolog PM1 and PM2 plates. The results from the PM1 and PM2 plates that suggested an inability to use a compound as a sole carbon source were verified by

streaking the strains on defined media containing these as sole carbon sources. Intriguingly, these results show that mutants with insertions in the ABC transporter transcript (SRmA783), the transcript containing *eryA* and *eryB* (SRmA782) as well as the transcript containing *eryC* (SRmA783) were unable to use adonitol or L-arabitol as a sole carbon source (Table 5). In contrast, the transcript that contains *tpiB* was only necessary for erythritol catabolism. In an effort to more precisely define which genes were necessary for the utilization of which sugar, other insertions in these transcripts were also tested. The majority of the strains carrying inserts in these transcripts were unable to use adonitol and L-arabitol in addition to erythritol (Table 5). This includes SRmA855 which contains an *eryD* mutation, suggesting that EryD also regulates the locus during adonitol or L-arabitol catabolism. SRmA787, containing an insertion in *eryC*, was able to use adonitol and L-arabitol as sole carbon sources (Table 5). It is noteworthy that the analysis also showed that although these strains were unable to grow on L-arabitol, they were still capable of utilizing D-arabitol (Table 5). It is also worth noting that Rm1021 could not grow using the closely related sugar alcohol xylitol as a sole carbon source (data not shown).

Lack of erythritol, adonitol, and arabitol utilization does not affect competition for nodule occupancy in *S. meliloti*

It has recently been shown that erythritol affects competition for nodule occupancy in *R. leguminosarum* (Yost *et al.*, 2006). In addition, erythritol utilization has been associated with avirulence in various *Brucella* strains (Köhler *et al.*, 2002). SRmA465 was used as a representative strain since it lacked the ability to take up erythritol and was unable to grow on adonitol or arabitol (Table 1). Two different inoculation ratios (mutant:wild-type) were tried and in every trial, the ratio of strains isolated from the nodules was identical to the inoculation ratio, suggesting that the ability to utilize erythritol, adonitol or arabitol does not play a role in competition for nodule occupancy (data not shown).

Table 5. Polyol utilization phenotypes of mutations across the erythritol locus

Growth is as follows: +, wild-type; +/-, marginal growth; -, no growth.

Strain	Genotype	Carbon source				
		Erythritol	Adonitol	L-Arabitol	D-Arabitol	Glycerol
Rm1021	Wild-type	+	+	+	+	+
SRmA885	<i>eryD</i>	-	-	-	+	+
SRmA785	<i>SMc01628</i>	-	-	-	+	+
SRmA782	<i>eryA</i>	-	-	-	+	+
SRmA781	<i>SMc01622</i>	-	-	-	+	+
SRmA779	<i>fucA1</i>	-	-	-	+	+
SRmA784	<i>eryB</i>	-	+/-	-	+	+
SRmA783	<i>SMc01619</i>	-	+/-	-	+	+
SRmA778	<i>SMc01618</i>	-	-	-	+	+
SRmA787	<i>eryC</i>	-	+	+	+	+
SRmA966	<i>SMc01615</i>	-	+	+	+	+
SRmA584	<i>tpiB</i>	-	+	+	+	+

DISCUSSION

Our genetic and physical data show that the erythritol locus in *S. meliloti* consists of five transcripts. Of the catabolic genes present, only *eryA*, *eryB*, *eryC* and *tpiB* appear to encode proteins that are necessary for erythritol catabolism (Tables 2 and 3). In addition, genes that encode the determinants for an ABC transporter are essential for the transport of erythritol into the cell (Fig. 3). It is of note that genes encoding Smb20349–51, which were predicted to be necessary for erythritol transport in *S. meliloti* on the basis of identity to the *R. leguminosarum* erythritol transporter (Yost *et al.*, 2006), do not have erythritol transport function based on our ability to show lack of erythritol transport in our mutants.

The original work outlining the catabolic pathway for erythritol catabolism provided evidence for five biochemical steps yielding dihydroxy-acetone phosphate (Sperry & Robertson, 1975b). Analyses done on *B. abortus* and *R. leguminosarum*, as well as the data presented here, only account for three of these genes as well as a dedicated triose-phosphate isomerase (Sangari *et al.*, 2000; Yost *et al.*, 2006). Complementation of each of *eryA*, *eryB*, *eryC* and *tpiB* clearly shows that the other genes at this locus do not encode enzymes that have the missing activities (Table 3). Although our limited mutagenesis did provide evidence for the transporters necessary for erythritol, it did not identify either of these two missing activities. It should also be noted that although a mutation in the gene annotated as *rpiB* (*SMc01613*) did not affect the ability to use erythritol as a sole carbon source, the corresponding mutant in *R. leguminosarum* was unable to use erythritol (Poysti & Oresnik, 2007; Yost *et al.*, 2006). It is not known at this time how *rpiB* does, or does not, contribute to erythritol catabolism in either *R. leguminosarum* or *S. meliloti*. Conjugation of pEL6 (containing the entire *S. meliloti* ery locus) into *R. leguminosarum* strain Rlt100, a strain that

does not have the capability of utilizing erythritol as a sole carbon source, allowed it to utilize erythritol (data not shown). We suggest that perhaps the biochemical steps that were originally described in *B. abortus*, that are not accounted for by the genes present in the erythritol locus, may be due to enzymes that have enzymic activity on a broad number of substrates and may not be specific for erythritol. Alternatively, some of the erythritol-associated proteins may have more than one enzymic activity.

The regulation of erythritol catabolic genes in *R. leguminosarum* and *B. abortus* has been shown to be carried out by EryD which has been annotated as a negative regulator that falls into a DeoR category (Sangari *et al.*, 2000; Yost *et al.*, 2006). Consistent with this annotation, an *R. leguminosarum* strain carrying an *eryD* mutation was not diminished in its ability to grow on erythritol (Yost *et al.*, 2006). In contrast, we found that *S. meliloti* carrying an *eryD* mutation was unable to use erythritol as a sole carbon source (Table 2). In addition, if an *eryD* mutation was moved into a strain containing a *tpiA* mutation, it was unable to phenotypically suppress the inability to utilize glycerol associated with a *tpiA* mutation if grown on glycerol with 0.5 mM erythritol (Poysti & Oresnik, 2007). Taken together, these data prompted a more thorough analysis of regulation that may be occurring at this locus.

Our data clearly show that EryD is a negative regulator (Table 4). In contrast, the transcription of the other operons at this locus appears to be at, or below, its basal level when *eryD* is absent (Table 4). These data are inconsistent with the hypothesis that *eryD* negatively regulates the genes encoding the ABC transporter, *eryA*, *eryB*, *eryC* and *tpiB*. The data support the involvement of a positive regulator in erythritol catabolism in *S. meliloti*.

BLAST analysis clearly showed that EryD falls into the SorC subclass of DeoR regulators. We find it of interest that

DalR, the regulator of arabinol utilization in *Klebsiella pneumoniae* is also considered a member of this family (Heuvel *et al.*, 1998). SorC has been shown to act as both a negative and a positive regulator of sorbitol utilization in *K. pneumoniae* (Wöhrl *et al.*, 1990). It has been shown that SorC is able to negatively regulate its own transcription. In addition Wöhrl *et al.* (1990) were also able to demonstrate lack of transcription of the sorbose operon in a non-polar background lacking *sorC*. Complementation of this mutation with a *sorC*⁺ allele restored gene expression of the sorbitol operon. More recently the crystal structure of SorC has been solved, and based on the structural model, a model of the positive and negative transcriptional regulation has been proposed (de Sanctis *et al.*, 2009). The SorC data are analogous to what we observed with *eryD* (Table 4). Attempts at complementing the *eryD* mutation with pBG2 and pBG18 (*eryD*⁺) gave ambiguous results; in some cases transcription was observed, whereas in other cases it was not (data not shown). Further work is being carried out to try to resolve this issue.

qRT-PCR data also showed that SMc01615 negatively affects the regulation its own transcript, as well as the transcription of *eryD* (Table 4). We note that SMc01615 contains a sugar-phosphate binding site (cl0039 sugarP_isomerase superfamily), suggesting that it does not respond directly to erythritol, but to a phosphorylated metabolite of the pathway. This is consistent with the data that show only basal levels of transcription of SMc01615 in an *eryA* background. It is also supported by observation that *tpiB* is only necessary for erythritol catabolism in *S. meliloti* and that a strain carrying a *tpiA* mutation had its inability to grow on glycerol phenotypically suppressed when it was grown on glycerol in the presence of 0.5 mM erythritol, but not 0.5 mM adonitol or L-arabitol (data not shown). The constitutive expression of the SMc01615 transcript in an SMc01615 background suggests that although EryD is required for its induction, the effect is probably indirect and is a consequence of its effect on the catabolic genes (Table 4). The effect of SMc01615 on the transcription of *eryD* however, is likely to be direct.

In SRmA782 (*eryA*), transcription at the erythritol locus was abolished (Table 4). These data strongly support the hypothesis that transcription is dependent upon a metabolite from the erythritol pathway since even transcription of *eryD* and SMc01615 were unable to be induced (Table 4). We note that EryD and SMc01615 contain different putative domains. EryD contains a domain that is classified as a part of the sugar-binding super family (cl04446) and SMc01615 contains a domain related to the sugar-phosphate isomerase super family (cl10039). Taken together, this suggests that the regulators may not recognize the same metabolite.

The apparent role of this locus in erythritol catabolism was expected since *eryA*, *eryB* and *eryC* are all homologues of genes previously shown to be necessary for erythritol catabolism in *R. leguminosarum* and *B. abortus* (Sangari

et al., 2000; Yost *et al.*, 2006). The role of the locus in adonitol and L-arabitol metabolism is surprising, as it was not suggested by the annotation of genes in the locus. The annotation suggests that the genes interspersed among the *ery* catabolic genes encode an oxidoreductase (SMc01622), an aldolase (*fucA1*), a putative class II aldolase/adducin protein (SMc01619), a sugar kinase (SMc01618) and a hydrolase (SMc01617). Based on the currently understood pathways of adonitol and arabinol catabolism as they were defined in *Aerobacter aerogenes* (Wood *et al.*, 1961), as well as what is known about polyol metabolism in *R. leguminosarum* (Primrose & Ronson, 1980; Ronson & Primrose, 1979; Stowers, 1985; Yost *et al.*, 2006), it is unclear which of these genes are necessary for the catabolism of either arabinol or adonitol. It may be pertinent to point out that orthologues of SMc01617–19 appear to form an independent operon in *Mesorhizobium loti* (*mlr3601*, *mlr3603* and *mlr3604*), suggesting that these genes may in fact be a separate catabolic entity (Kaneko *et al.*, 2000).

An inability to catabolize erythritol has been shown to affect competition for nodule occupancy in *R. leguminosarum* bv. *viciae* (Fry *et al.*, 2001; Yost *et al.*, 2006), whereas the ability to catabolize adonitol in *R. leguminosarum* bv. *trifolii* did not affect competition for nodule occupancy (Oresnik *et al.*, 1998). Competition experiments indicated that a strain unable to transport erythritol, adonitol and L-arabitol was as effective as the wild-type at competing for nodule occupancy on alfalfa. It would be of interest to determine whether the ability to catabolize erythritol by other rhizobia affects competition for nodule occupancy or whether there are host-plant-specific effects of erythritol catabolism on competition.

We are currently investigating the erythritol locus with respect to its make-up, gene arrangement and polyol catabolic ability throughout the rhizobia and other bacterial species. In addition, we are attempting to determine the precise functions encoded by the genes that are necessary for adonitol and arabinol catabolism in *S. meliloti*. It is hoped that defining the roles of these genes will be of benefit to both the development of *S. meliloti* as a model organism for the study of plant-microbe interactions as well as to provide insight into basic metabolic pathways.

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