The genes for erythritol catabolism are organized as an inducible operon in *Brucella abortus*

Félix J. Sangari, Jesús Agüero and Juan M. García-Lobo

Erythritol utilization is a characteristic of pathogenic *Brucella abortus* strains. The attenuated vaccine strain B19 is the only *Brucella* strain that is inhibited by erythritol, so a role for erythritol metabolism in virulence is suspected. A chromosomal fragment from the pathogenic strain *B. abortus* 2308 containing genes for the utilization of erythritol was cloned taking advantage of an erythritol-sensitive Tn5 insertion mutant. The nucleotide sequence of the complete 7714 bp fragment was determined. Four ORFs were identified in the sequence. The four genes were closely spaced, suggesting that they were organized as a single operon (the *ery* operon). The first gene (*eryA*) encoded a 519 aa putative erythritol kinase. The second gene (*eryB*) encoded an erythritol phosphate dehydrogenase. The function of the third gene (*eryC*) product was tentatively assigned as D-erythrulose-1-phosphate dehydrogenase and the fourth gene (*eryD*) encoded a regulator of *ery* operon expression. The operon promoter was located 5' to *eryA*, and contained an IHF (integration host factor) binding site. Transcription from this promoter was repressed by EryD, and stimulated by erythritol. Functional IHF was required for expression of the operon in *Escherichia coli*, suggesting a role for IHF in its regulation in *B. abortus*. The results obtained will be helpful in clarifying the role of erythritol metabolism in the virulence of *Brucella* spp.

**Keywords**: erythritol operon, *Brucella abortus*

INTRODUCTION

*Brucella abortus* is a Gram-negative pathogenic bacterium which causes bovine brucellosis. *B. abortus* has a tropism for the reproductive organs, colonizing the placenta and fetus of pregnant cows and thus causing abortions. The preferential use of erythritol is characteristic of the genus *Brucella*. Erythritol is used by *Brucella* in preference to glucose (Anderson & Smith, 1965) and erythritol promotes the growth of some *Brucella* strains (Meyer, 1967). The metabolic pathway for degradation of erythritol in *Brucella* was described by Sperry & Robertson (1975a). *B. abortus* strain B19 is a spontaneous attenuated mutant widely used to vaccinate cattle. Erythritol inhibits, rather than promoting, the growth of B19. Sperry & Robertson (1975b) reported the absence in strain B19 of D-erythrulose-1-phosphate dehydrogenase, a critical enzyme in the erythritol catabolic pathway. This defect causes both the accumulation of the toxic intermediate D-erythrulose 1-phosphate and a depletion of ATP levels, leading to inhibition of bacterial growth. Erythritol metabolism is the best known and most important difference between B19 and virulent *B. abortus* strains. Consequently, a correlation has been proposed between the capacity of *B. abortus* to metabolize erythritol and the virulence of this species. Erythritol has been found in the bovine placenta, and a chemotactic role has been attributed to this polyalcohol that would explain the specific localization of *B. abortus* in this organ (Smith et al., 1962). Several reports have claimed a correlation between erythritol oxidation and virulence in different animal models (Meyer, 1966).

**Abbreviation**: IHF, integration host factor.

The GenBank accession number for the sequence reported in this paper is U57100.
The role of erythritol metabolism as a virulence factor remains obscure. This prompted us to undertake a genetic characterization of the metabolism of erythritol and its contribution to virulence in B. abortus. As a first step we generated a library of transposon insertion mutants of B. abortus strain 2308 as previously described (Sangari & Aguero, 1991). A mutant was isolated (mutant 227) that did not metabolize erythritol and was inhibited by this compound, mimicking the behaviour of the vaccine strain B19. This insertion mutant was used to clone the ery::Tn5 region from its chromosome, and then the corresponding regions from both strains 2308 and B19. By comparison of these regions a deletion was found in the chromosome of the B19 strain, allowing the development of a PCR assay for the rapid and unequivocal differentiation of the B19 vaccine strain from other Brucella strains (Sangari & Agüero, 1991; Sangari et al., 1994). The transposon insertion mutants in the ery genes were used to demonstrate that oxidation of erythritol was not essential for virulence in a mouse model of B. abortus infection (Sangari et al., 1998). The aim of the work described here was to characterize the complete ery region from B. abortus 2308 and gain some insight into its regulation, thus enabling further analysis of the association between erythritol catabolism and virulence in Brucella.

**METHODS**

**Bacterial strains, plasmids, and growth conditions.** Bacterial strains and plasmids used in this work are listed in Table 1. B. abortus was grown on tryptose broth (TB) or tryptose agar (TA) (Difco). Plates were usually incubated at 37 °C, under a 5% CO₂ atmosphere. Erythritol (Sigma) was added to the growth medium at 1% as a nutritional supplement or at 1 mg ml⁻¹ for induction of the ery operon. Escherichia coli strains were usually grown on Luria–Bertani broth or plates. Concentrations of antibiotics in the agar plates were as follows: ampicillin, 50 μg ml⁻¹; chloramphenicol, 25 μg ml⁻¹; kanamycin, 50 μg ml⁻¹; nalidixic acid, 25 μg ml⁻¹.

**Genetic and DNA methods.** E. coli transformation was

---

**Table 1. Bacterial strains and plasmids**

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Relevant characteristics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DH5α</td>
<td>Δ80dlacZ AM15 Δ(argF–lacZYA)U169 deoR endA1 gyrA96 bsdR17 recA1 relA1 supE44 thi-1</td>
<td>Life Technologies</td>
</tr>
<tr>
<td>S17.1</td>
<td>thi pro his recA RP4-2-Tc::Mu Km::Tn7</td>
<td>Simon et al. (1983)</td>
</tr>
<tr>
<td>RU4404</td>
<td>MM294 [thi-1 endA1 bsdR17 supE44::Tn1725</td>
<td>Ullben &amp; Schmitt (1986)</td>
</tr>
<tr>
<td>MC294</td>
<td>ara argE_53 Δ(lac–pro) gyrA metB Δ(recA) rpoB srl::Tn10 supF thi</td>
<td>Gamas et al. (1986)</td>
</tr>
<tr>
<td>MC296</td>
<td>MC294 bimAA82::Tn10 hipA3:cam</td>
<td>Gamas et al. (1986)</td>
</tr>
<tr>
<td><strong>B. abortus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2308</td>
<td>Virulent strain</td>
<td></td>
</tr>
<tr>
<td>2308Nx</td>
<td>Spontaneous nalidixic acid resistant mutant</td>
<td>Sangari &amp; Agüero (1991)</td>
</tr>
<tr>
<td>B19</td>
<td>Vaccine strain</td>
<td></td>
</tr>
<tr>
<td>B19Nx</td>
<td>Spontaneous nalidixic acid resistant mutant</td>
<td>Sangari et al. (1994)</td>
</tr>
<tr>
<td>FJS-2</td>
<td>2308Nx [eryD::Km]</td>
<td>This work</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pBluescript II SK(+)</td>
<td>Ap⁺</td>
<td>Stratagene</td>
</tr>
<tr>
<td>pKT231</td>
<td>Km⁺ Sm⁺</td>
<td>Bagdasarian et al. (1981)</td>
</tr>
<tr>
<td>pUC4K</td>
<td>Km⁺</td>
<td>Pharmacia</td>
</tr>
<tr>
<td>pKOK.4</td>
<td>Ap⁺ Cm⁺ Tc⁺</td>
<td>Kokotek &amp; Lotz (1991)</td>
</tr>
<tr>
<td>pSU6004</td>
<td>7·7 kb EcoRI ery fragment from 2308 in pBluescript II SK(+)</td>
<td>Sangari &amp; Agüero (1994)</td>
</tr>
<tr>
<td>pSU6010</td>
<td>Same insert cloned in pKT231</td>
<td>Sangari et al. (1994)</td>
</tr>
<tr>
<td>pSU6104</td>
<td>eryA in pBluescript II SK(+)</td>
<td>This work</td>
</tr>
<tr>
<td>pSU6099</td>
<td>eryAB in pBluescript II SK(+)</td>
<td>This work</td>
</tr>
<tr>
<td>pSU6100</td>
<td>eryABC in pBluescript II SK(+)</td>
<td>This work</td>
</tr>
<tr>
<td>pSU6111</td>
<td>eryABCD in pBluescript II SK(+)</td>
<td>This work</td>
</tr>
</tbody>
</table>
performed either by the calcium chloride method (Sambrook et al., 1989), or by electroporation (Dower et al., 1988) using a Bio-Rad Gene-Pulser apparatus. Conjugation between E. coli S17.1 containing mobilizable plasmids and B. abortus was performed in solid medium on nitrocellulose filters for 6 h (Sangari & Aguero, 1991). Transconjugants were obtained on selective plates. Restriction endonucleases, ligase and other DNA enzymes were used according to the manufacturers’ directions. DNA sequencing was performed by the dideoxy chain-termination method (Sanger et al., 1977) with plasmid templates using a Taq DNA polymerase based kit (Promega).

### Transposon mutagenesis

Plasmid pSU6010 (Table 1), consisting of the complete ery region from B. abortus 2308 in a 7714 bp EcoRI fragment cloned in the broad-host-range, mobilizable vector pKT231 (Bagdasarian et al., 1981), was able to complement the erythritol susceptibility of both B. abortus strain B19 and the ery::Tn5 mutant 227 (Sangari et al., 1994). This plasmid was subjected to mutagenesis with the chloramphenicol-resistance transposon Tn1725 to map the position of ery genes precisely. To do this, pSU6010 was introduced into E. coli strain RU4404 (Ubben & Schmitt, 1986), carrying Tn1725 in the chromosome. After 2 d growth at 30 °C, plasmid DNA was isolated and used to transform E. coli DH5a to chloramphenicol resistance. Plasmid DNA was purified from individual transformants and the location of the transposon was mapped with EcoRI and HindIII.

#### Construction of a B. abortus eryD mutant

To construct a B. abortus eryD mutant by allelic replacement, we cloned a 2 kb NruI–HindIII fragment containing eryD from the chromosome of strain 2308 (Sangari et al., 1994) into pBluescript SK. This plasmid was called pSU6101 (Fig. 1). The central 0.5 kb BstFI fragment from the eryD insert in pSU6101 was then replaced with a 1.2 kb kanamycin-resistance cassette from pUC4K. The resulting plasmid, pSU6103, contained a deleted eryD allele interrupted by the kanamycin-resistance cassette. The insert in pSU6103 was cloned into the plasmid pKOK-A, which can be transferred by conjugation into B. abortus, but which does not replicate in this species. In this way the plasmid pSU6106 was obtained and transferred into B. abortus 2308Nx. Kanamycin-resistant transconjugants were analysed by Southern blot hybridization with an eryD probe to check for replacement of the locus. One colony with the correct genomic structure (strain FJS-2) was selected for further work. The construction procedure is shown diagrammatically in Fig. 1.

#### Phenotypic expression of ery genes in E. coli

E. coli cells containing ery recombinant plasmids were plated on MacConkey agar base supplemented with 1% erythritol. Colonies able to utilize erythritol were red on these plates.

### Primer extension mapping of the 5’ end of ery RNA

Total RNA was prepared from 10 ml cultures as described previously (Garrido et al., 1993). For primer extension studies, RNA was used as template for the synthesis of cDNA by AMV reverse transcriptase (Boehringer Mannheim). Synthetic oligonucleotide primer M2990 (Table 2) was 5’-end labelled with polynucleotide kinase and [γ-32P]ATP. The products of the extension reactions were analysed in 6% urea-polyacrylamide gels. A sequencing ladder using the same primer was run in the gel beside the extension products to map the 5’ end of the mRNA.

### Sequence analysis and comparison

The DNA sequence was analysed with the GCG programs (Devereux et al., 1984) in the EMBNET node at the Centro Nacional de Biotecnologia, Madrid. Sequence comparisons against databases were done with the BLAST programs (Altschul et al., 1997) at the NCBI BLAST server. Analysis of DNA curvature was performed with the program DNASTAR.

### RESULTS

#### Tn1725 mutagenesis of the ery region

A total of 80 independent Tn1725 insertions in pSU6010 were analysed. The location of the transposon in each individual mutant was determined by mapping with EcoRI and HindIII (Fig. 2). In 38 of them (pSU6022 to pSU6059) the transposon was found integrated into the Brucella DNA insert. In order to evaluate the effect of Tn1725 insertion on the Ery phenotype, each mutant was introduced by conjugation into B. abortus B19 and the transconjugants were checked for their ability to grow on erythritol-containing plates. The results of this complementation experiment are also summarized in Fig. 2.

#### Nucleotide sequence of the ery region

The nucleotide sequence of the complete EcoRI insert in pSU6010 was determined on both DNA strands. Three approaches were used for sequencing: (i) sequencing of Tn1725 insertions using specific primers at the trans-

---

### Table 2. Synthetic oligonucleotides

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2990</td>
<td>CAGGTCGCTCGCAGGCCCTTTCC</td>
<td>ery mRNA primer extension</td>
</tr>
<tr>
<td>eryA.1</td>
<td>CGCATCACCCTGTTGCT</td>
<td>Sequencing eryA</td>
</tr>
<tr>
<td>eryA.2</td>
<td>CTTCGCGATAGGCAAAGA</td>
<td>Sequencing eryA</td>
</tr>
<tr>
<td>Tn1725.I</td>
<td>GAGCTGTACGAGAACCCG</td>
<td>Sequencing Tn1725 insertions</td>
</tr>
<tr>
<td>Tn1725.II</td>
<td>CCGTACATGAGGCCGAA</td>
<td>Sequencing Tn1725 insertions</td>
</tr>
</tbody>
</table>

---

489
poson ends, (ii) subcloning of specific restriction fragments that were sequenced with universal primers, and (iii) use of synthetic oligonucleotides as primers to sequence the remaining gaps (Table 2). The main features of the sequence are described below and shown in Fig. 2.

The region between nucleotides 1 and 376 showed an A + T content much higher than the 41 mol % mean of the *Brucella* genome. A sequence identical to the integration host factor (IHF) binding site (Craig & Nash, 1984) was found in this region (116–129). The sequence $252\text{TATACAN}_{18}\text{TGGTAT}_{281}$ (P1) was similar to the canonical *E. coli* promoter. Furthermore, some stretches of alternating As and Ts were suggestive of the existence of intrinsic curvature by analysis with the program DNASTAR (data not shown). All these data strongly suggested that this region could be involved in the transcriptional start and regulation of expression of downstream genes.

---

**Fig. 1.** Schematic diagram of the construction of an *eryD* mutant by allelic replacement in strain 2308Nx. A *Nru*I–*Hind*III fragment containing *eryD* was cloned in pBluescript SK (pSU6101), and an internal *Bal*I fragment was replaced with a kanamycin-resistance cassette, giving pSU6103. The interrupted region was transferred to the suicide plasmid pOK4 (pSU6106), and used to produce the *eryD* mutant strain FJS-2 by homologous recombination.

**Fig. 2.** Physical map of the EcoRI fragment containing the *ery* region from the chromosome of *B. abortus* 2308 cloned in pSU6004. The arrows under the map point to the positions of different Tn1725 insertions in that plasmid. These insertions were named pSU6022 to pSU6059, and they are identified in the figure by the last two digits of their plasmid numbers. A plus sign below the arrow indicates that the plasmid complemented the *Ery* − phenotype of strain B19; a minus sign indicates lack of complementation. The position of the *ery* ORFs as well as the *ery* (P1) promoter and terminator is shown in the lower part of the figure.
Four ORFs were identified in the region spanning basepairs 377 to 5400. The eryA gene could start either at the GTG codon at bp 377 or at the ATG at basepair 386. The presence of the sequence GAAAG, similar to the E. coli ribosome-binding site, 3 bp upstream of the GTG codon suggested that this was the initiation codon of eryA. The stop codon for this gene was found at position 1934. Translation of this sequence would result in a 519 aa polypeptide with a calculated molecular mass of 54.4 kDa. The next gene in the sequence (eryB) would start at the ATG at basepair 1949 and its stop codon was found at basepair 3455. This gene would translate into a 502 aa polypeptide with a calculated molecular mass of 56.2 kDa. The eryC gene started at 3465 and ended at 4392. Its product would be 309 aa long with a molecular mass of 35 kDa. Finally, eryD was found between positions 4422 and 5370; its translated product would be a protein of 316 aa with a molecular mass of 33.5 kDa. Genes eryA, eryB and eryC were closely spaced with very short intergenic regions in which sequences resembling the E. coli ribosome-binding site were found, and there were only 30 bp between eryC and eryD. A sequence found 3’ to eryD between basepairs 5372 and 5412 was able to fold as a stem–loop structure and had some analogies with transcription terminators. These data suggested that the four genes eryABCD could constitute a single transcriptional unit. Three additional genes, encoding a triose phosphate isomerase, a subunit of the galactose-6-phosphate isomerase and a regulatory protein belonging to the DeoR family, were found in the sequence downstream of the ery operon.

**Analysis of the ery gene products by sequence similarity**

The relationship of the polypeptides deduced from the nucleotide sequence of the ery region to known gene products was determined using the program TBLASTN. The results of these comparisons are summarized in Table 3.

<table>
<thead>
<tr>
<th>ORF</th>
<th>Size (bp)</th>
<th>Closest homologous gene/gene product (accession no.)</th>
<th>aa identity (%)/similarity (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>EryA</td>
<td>519</td>
<td>E. coli xylB/xylulose kinase (X04691)</td>
<td>30/5/39/0</td>
</tr>
<tr>
<td>EryB</td>
<td>502</td>
<td>E. coli glpD/glycerol-3-phosphate dehydrogenase (M55989)</td>
<td>50/4/68/5</td>
</tr>
<tr>
<td>EryC</td>
<td>309</td>
<td>Alcaligenes hydrogenophilus hupL/hydrogenase (S56898)</td>
<td>38/9/51/8 †</td>
</tr>
<tr>
<td>EryD</td>
<td>316</td>
<td>Rhodobacter sphaeroides smoC/operon regulator (AF018073)</td>
<td>36/0/48/7</td>
</tr>
</tbody>
</table>

*Klebsiella pneumoniae dalR/operon regulator (AF04525) 30/7/42/0

*Percentage identity and similarity were determined using the GAP program from the Genetics Computer Group, with gap weight and gap length values of 8 and 2 respectively.

†Comparison limited to a 57 aa region containing the best fit between the two proteins.

The tblastn program identified homology between the eryA gene product and several bacterial xylulose kinases. The closest homologue to EryA was the product of the E. coli gene xylB, encoding a xylulose kinase. Homology of EryA with bacterial glycerol kinases was also detected. A motif search in the EryA sequence against PROSITE revealed the presence of signature 2 of the FGGY family of carbohydrate kinases, PS00445 (Reizer et al., 1991). This suggested that the eryA gene product could be a kinase for erythritol.

EryB was found to be a homologue of aerobic glycerol-3-phosphate dehydrogenases. The greatest identity (50-4%) was with the enzyme from E. coli. EryB had the PROSITE signature of FAD-dependent glycerol-3-phosphate dehydrogenases, PS00977. These findings and the structural similarity between glycerol phosphate and erythritol phosphate suggested that EryB was the erythritol phosphate dehydrogenase needed for the catabolism of erythritol.

We failed to find any protein in the database with extended similarity to the complete EryC. The most significant match corresponded to a limited region (57 aa) of the large subunit (HupL) of Alcaligenes hydrogenophilus hydrogenase (accession number S56898).

EryD showed similarity to several DNA-binding regulatory proteins. The closest homologue of EryD was SmoC, the regulator of a Rhodobacter sphaeroides operon involved in polyol transport and metabolism. Residues 21–42 of EryD were identified with a 71% probability as a helix–turn–helix motif using the method described by Dodd & Egan (1990). This finding suggested that EryD was a DNA-binding protein with a regulatory function.

**Identification of the ery operon promoter**

Functionality of the putative promoter identified by sequence analysis was studied by primer extension.
Synthetic oligonucleotide M2990 (Table 2), complementary to bases 314–335 of the ery sequence, was used to identify the transcriptional start point. The results of the primer extension study are shown in Fig. 3. A major band was obtained whose size indicated that the putative transcriptional start point was the residue G291, located 10 bp downstream of the promoter. This result confirmed that P1 was a functional promoter that directs the transcription of the ery operon.

**Expression of ery genes in E. coli**

Since wild-type E. coli cells are unable to utilize erythritol we introduced recombinant plasmids containing different genes from the ery operon into E. coli HB101 and studied the ability of the recombinants to use erythritol. Using this assay and the set of plasmids described in Fig. 4, we determined that the minimal region required for degradation of erythritol in E. coli was eryABC (Fig. 4). In addition, plasmid pSU6004 was introduced into several E. coli strains to investigate the effects of various E. coli genes on ery expression. Strain MC296, a himA hip (IHF−) double mutant did not utilize erythritol upon introduction of pSU6004, whereas the isogenic strain with the wild-type IHF alleles (MC294) did. This finding correlated with the presence of an IHF-binding site in the ery control region and strongly suggested a role for IHF in the regulation of expression of the ery operon. E. coli DH5α also produced white colonies on McConkey-erythritol plates when transformed with the ery plasmids. The relA mutation could be responsible for the poor expression of the ery operon in this strain.

**DISCUSSION**

Erythritol metabolism in B. abortus has been studied from a biochemical perspective. Degradation of the polyalcohol starts with erythritol phosphorylation and is followed by a dehydrogenation step that produces D-erythrulose 1-phosphate. Two more dehydrogenation steps and a decarboxylation reaction are required to finally produce dihydroxyacetone phosphate and carbon dioxide (Sperry & Robertson, 1975a). We have sequenced a genomic DNA fragment from B. abortus containing four genes organized as an operon that we
have called the *ery* operon. Introduction of different combinations of these genes into *E. coli* indicated that *eryAB* and *C* were sufficient for erythritol degradation. The similarity of the EryA and EryB proteins to sugar kinases and glycerol-3-phosphate dehydrogenase, respectively, fit the biochemical data. Accordingly we assigned the function of erythritol kinase and erythritol phosphate dehydrogenase to EryA and EryB, respectively. A possible discrepancy in this identification would be that biochemical data have indicated the use of NAD as the electron acceptor for the dehydrogenation step, whereas the close identity of EryB with aerobic glycerol-3-phosphate dehydrogenase to EryA and EryB, respectively, fit the biochemical data. Accordingly we assigned the function of erythritol kinase and erythritol phosphate dehydrogenase to EryA and EryB, respectively. A possible discrepancy in this identification would be that biochemical data have indicated the use of NAD as the electron acceptor for the dehydrogenation step, whereas the close identity of EryB with aerobic glycerol-3-phosphate dehydrogenase, and its sequence signature, suggested the use of FAD.

We have been unable to assign a function to EryC by sequence comparison. A clue to EryC function was suggested the use of FAD. The similarity of the EryA and EryB proteins to sugar kinases and glycerol-3-phosphate dehydrogenase, respectively, fit the biochemical data. Accordingly we assigned the function of erythritol kinase and erythritol phosphate dehydrogenase to EryA and EryB, respectively. A possible discrepancy in this identification would be that biochemical data have indicated the use of NAD as the electron acceptor for the dehydrogenation step, whereas the close identity of EryB with aerobic glycerol-3-phosphate dehydrogenase, and its sequence signature, suggested the use of FAD.

Sequence analysis and comparison showed that EryD was a protein with a helix–turn–helix motif and probably possessed some regulatory function. To obtain further evidence for this, we constructed an *eryD* mutant (FJS-2) and compared the levels of *ery* transcription in this mutant and a wild-type strain. Transcription from the *ery* promoter (P1) was more active in both the mutant FJS-2 and strain B19 than in strain 2308. This result strongly suggested a repressor function for EryD. The increase in *ery* mRNA level induced by erythritol in strain 2308 indicated an inducing function for erythritol, probably through binding to EryD. Many transcription repressors with a helix–turn–helix motif bind sugars that function as inducers. In addition to control by EryD, we have found an IHF-binding site upstream of the P1 promoter and results suggested that IHF was required for *ery* expression in *E. coli*. Although IHF has not yet been described in *Brucella*, it is generally accepted that all bacteria must contain a protein of this type. The role of IHF as a transcriptional regulator in other species has been well documented (Perez-Martin et al., 1994).
The genes in the ery operon described here encode three enzymes that are able to transform erythritol into 3-keto-L-erythrose 4-phosphate according to the pathway described by Sperry & Robertson (1975a). E. coli cells provided with these three enzymes were capable of erythritol utilization. This observation suggests that the enzymes for degradation steps beyond 3-keto-L-erythrose 4-phosphate may be organized in other pathways, and their genes found at other chromosomal locations. Since this is the first description of an operon for erythritol utilization, comparisons are not possible. However, the analysis of operons for polyl utilization (Heuel et al., 1998) highlights the lack of a gene for erythritol transport. Erythritol was shown to be a substrate for the E. coli glycerol facilitator GlpF (Heller et al., 1980). Furthermore it is known that B. abortus B19 mutants tolerant to erythritol arise frequently, and that those mutants show impaired ability to grow on glycerol culture media (Sangari et al., 1996). Thus it is possible that erythritol is transported into Brucella through a GlpF analogue, and later phosphorylated by erythritol kinase, trapping the polyl in the bacterial cytoplasm. The results reported here explain the utilization of erythritol by B. abortus, as well as the inhibition of growth in strain B19 by overproduction of the kinase EryA and the dehydrogenase EryB. The growth-promoting effect of erythritol has not been addressed in this work but there could be a link between erythritol and the aromatic pathway. Erythrose 4-phosphate, the precursor for the biosynthetic pathway for aromatic compounds in bacteria, may be easily obtained from the intermediates of erythritol catabolism. Thus erythritol may be crucial to obtain molecules such as aromatic amino acids and catechols, which play important physiological roles in Brucella, providing a connection between erythritol metabolism and virulence in B. abortus.

ACKNOWLEDGEMENTS

This work was supported by grants BIO96-1398-C02-02 and BIO98-0674 from the Spanish ‘Plan Nacional de I+D’. We thank José Pérez-Martin for the computer analysis of DNA bending.

REFERENCES


Received 21 June 1999; revised 10 October 1999; accepted 1 November 1999.