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Cloning and Characterization of the Glucokinase Gene of *Brucella abortus* 19 and Identification of Three Other Genes

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A clone from *Brucella abortus* 19 complemented an *Escherichia coli* strain deficient in phosphorylation of glucose. Open reading frames similar to *E. coli mepA*, *glk*, and genes encoding ATP-coupled exporters were found in the sequence. A fourth affected growth on minimal media of the *ptsI glk* strain with various carbon sources.

Rest and Robertson (12) studied glucose transport in *Brucella abortus* and found an active transport system and an ATP (but not phosphoenolpyruvate)-coupled glucokinase. The present paper describes the cloning and sequence of the glucokinase gene from *B. abortus*. In addition to this gene, three other open reading frames (ORFs) were found; one was similar to *mepA*, encoding the *Escherichia coli* murein endopeptidase, and another was similar to an ATP-coupled transporter gene.

E. coli RE457 (strain DF52 [6] with the Tn*10*-derived *recA-srlR* deletion of JC10289 [2]) is deficient in the ability to phosphorylate glucose because of mutations in enzyme I of the phosphotransferase system and in glucokinase. Approximately 5 of the 2,500 plasmids in the *B. abortus* 19 pUC9 DNA library (4) caused RE457 to indicate utilization of glucose on MacConkey (Difco) plates. Strain RE457 carrying one of the complementing plasmids, pRE65, after growth overnight in LB (11) at 37°C showed greatly increased levels of glucokinase activity (12) compared with RE457 carrying pBluescript (16.2 ± 0.2 [n = 3] versus 0.5 ± 0.02 [n = 9] μmol/min/mg of protein). Inversion of the insert relative to the vector had no effect on complementation.

On the basis of the restriction map of pRE65 (Fig. 1), various fragments were subcloned in pBluescript SK (Stratagene). The *Eco*RI-*Bam*HI fragment (pRE94) and the *Sma*I-*Bam*HI fragment (pRE103) complemented the glucose deficiency in RE457 on MacConkey medium and restored glucokinase activity. Deletions from either end of subclone pRE94 made by using exonuclease III (7) further localized the area complementing the glucose utilization defect and glucokinase activity. Two mini-Tn*10*(Kan) insertions isolated by using λNK1316 (9) that abolished the ability to complement RE457 were located in this region (Fig. 1).

As expected for *ptsI* strains, growth on glucose minimal medium was not complemented by these plasmids; however,

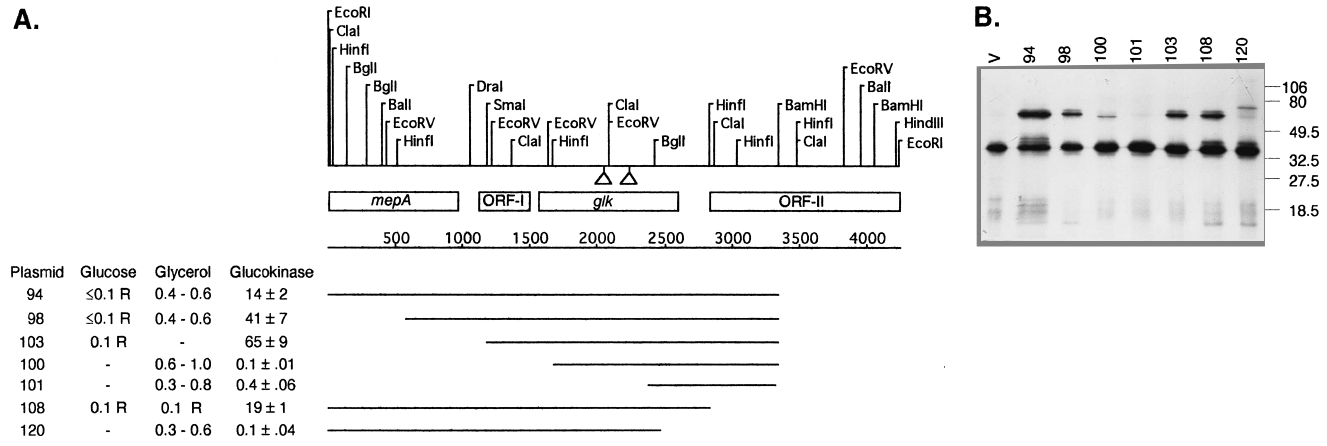

FIG. 1. Map of pRE65, subclones, and deletions and proteins labeled in maxicells containing these plasmids. (A) The top line is a restriction map of the *Brucella* DNA insert of pRE65 determined from the sequence. Sites for cleavage by *Bam*HI, *Eco*RI, *Hinf*1, *Bgl*I, and *Sma*I agree with digests. Other enzymes were not tried. The two triangles show the sites of mini-Tn*10*(Kan) insertions abolishing *glk*-complementing activity. Inferred ORFs are indicated. The DNA contained in subclones and deletions made from both ends of pRE94 by using exonuclease III are indicated below. The phenotype of strain RE457 containing these plasmids is shown on the left. For glucose and glycerol, the colony size in millimeters after 7 days of growth at 37°C on the indicated minimal medium is given. −, colonies barely visible with magnification (seen with RE457 with pBluescript alone on glucose); R, some additional colonies of a size comparable to those of XL1-blue carrying pBluescript (wild-type control; glucose, 1.2 to 2.0 mm; glycerol, 0.4 to 0.6 mm) that appeared during the incubation. For glucokinase, the average specific activity ± standard error in micromoles per minute per milligram of protein is indicated. (B) Sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis of maxicells carrying pBluescript (V) or other plasmids as indicated. Positions of the prestained protein standards and their apparent molecular masses in kilodaltons are indicated on the right.

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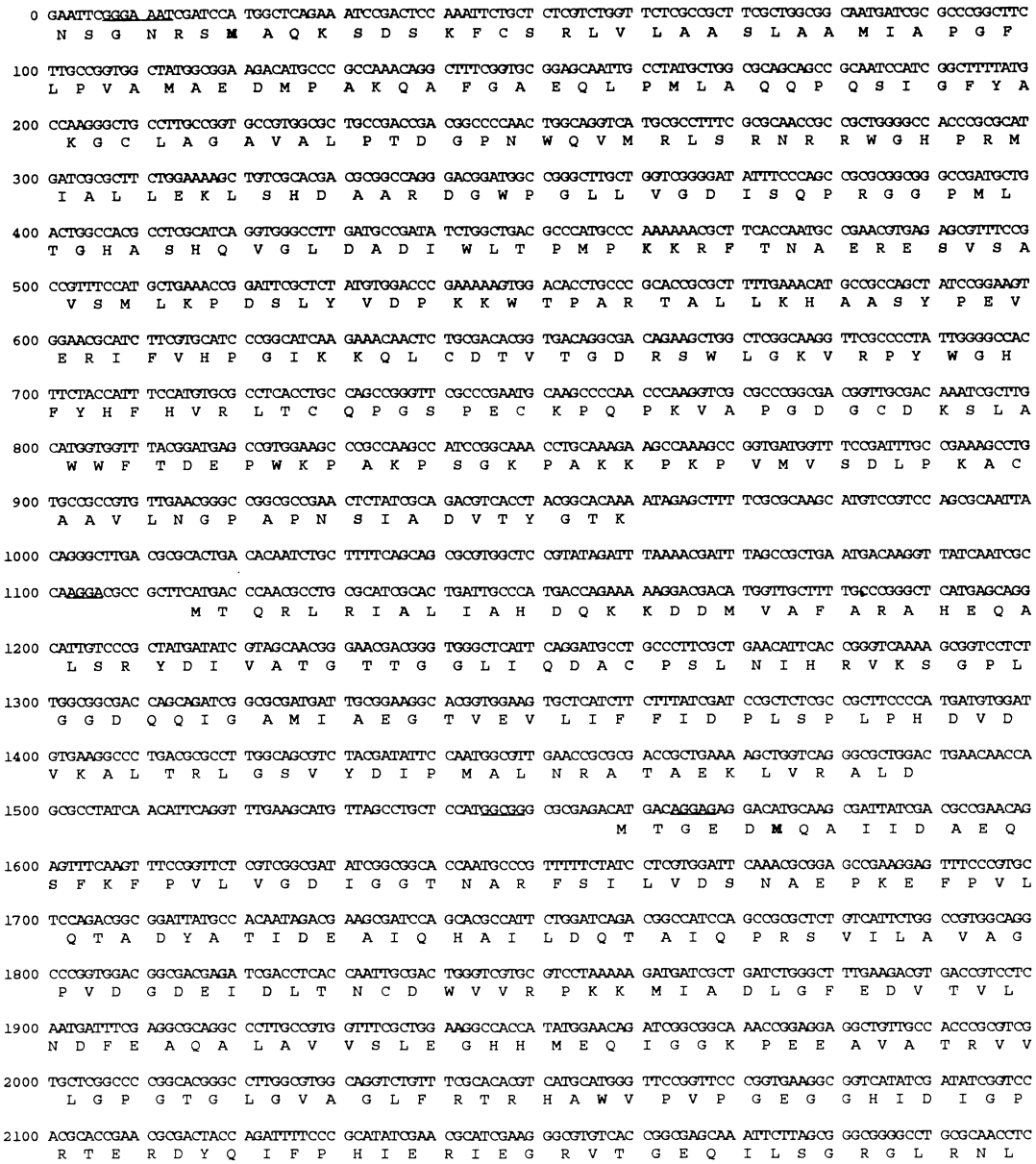

FIG. 2. Nucleotide and predicted amino acid sequences of the insert of plasmid pRE65. The upper line is the base sequence in the same orientation as in Fig. 1, with nucleotide numbers to the left. Underneath are the amino acid sequences in the one-letter code for the ORFs detected. All indicated ORFs are N terminal to C terminal. Possible alternative translation starts are indicated by boldface in the *mepA* and *glk* reading frames. Underlined sequences at the start of *mepA*, ORF-I, and *glk* are potential Shine-Dalgarno sequences. A possible termination sequence between *glk* and ORF-II is indicated by asterisks.

plasmids complementing glucokinase activity apparently allowed easy mutation to growth (Fig. 1). Because of the effects of *ptsI* mutations on growth on other carbon sources (13), growth on glycerol minimal medium was tested. RE457 grew on glycerol minimal medium unless it contained subclone pRE103. Growth was slower with pRE108, but faster-growing colonies appeared with high frequency.

Proteins coded by various plasmids were labeled by the maxicell procedure (15), using strain CSR603 (14). Three major proteins were seen for pRE94 (Fig. 1), one of which, also seen with pBluescript, had the molecular weight expected for β-lactamase. Another, fainter band appeared just above this band (33,000 Da) and was absent from all of the deletions from the *Eco*RI side of pRE94 (e.g., pRE98). The third band had an apparent molecular weight of 51,000 and was absent from deletions without glucokinase activity (pRE100, pRE101, and pRE120). Faint bands of <18,000 Da were seen, the smallest of which was missing in pRE100, pRE101, and pRE103.

The nucleotide sequence of the 4,241-bp insert in pRE65 was determined by a combination of manual and automated methods, using the deletions constructed as described above and oligonucleotide primers (Fig. 2). The ORFs detected by Ecoparse (10) could be assigned to glucokinase and the other proteins seen in maxicells, except for an incomplete one on the 3′ end. The 33,000-Da protein must be coded by the ORF at the 5′ end. It is similar to (60% similarity, 43% identity) the *E. coli* penicillin-insensitive murein endopeptidase (encoded by *mepA*) (8). Translation may start at position 19, which has a

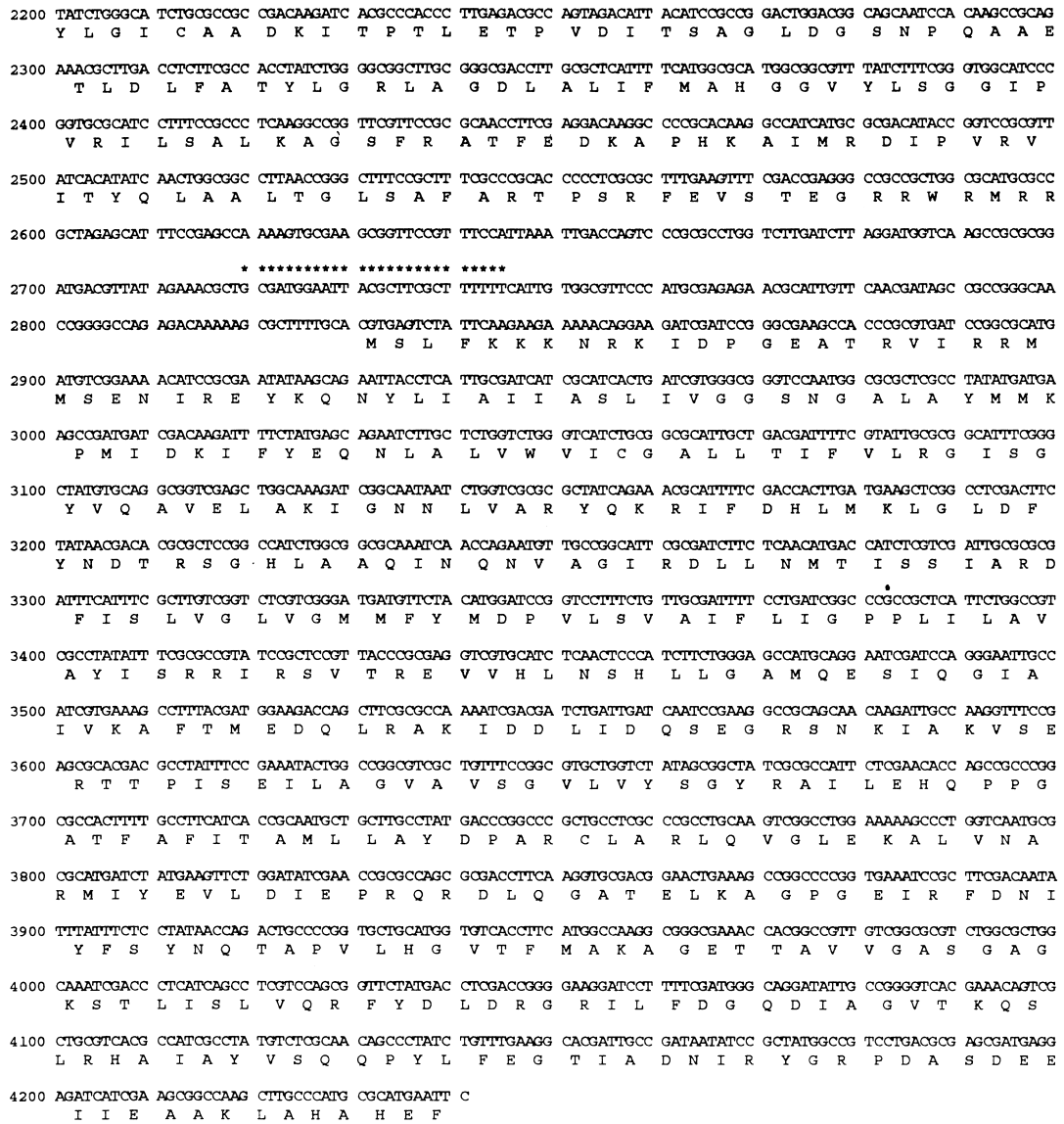

FIG. 2—*Continued.*

reasonable Shine-Dalgarno sequence, or it may start at the β-galactosidase start on the vector, which is in frame with the gene. This is followed by a small ORF in the right position to direct the synthesis of the <18,000 Da protein. Next is the *glk* gene, coding for the 51,000-Da protein, which is similar to (58% similarity, 35% identity) the *Zymomonas mobilis glk* gene (1) and the *E. coli glk* gene (16). There are two possible ATG codons following potential Shine-Dalgarno sequences, six codons apart (Fig. 2). Finally comes an incomplete ORF coding for a protein with similarities to a number of ATP-coupled transporters. It contains one of the two characteristic ATP-binding sites (17) and potential transmembrane segments in the N-terminal region, characteristic of the exporter family (5). No maxicell protein corresponds to this ORF. A stem-loop structure followed by a series of thymines is found starting at position 2720, following the *glk* ORF, and may be a transcription terminator.

A gene that complements a glucokinase deficiency in *E. coli*, both in vivo and in vitro, has been isolated from *B. abortus*. This gene directs the synthesis in maxicells of a protein with a molecular weight of 51,000 and a pI near neutrality (data not shown). The size of the gene, as determined by the ORF, is 1,044 bp, and it predicts a protein with a molecular weight of 37,558 and a pI of 4.93. It is not clear why there should be such a large discrepancy between the sizes and pIs of the predicted and observed proteins. Though no transcription start signals were found, this gene is probably transcribed from its own promoter, because inversion of the insert had no effect on complementation. The genes for glucokinase from *Pseudomonas aeruginosa* (3) and *Z. mobilis* (1) are both found clustered with genes for glucose transport and metabolism, but the genes from *B. abortus* are apparently not clustered in the same way.

The region 5′ of the glucokinase ORF appears to affect growth of RE457 on minimal media with carbon sources other than glucose, and it apparently codes for a small protein. Its predicted size is 13,598 Da, with a pI of 6.05, consistent with the maxicell results. It has a stretch of hydrophobic amino acids and so may be a membrane protein. It is possible that it

prevents glucokinase from inhibiting growth because deletions from the 5′ end affected in both ORF-I and glucokinase (pRE100) do not affect growth of RE457 on glycerol. Deletions from the 3′ end that end near the glucokinase gene (pRE108) also affect growth on glycerol, but in a different way.

Nucleotide sequence accession number. This sequence reported has been deposited with GenBank and assigned accession number U21919.

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