Cloning, Nucleotide Sequence, and Expression of the *Brucella melitensis* suc*B* Gene Coding for an Immunogenic Dihydrolipoamide Succinyltransferase Homologous Protein

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The *Brucella melitensis* suc*B* gene encoding the dihydrolipoamide succinyltransferase (E2o) enzyme (previously identified as an immunogenic protein in infected sheep) was cloned and sequenced. The amino acid sequence predicted from the cloned gene revealed 88.8 and 51.2% identity to the dihydrolipoamide succinyltransferase SucB protein from *Brucella abortus* and *Escherichia coli*, respectively. Sera from naturally infected sheep showed antibody reactivity against the recombinant SucB protein.

*Brucella* spp. are gram-negative, facultative intracellular bacterial pathogens that cause brucellosis, an infectious disease affecting animals and humans. *Brucella melitensis* is the most important species involved in ovine and caprine brucellosis, which is characterized by abortion, low production, and infertility in infected animals. *B. melitensis* is also the most pathogenic species for humans.

One of the principal aims in brucellosis research is the identification of *Brucella* antigens eliciting humoral and/or cell-mediated responses, which might be of interest for the development of diagnostic tests or subcellular vaccines that avoid the drawbacks of those currently used. *B. melitensis* Rev1, a live attenuated vaccine strain that is currently used in sheep and goats, has been successful in disease eradication and control programs in some countries (1). However, there have been significant problems associated with its use. The most important among them are the residual virulence of Rev1 for humans and the development of agglutinating antibodies in animals vaccinated as adults which are indistinguishable from those elicited by natural infection (8). The construction of new brucellosis vaccines and associated diagnostic tests lacking these undesirable properties would be of great interest to veterinary medicine.

A number of immunogenic proteins have been previously identified by immunoblotting, such as the BP26 protein, and are currently being considered for the development of new diagnostic tests for ovine brucellosis (3, 6, 7, 10). Recently, two-dimensional electrophoresis, immunoblotting, and N-terminal microsequencing have considerably facilitated the identification of immunogenic proteins in ovine brucellosis (15, 16). Among the proteins identified by these methods, one with an apparent molecular mass of 45 kDa was recognized by sera from *Brucella*-infected sheep, and its N-terminal sequence showed homology to a dihydrolipoamide succinyltransferase (SucB) described in many bacteria (2, 5, 9, 13, 19). A monoclonal antibody (MAb) was raised against this protein (16) to allow easy screening of genomic libraries to clone the corresponding gene. The present report describes the cloning and the nucleotide sequence of the gene termed *sucB* encoding dihydrolipoamide succinyltransferase (E2o), an enzyme of the α-ketoglutarate dehydrogenase complex, and its expression in *Escherichia coli*.

**Specificity of the anti-SucB MAb.** The MAb raised against *Brucella* SucB did not cross-react with *E. coli* and other bacteria closely genetically related to *Brucella*, such as *Ochrobactrum anthropi*, *Phyllobacterium rubiacearum*, *Rhizobium leguminosarum*, and *Agrobacterium tumefaciens* (20; data not shown). Thus, the MAb appeared to be specific for *Brucella* and therefore particularly useful for screening genomic libraries constructed in *E. coli*.

**Cloning of the *B. melitensis* suc*B* gene and its expression in *E. coli*.** A *B. melitensis* 16M genomic library was constructed in lambdaGEM-12 XhoI half-site arms (Promega, Madison, Wis.) by following the instructions of the manufacturer. Briefly, *B. melitensis* 16M DNA, extracted and purified as described previously (17), was partially digested for 30 min at 37°C with Sau3 AI (Promega) at 0.014 U/μg of DNA, the enzyme concentration giving the highest percentage of fragments ranging from 15 to 23 kb. DNA fragments were partially filled in with dGTP and dATP, by using Klenow DNA polymerase (Promega), ligated with T4 DNA ligase (Promega) to lambda-GEM-12 digested with XhoI, and partially filled in with dTTP and dCTP. Recombinant phage DNA was packaged in vitro with the Packagene System (Promega), and the library was titrated by determination of the number of PFU that appeared after infection of *E. coli* KW251 cells (Promega). Recombinant phages were transferred to nitrocellulose filters, and phages expressing the *sucB* gene were identified by reactivity with the anti-SucB MAb. DNA of a positive phage was extracted from culture supernatants of *E. coli* KW251 cells infected with the phage and cultured until lysis was observed. Phage DNA was then cut with NotI, BamHI, EcoRI, or SacI, and restriction fragments...
were ligated into pGEM-5Zf (Promega) cut with NotI or into pGEM-7Zf+ (Promega) cut with BamHI, EcoRI, or SacI, respectively. Competent E. coli JM109 cells (Promega) were transformed with recombinant plasmid DNA as described previously, and bacteria were spread on Luria-Bertani (LB) broth-ampicillin (50 μg/ml) plates containing isopropyl-1-thio-
β-d-galactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside (X-Gal). D. E. coli JM109 colonies bearing recombinant plasmids were transferred to nitrocel-
lulose, lysed with 10% sodium dodecyl sulfate (SDS), and screened with the anti-SucB MAb by a colony blotting tech-
nique. One positive colony was found bearing a plasmid with a NotI fragment from this insert that was overproduced in
E. coli (Promega) cut with BamHI, resulting in plasmid pMZ4503. Expression of SucB as demonstrated by
Coomassie blue staining (data not shown). Control
E. coli JM109 cells carrying the control vector pGEM7Zf showed no reaction at all
E. coli with sera from naturally infected sheep (lanes 4 to 10), all ad-
sorbed with E. coli JM109 cells carrying the control vector pGEM7Zf+. Lane 2, immunoblotting after SDS-PAGE of E. coli JM109 carrying the control vector pGEM7Zf+ with anti-SucB MAb.

![Image of SucB](image)

**FIG. 1.** Immunoblotting after SDS-PAGE of E. coli (pMZ4503) cells expressing the sucB gene of B. melitensis 16M with anti-SucB MAb (lane 1), with sera from brucellosis-negative sheep (lanes 3 and 4), and with sera from naturally infected sheep (lanes 4 to 10), all ad-
sorbed with E. coli JM109 cells carrying the control vector pGEM7Zf+.

Lane 2, immunoblotting after SDS-PAGE of E. coli JM109 carrying the control vector pGEM7Zf+ with anti-SucB MAb.

ORFs encoded proteins homologous to SucA, SucB, and LpdA previously identified in E. coli (12). The first partial ORF contained 713 codons with 80.7% amino acid sequence identity to the sucA gene product of B. abortus S19 (GenBank accession no. AF07932) and 41.7% identity to the sucA-en-
coded E1o protein of E. coli (GenBank accession no. X00661). There was a complete ORF immediately downstream of the partial sucA gene that contains 409 codons with 88% amino acid sequence identity to the sucB gene product of B. abortus
strain S19 and 51.2% identity to the sucB-encoded E2o protein of E. coli (GenBank accession no. X00664) (13). The N-termi-
nal amino acid sequence of the protein deduced from the nucleotide sequence matched the first 14 amino acids of the protein identified by two-dimensional electrophoresis and N-termi
nal microsequencing (16). The differences between the B. abortus S19 and B. melitensis 16M sucB genes consisted of the following: single nucleotide substitutions; one, two, or three nucleotide deletions; one nucleotide addition; and, most importantly, a 42-bp deletion in B. abortus S19 sucB (Fig. 2). These nucleotide substitutions and additions in the B. abortus S19 sucB gene relative to the B. melitensis 16M sucB gene altered the predicted amino acid sequence for many amino acids. The 42-bp deletion (coding for 14 amino acids) could possibly have a greater importance and perhaps cause an antigenic shift, as previously described for other proteins (4).

The nucleotide sequence of the sucB downstream region revealed a third ORF, ORF1, which is 639 nucleotides long, starts 61 nucleotides downstream of sucB, and codes for a
protein with 29% amino acid identity to a putative amino acid efflux-like protein of E. coli (GenBank accession no. P27846) (21). The last ORF is a sequence homologous to the lpdA gene coding for a dihydrolipoamide dehydrogenase and starts 2,762 nucleotides downstream of sucB.

The genetic organization of the 6.5-kbp suc region of the B. melitensis chromosome is summarized in Fig. 3. This organ-
ization appears to be similar to that of Rhodobacter capsulatus and Rhizobium leguminosarum, in which the lpd gene is also located downstream of the sucB gene (5, 19). This organization is different from that of E. coli, in which sucC and sucB are located between the sucB and lpdA genes (12, 14). The close phylogenetic relationship between Brucella, Rhodobacter capsulatus, and Rhizobium leguminosarum could explain this sim-
ilar organization.

**Serum activities.** The antibody responses of naturally infect-
ed sheep against recombinant SucB protein were analyzed. pMZ4503-transformed E. coli cells were cultured in LB broth, and total cell protein extracts were subjected to SDS-polyacryl-
amide gel electrophoresis (PAGE) followed by Western blot-
ting as described previously (22). All sera have been adsorbed with E. coli JM109 carrying plasmid pGEM7Zf+ to remove nonspecific antibodies reacting with proteins from E. coli. Figure 1 shows the positive reaction with infected sheep sera of the recombinant SucB protein showing an apparent molecular mass of 45 kDa (lanes 5 to 10) corresponding to the SucB protein migration identified by using a MAb against the recombinant protein (lane 1). Antibody responses against the recombinant SucB protein were detected in all naturally in-
fected sheep and not in Brucella-free sheep (lanes 3 and 4).

In other intracellular pathogens, such as Coxiella burnetii, SucB has also been found as immunogenic protein reacting...
FIG. 2. Alignment of the B. melitensis (B. mel) 16M sucB and B. abortus (B. ab) S19 sucB nucleotide sequences. Identical nucleotides are indicated by stars, and gaps are indicated by hyphens.
with sera from Q fever patients (9). Possibly, recombinant SucB could be used in association with other immunogenic proteins such as BP26 for the serodiagnosis of ovine brucellosis (3, 7, 10, 18).

**Nucleotide sequence accession number.** The nucleotide sequence of the *B. melitensis* 16M sucB gene and flanking regions has been deposited in GenBank under accession no. AF235020.

**REFERENCES**


