INFECTION AND IMMUNITY, June 1992, p. 2361–2367
0019-9567/92/062361-07$02.00/0

Vol. 60, No. 6

Cloning and Nucleotide Sequence of the Gene Encoding the 136-Kilodalton Surface Protein (Muramidase-Released Protein) of *Streptococcus suis* Type 2

HILDE E. SMITH,[1]* URI VECHT,[2] ARNO L. J. GIELKENS,[1] AND MARI A. SMITS[1]

Departments of Molecular Biology[1] and Bacteriology,[2] DLO-Central Veterinary Institute, P.O. Box 65, 8200 AB Lelystad, The Netherlands

Received 11 November 1991/Accepted 16 March 1992

We cloned and sequenced the gene encoding the muramidase-released protein (MRP) of a pathogenic *Streptococcus suis* type 2 strain to determine whether its amino acid sequence resembles that of proteins with known functions and to determine its function in virulence. The complete nucleotide sequence composing the gene and the regions flanking it was determined. The deduced amino acid sequence revealed the presence of a signal peptide at the N terminus and a cell envelope anchor at the C terminus, both of which resembled similar regions in several other surface proteins from gram-positive bacteria. The processed form of MRP has a length of 1,209 amino acids and a calculated molecular weight of 131,094. A highly repetitive region preceded the envelope anchor. The repeated units were preceded by a proline-rich stretch of amino acids (26 of 86). No overall homologies were observed between the amino acid sequence of MRP and protein sequences in the EMBL data bank. A particular region within the amino acid sequence, however, showed some similarity with the fibronectin-binding protein of *Staphylococcus aureus*. Binding of MRP to human fibronectin, however, could not be confirmed.

Streptococcus suis type 2 infections are a common cause of septicemia, arthritis, meningitis, and sudden death in young pigs (3, 29) and meningitis in humans (2). During the last few years, *S. suis* type 2 infections have become a major problem in almost all countries with an intensive pig industry. Attempts to control the disease are hampered by the lack of effective vaccines and suitable diagnostics.

In recent studies, we identified three phenotypes of *S. suis* type 2 strains, each of which caused specific pathological and clinical signs (31, 32). Some strains produced both a 136-kDa cell envelope-associated protein, muramidase-released protein (MRP), and a 110-kDa extracellular protein factor (EF). Strains of this phenotype (MRP^+ EF^+) were frequently isolated from organs of diseased pigs and were associated with severe clinical signs of disease after experimental infection. We also identified strains that did not produce MRP and EF (MRP^- EF^-). Strains of this phenotype were frequently isolated from tonsils of healthy pigs and were nonpathogenic after experimental infection. Finally, we detected strains that produced MRP but not the 110-kDa EF. These strains produce EF-related (EF*) proteins of various sizes (>150 kDa). These EF-related proteins are encoded by *ef* genes that contain insertions of various sizes (25). Most of these MRP^+ EF* strains were isolated from human patients and did not cause disease in young pigs after experimental infection. Both MRP and EF are recognized by convalescent antisera from pigs infected with sublethal doses of MRP^+ EF^+ strains.

We cloned and sequenced the gene encoding MRP of a pathogenic *S. suis* type 2 strain to determine whether its amino acid sequence resembles that of proteins with known functions and to determine its function in virulence. We found little overall homology to amino acid sequences of known proteins. A subsequence of MRP, however, showed some similarity with the fibronectin-binding protein of *Staphylococcus aureus*. Binding of MRP to human fibronectin, however, could not be confirmed.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *Escherichia coli* JM101 [*supE thi* Δ (*lac-proAB*) (F′ *traD36 lacI*^{q}ZΔM15)] (16) was used as host for recombinant plasmid DNA. *E. coli* LE392 [F^- *hsdR574* (r_K^- m_K^+) *supE44 supF58 lacY1* or Δ(*lac1ZY*)*6 galK2 galT22 melB1 trpR55*] (18) was used as a host for recombinant bacteriophage. The pathogenic MRP^+ EF^+ strain D282 of *S. suis* type 2 (28) was used for isolating chromosomal DNA. *E. coli* strains were grown on L broth (17). Solid LB medium contained 1.5% agar. Ampicillin was added as needed to a final concentration of 50 μg/ml. *S. suis* strains were grown in Todd-Hewitt broth (Oxoid, Ltd., London, England).

DNA techniques. Routine DNA manipulations were performed as described by Maniatis et al. (15).

Southern blotting and hybridization. DNA was transferred to GeneScreen Plus membranes (New England Nuclear Corp., Dreieich, Germany) as described by Maniatis et al. (15). DNA probes were labeled with [α-^{32}P]dCTP (3,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) by use of a random primed labeling kit (Boehringer GmbH, Mannheim, Germany). The blots were hybridized with DNA probes as recommended by the supplier of the GeneScreen Plus membranes. After hybridization, the membranes were washed twice with a solution of 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M Na citrate, pH 7.0) for 5 min at room temperature and twice with a solution of 0.1× SSC plus 0.5% sodium dodecyl sulfate for 30 min at 65°C.

Construction and immunological screening of DNA library. A DNA library of *S. suis* type 2 strain D282 was constructed in λGEM11, according to the methods recommended by the manufacturer of the cloning vector (Promega, Madison,

* Corresponding author.

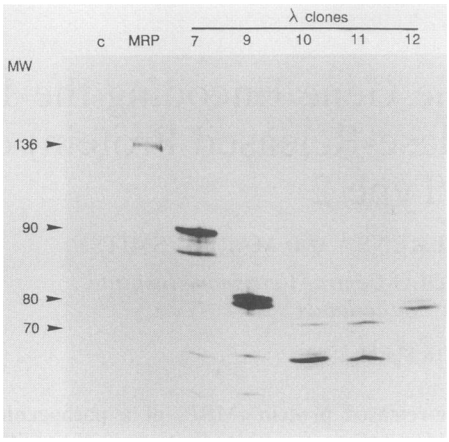

FIG. 1. Western blot analysis of proteins eluted from plaques of recombinant bacteriophages screened with anti-MRP MAbs. Ten plaques of each recombinant bacteriophage were collected from an agar plate. The proteins in the plaques were eluted with 200 μl of Laemmli buffer for 24 h at 4°C. Samples of 5 μl were applied to the gel. Lanes: c, proteins extracted from the cell wall of an MRP-negative *S. suis* strain; MRP, proteins extracted from the cell wall of an MRP-positive strain, D282; 7, proteins extracted from clone 7; 9, clone 9; 10, clone 10; 11, clone 11; 12, clone 12; MW (10^3) is indicated.

Wis.). Recombinant phages were plated on *E. coli* LE392 and incubated for 16 h at 37°C. Nitrocellulose filters (Schleicher & Schuell, Inc., Dassel, Germany) were placed on the plaques, and the plates were further incubated for 2 h at 37°C. Recombinants that produce MRP were visualized by use of anti-MRP monoclonal antibodies (MAbs) (30). Bound antibodies were detected with anti-mouse serum conjugated with alkaline phosphatase (Zymed Laboratories, Inc., San Francisco, Calif.) as described by Maniatis et al. (15). Selected clones were purified by several rounds of single-plaque isolation and immunological screening.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot (immunoblot) analysis. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in which 4% stacking and 6% separation gels were used (14). The separated proteins were transferred to nitrocellulose by use of a Semi-Dry transfer cell (Bio-Rad Laboratories, Richmond, Calif.). Specific proteins were visualized by use of anti-MRP polyclonal antibodies (31) or anti-MRP MAbs (30) and anti-rabbit or anti-mouse serum conjugated with alkaline phosphatase (Zymed Laboratories).

Binding to fibronectin. To detect binding of proteins to fibronectin, we incubated Western blots with human plasma fibronectin (Sigma Chemical Co., St. Louis, Mo.) (25 μg/ml) for 2 h at 37°C (1, 26). Fibronectin-binding proteins were visualized by use of rabbit antifibronectin immunoglobulins conjugated with peroxidase (DAKOPATTS, Glostrup, Denmark).

Nucleotide sequence analysis. DNA sequences were determined by the dideoxy-chain termination method (21). DNA and protein sequences were analyzed by two software systems: PCGENE (Intelligenetics Corp., Mountain View, Calif.) and Wisconsin GCG (University of Wisconsin). The *mrp* sequence has been assigned GenBank accession number X64450.

RESULTS

Construction and screening of the library. Chromosomal DNA isolated from strain D282 of *S. suis* type 2 was partially digested with the restriction enzyme *Sau*3A. A DNA library was then constructed in the bacteriophage λGEM11 replacement vector. We obtained approximately 5×10^5 recombinants per μg of DNA. An MAb directed against MRP was used to screen 1,400 recombinant plaques for the presence of antigenic determinants of MRP. Five recombinant plaques were reactive with the MAb.

Characterization of immunoreactive recombinants. To study the expression of MRP by the five selected recombinant bacteriophages, we used Western blotting to analyze the proteins eluted from the plaques. All five recombinants encoded proteins that were recognized by MAbs directed against MRP (Fig. 1). These proteins, however, had lower molecular weights (MW) than the MRP. Two clones encoded a protein of approximately 70 kDa (Fig. 1, clones 10 and 11), two clones encoded a protein of approximately 80 kDa (Fig. 1, clones 9 and 12), and one clone encoded a protein of approximately 90 kDa (Fig. 1, clone 7). Therefore, we concluded that the five recombinants did not contain the complete genetic information for MRP.

To confirm this conclusion, we used restriction enzyme analysis to compare the DNA inserts of the five recombinants (data not shown). All clones shared a DNA region of about 17 kb (Fig. 2A). The DNA inserts differed, however, at the 3′ and 5′ ends. The variation in length at the 3′ ends of

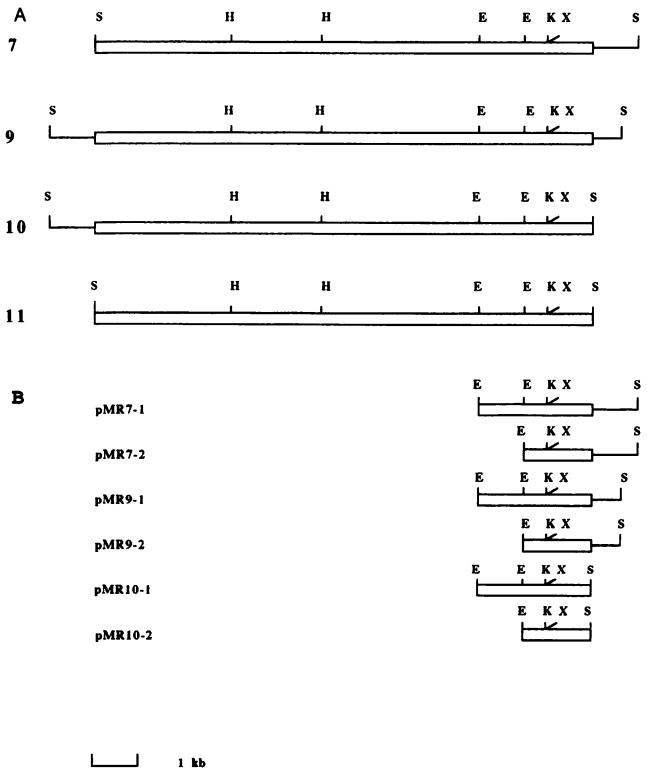

FIG. 2. (A) Restriction maps of the DNA inserts of putative MRP-positive recombinant bacteriophages. The bar indicates the DNA region common to all these clones. Restriction sites: E, *Eco*RI; H, *Hin*dIII; X, *Xba*I; K, *Kpn*I; and S, *Sac*I. The *Sac*I sites are derived from the lambda vector. (B) Parts of the DNA inserts subcloned in the plasmid vector pKUN19 (13).

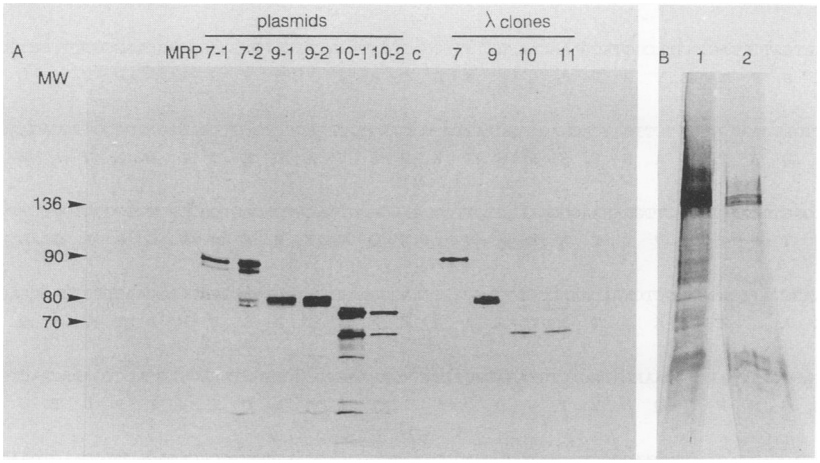

FIG. 3. (A) Western blot analysis of proteins encoded by recombinant plasmids and recombinant bacteriophages screened with anti-MRP MAbs. Overnight cultures (1 ml) of *E. coli* cells containing the various recombinant plasmids were centrifuged for 5 min in an Eppendorf centrifuge. The pellets were resuspended in 1 ml of Laemmli buffer and incubated for 5 min at 100°C. Samples of 5 μl were applied to the gel. Ten plaques of each recombinant bacteriophage were collected from an agar plate. The proteins in the plaques were eluted with 200 μl of Laemmli buffer for 24 h at 4°C. Samples of 5 μl were applied to the gel. Lanes: MRP, proteins extracted from the cell wall of the MRP-positive strain D282; 7-1, pMR7-1; 7-2, pMR7-2; 9-1, pMR9-1; 9-2, pMR9-2; 10-1, pMR10-1; 10-2, pMR10-2; c, λGEM11 with control insert; 7, lambda clone 7; 9, lambda clone 9; 10, lambda clone 10; 11, lambda clone 11. (B) Western blot analysis of proteins encoded by pMR11 screened with anti-MRP polyclonal antibodies. Lane 1, proteins extracted from the cell wall of the MRP-positive strain D282; lane 2, pMR11-encoded proteins. MW (10^3) is indicated.

the inserts correlated well with the variation in MW of the truncated MRPs (cf. Fig. 1 and Fig. 2A). This correlation indicates that MRP-encoding sequences were located at the 3′ end of the DNA inserts. To confirm this, we subcloned fragments derived from the 3′ ends of the DNA inserts of clones 7, 9, and 10 (Fig. 2B) into plasmid vector pKUN19 (13). These constructs encoded truncated MRPs that were indistinguishable from the truncated MRPs encoded by the recombinant phages (Fig. 3A). Deletion of the 0.7-kb *Eco*RI-*Kpn*I fragment from these constructs stopped the expression of the truncated MRPs (data not shown). This suggests that the expression of *mrp* is initiated from the 0.7-kb *Eco*RI-*Kpn*I fragment.

Cloning of the complete *mrp* gene. To obtain the complete gene for MRP, we hybridized the ^{32}P-labeled *Kpn*I-*Sac*I fragment of pMR7-2 (Fig. 2B) with *Eco*RI- or *Kpn*I-digested chromosomal DNA of strain D282 of *S. suis* type 2. An *Eco*RI fragment of 7 kb and a *Kpn*I fragment of 7 kb hybridized with the probe (data not shown). Because of its size, the *Eco*RI fragment was expected to contain the complete *mrp* gene, and because the expression of *mrp* is initiated from the 0.7-kb *Eco*RI-*Kpn*I fragment, the *Kpn*I fragment was expected to contain only the 3′ end of the gene. We isolated fragments ranging from 6 to 8 kb from *Eco*RI- and *Kpn*I-digested chromosomal DNA, ligated those fragments into the *Eco*RI or *Kpn*I site of pKUN19, and transformed the ligation mixtures into *E. coli* JM101. Thirteen of 50 selected recombinant clones obtained with the *Kpn*I fragments hybridized with an MRP (DNA) probe. All these recombinant clones contained a plasmid (pMR-C) with a 7-kb *Kpn*I insert. In contrast, of 2,500 selected recombinant clones obtained with *Eco*RI fragments, none hybridized with the probe. Since the 7-kb *Eco*RI fragment is expected to contain the complete *mrp* gene, this finding suggests that expression of MRP is toxic in *E. coli*. Nevertheless, we were able to construct a plasmid (pMR11) with the entire *mrp* gene. To do this, we combined the 5′ end of the *mrp* gene (isolated from pMR7-2) and the 3′ end of the gene (isolated from pMR-C). The copy number of this plasmid appeared to be strongly reduced, about 20 times, compared with the copy number of pKUN19. The low copy number presumably reduced the toxic effects of high-level expression of MRP in *E. coli* to tolerable levels. The proteins produced by *E. coli* cells containing pMR11 were analyzed by Western blotting. As expected, these cells produced a 136-kDa protein that comigrated with MRP and that was recognized by polyclonal antibodies directed against MRP (Fig. 3B).

Nucleotide sequence of the *mrp* gene. We determined the nucleotide sequence of a 4.6-kb *Eco*RI-*Hin*dIII fragment containing the entire *mrp* gene and the regions flanking it. Analysis of the sequence (Fig. 4) revealed an open reading frame of 3,768 nucleotides coding for a polypeptide of 1,256 amino acids (with a calculated MW of 135,794). The putative ATG start codon is preceded by a sequence that is similar to ribosome binding sites in several types of gram-positive bacteria (11). Moreover, the nucleotide sequence upstream of *mrp* resembles the −35 and −10 consensus sequences of promoters commonly found in gram-positive bacteria. Downstream of the *mrp* gene, a region showing extended dyad symmetry can be detected. The potential hairpin structure in the corresponding mRNA has a 12-bp stem separated by a 6-bp loop ($\Delta G = -15.9$ kcal/mol, calculated according to the rules of Tinoco et al. [27]). This potential transcription terminator lacks the characteristic features of a rho-independent terminator (20).

Amino acid sequence of MRP. MRP is a cell envelope-associated protein and must be translocated across the cytoplasmic membrane. The protein must therefore contain a signal peptide. Indeed, the first 47 amino acids of the MRP have the characteristics of a typical signal peptide. An N-terminal part that contains seven positively charged residues is followed by a hydrophobic core of 21 amino acids and a putative signal peptidase cleavage site (33) (vertical arrow in Fig. 4). Cleavage of the signal peptide would result in a mature protein with an MW of 131,094, which is close to the MW (136,000) of MRP, estimated from sodium dodecyl

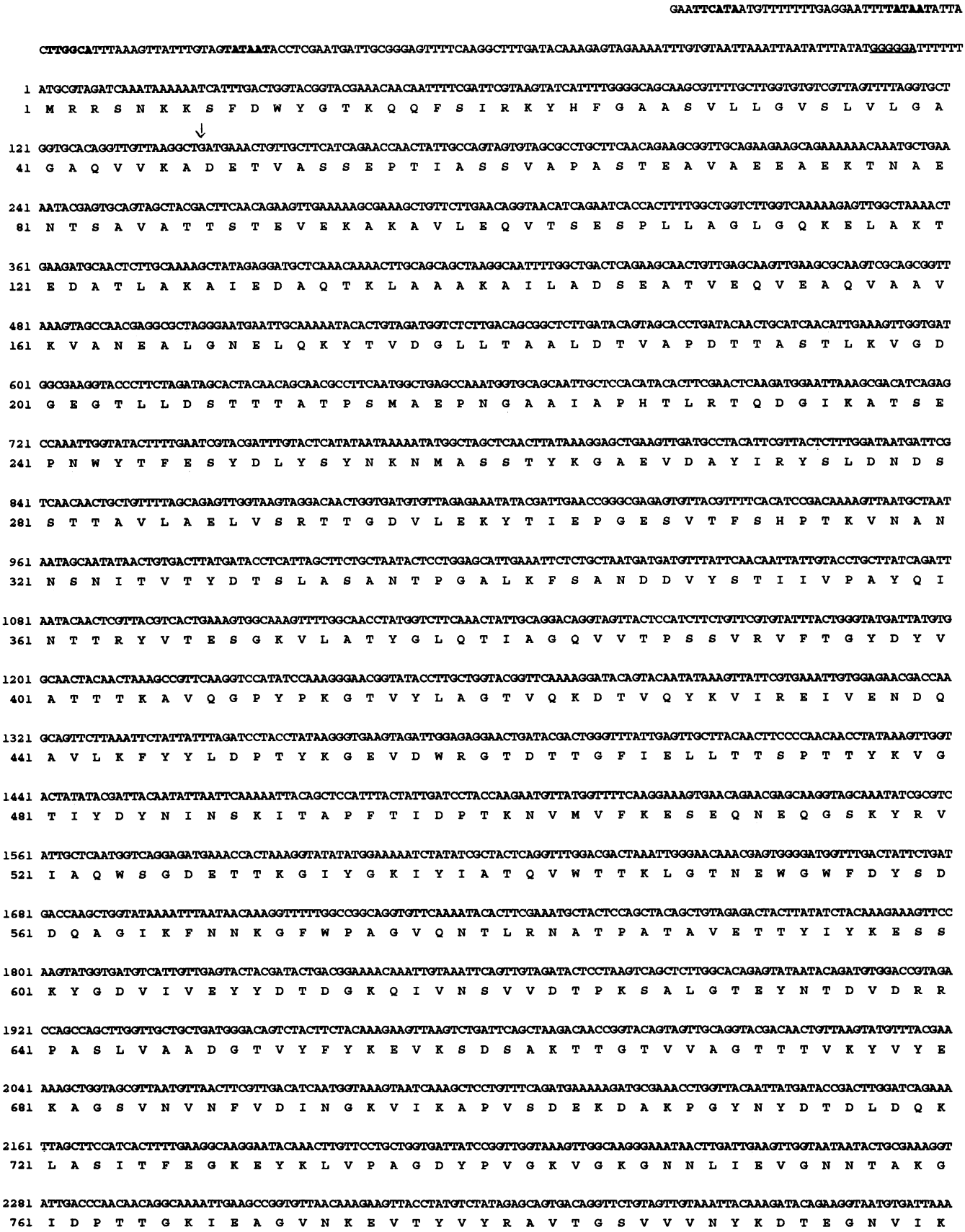

FIG. 4. Nucleotide sequence of the 4-kb *Eco*RI-*Sac*I fragment containing the *mrp* gene of *S. suis* type 2 and the deduced amino acid sequence of MRP. The putative ribosome binding site is underlined. Nucleotides in boldface type indicate the −35 and −10 regions of the putative promoter sequences. The horizontal arrowheads indicate the putative termination signal. The vertical arrow indicates the potential signal peptidase cleavage site. P indicates the beginning of the proline-rich region. R1, R2, and R3 indicate the beginning of the repeat units, and E indicates the beginning of the anchor domain.

```
2401 GATCCAGAAACGGATGTGTCTGATGCACCGGTTGGAGATGCTTATACTACAACTGACAAGAAACCAAACGAAATCATCACAAAAGATGGATCACGCTATGTTCTTGTTCCATCTAAGACA
 801 D  P  E  T  D  V  S  D  A  P  V  G  D  A  Y  T  T  T  D  K  K  P  N  E  I  I  T  K  D  G  S  R  Y  V  L  V  P  S  K  T
                                                                                         ----> P
2521 GATGGTGAGGAAAATGGTAAAGTTATCGAAGGAACAATCACAGTAACTTATGTTTACCAGAAAGTTGCAAACTGGATTCCAGAGATTCCAAATGTACCAGAAACAGACCGTCCAAAAGTA
 841 D  G  E  E  N  G  K  V  I  E  G  T  I  T  V  T  Y  V  Y  Q  K  V  A  N  W  I  P  E  I  P  N  V  P  E  T  D  R  P  K  V

2641 CCTTACCCATTTGACCCAACAGAGCCAGACGAGCCAATCGATCCAACGACACCAGGAACAAATGGCGAGGTTCCAAATATTCCTTACGTTCCAGGATATACACCGGTTGATCCTAAGGAT
 881 P  Y  P  F  D  P  T  E  P  D  E  P  I  D  P  T  T  P  G  T  N  G  E  V  P  N  I  P  Y  V  P  G  Y  T  P  V  D  P  K  D
                                                                                                             ----> R1
2761 AACACGCCGTTGAAACCAATTGATCCAAATGATCCAGGTAAGGGTTATGTACCACCAACACCAGAAAATCCAGGTGTTGATACACCAATTCCTTATGTTCCAGTTAAAAAAGTCGTAACT
 921 N  T  P  L  K  P  I  D  P  N  D  P  G  K  G  Y  V  P  P  T  P  E  N  P  G  V  D  T  P  I  P  Y  V  P  V  K  K  V  V  T

2881 AACCACGTTGATGAAGAGGGTAACCCTATTGCACCGCAAGAAGAGGGAACAAAACCAAACAAATCAATCCCAGGTTACGAGTTCACAGGTAAAACTGTTACTGACGAAGATGGCAACACA
 961 N  H  V  D  E  E  G  N  P  I  A  P  Q  E  E  G  T  K  P  N  K  S  I  P  G  Y  E  F  T  G  K  T  V  T  D  E  D  G  N  T

3001 ACTCACATCTACAAGAAAACACCAGAAGTTAAGAATGGTACAGTTGTTGTTAACTATGTAACAGAAGATGGCACAGTTATCAAGGAACCTGTAACAGATACACCAACTTCTCCAGAAGGC
1001 T  H  I  Y  K  K  T  P  E  V  K  N  G  T  V  V  V  N  Y  V  T  E  D  G  T  V  I  K  E  P  V  T  D  T  P  T  S  P  E  G

3121 ACACCATACGACACTACAGACAACAAACCTAAGACAATCACTTTCAAAGGTGAAGAGTATGAATTGGTTCGTGTTGACGGTACAGAAAACGGTAAAGTTGTAGAAGGTGAAACAGTTGTG
1041 T  P  Y  D  T  T  D  N  K  P  K  T  I  T  F  K  G  E  E  Y  E  L  V  R  V  D  G  T  E  N  G  K  V  V  E  G  E  T  V  V
                        ----> R2
3241 ACTTACGTTTACCGTAAAGTCGAAACACCTGCTAAGAAAGTTGTAACTAACCACGTTGATGAAGAGGGTAACCCTGTTGCGCCGCAAGAAGAGGGAACAAAACCAAACAAATCAATCCCA
1081 T  Y  V  Y  R  K  V  E  T  P  A  K  K  V  V  T  N  H  V  D  E  E  G  N  P  V  A  P  Q  E  E  G  T  K  P  N  K  S  I  P
                                                                           ----> R3
3361 GGTTACGAATTTACAGGTAAAACTGTTACTGACGAAGATGGCAACACAACTCACATCTACAAGAAAACACCTGCTAAGAAAGTTGTGACTAACCACGTTGATGAAGAAGGTAACCCTATT
1121 G  Y  E  F  T  G  K  T  V  T  D  E  D  G  N  T  T  H  I  Y  K  K  T  P  A  K  K  V  V  T  N  H  V  D  E  E  G  N  P  I

3481 GCTCCACAAGAGGATGGGACAACACCAAAACGTCAAATTTCAGGTTACGAGTATGTGCGTACTGTAGTTGATGAAGAAGGTAACACGACACATATTTATCGCAAACTTTCTAATAAACCA
1161 A  P  Q  E  D  G  T  T  P  K  R  Q  I  S  G  Y  E  Y  V  R  T  V  V  D  E  E  G  N  T  T  H  I  Y  R  K  L  S  N  K  P
                                                                           ----> E
3601 ACAACACCTGAGAAGGAAACTCCTGCAAAACCTCAAGCAGGTAAAACCGCTTCAGGTAAAGCTCAATTGCCAAATACTGGTGAGGCTTCATCTGTGGCAGGTGCGCTTGGTACAGCAATG
1201 T  T  P  E  K  E  T  P  A  K  P  Q  A  G  K  T  A  S  G  K  A  Q  L  P  N  T  G  E  A  S  S  V  A  G  A  L  G  T  A  M

3721 CTTGTCGCAACACTTGCGTTTGCAAGAAAACGTCGTCGTAACGAAGATTAGTCAAAATTCTTTATACAGACTTTATTCCCCCACATAGAAAGTATAAGAATTGTACGTAACATGCAGGAT
1241 L  V  A  T  L  A  F  A  R  K  R  R  R  N  E  D  -

     TGCCTTTCCGAAAAAAATGAGGCTGGGCAAAAAGTCCAGAGTTACATCTTAGAGTTCGCTCCATTTCCAACCTCCAACAGTCACTACTCTGACTGTTGGAGCTGTGTGGGGGTGGGAGACG
                                                                                 >>>>>>>>>>>>      <<<<<<<<<<<<
```

FIG. 4—*Continued.*

sulfate-polyacrylamide gels (31). A second hydrophobic region of 20 amino acids was identified at the C terminus of the protein. If this region is analogous to other envelope-associated proteins of gram-positive bacteria (4, 5, 7, 8, 10, 12, 22, 23, 34), it is probably a cell membrane anchor. A short, highly charged region and a region with the LP-X-TGE amino acid sequence, two regions that flank the presumed cell membrane anchor, are also highly conserved among surface proteins of gram-positive bacteria (Fig. 5). The amino acid sequence LP-X-TGE is putatively involved in cell wall binding.

Several other regions were identified in the MRP sequence. The mature form of MRP starts with a unique N-terminal sequence of 819 amino acids. This region is followed by a stretch of amino acids that is rich in proline residues: of 86 amino acids, 26 are proline residues. This region is followed by three repeated units of 54 amino acids. The first unit is separated from the second by 77 amino acids, but the second and third units are contiguous. The sequences of the first and second units are highly conserved, whereas that of the third varies. The third repeated unit is followed by the envelope anchor sequence. There was little homology between the MRP sequence and the protein sequences of the EMBL data library. One subsequence of MRP, amino acid residues 619 to 985, however, shared some similarity (17.2% identity in a 377-amino-acid sequence) with a sequence of the fibronectin-binding protein of *S. aureus* (23).

Binding to fibronectin. We tested whether MRP had the capacity to bind to human fibronectin. Therefore, Western blots containing proteins of cell lysates of *S. suis* type 2 strain D282, *E. coli* JM101 containing plasmid pMR11, and

MRP	LP	N	TG	E	----	-	ASSVAGALGTAML	V	ATLAFA	RKRRR	NED*
M6	LP	S	TG	E	TA-N	P	FFTAAALTVMATA	G	VAAVV-	KRK--	EEN*
A	LP	E	TG	E	--EN	P	LIGTTVFGGLSLA	G	AALLAG	RRR--	EL*
G	LP	S	TG	E	GS-N	P	FFTAAALAVMAGA	G	ALAVAS	KRK--	ED*
AP4	LP	S	TG	E	TA-N	P	FFTAAAATVMVSA	G	MLAL--	KRK--	EEN*
LP	LP	K	TG	E	TTER	P	AFGFLGVIVVSLM	G	VLGV--	KRK--	QREE*
WapA	LP	S	TG	E	-QAG	L	LLTTVGLVIVAVA	G	VYFY--	RTRR-	----*
T6	LP	S	TG	S	IGTY	L	FKAIGSAAMIGAI	G	IYIV--	RRK--	A*
Fn-BP	LP	E	TG	G	-EES	T	NKGMLFGGLFSIL	G	LALL--	RRNKK	NHKA*

FIG. 5. Homology between the amino acid sequences at the C terminus of MRP and several cell envelope-associated proteins of gram-positive bacteria. The amino acid sequence of *S. suis* MRP was compared with those of M6 protein of *Streptococcus pyogenes* (12), protein A of *S. aureus* (10), protein G of group G streptococci (4), AP4 of *S. pyogenes* (8), LP (lactococcus proteinase) of *Lactococcus lactis* (34), WapA of *Streptococcus mutans* (5), T6 of *S. pyogenes* (22), and Fn-BP (fibronectin-binding protein) of *S. aureus* (23).

S. aureus Cowan I were probed with human fibronectin. Binding of human fibronectin to the fibronectin-binding protein of *S. aureus* could clearly be detected. In contrast, we could not detect binding of human fibronectin to MRP (data not shown).

DISCUSSION

This article describes the cloning and characterization of the *S. suis* type 2 *mrp* gene. The protein encoded by this gene is associated with the cell envelope of *S. suis* (28, 31). Attempts to clone the entire gene directly in phage lambda or in plasmids were unsuccessful. Nevertheless, we were able to reconstitute the intact *mrp* gene from fragments which contained the 5′ and 3′ ends of the gene. *E. coli* cells that contained the entire *mrp* gene produced a protein that was identical in size to the MRP of *S. suis*. The level of expression of MRP was rather low, however. This is probably due to a considerable reduction of the copy number of the plasmid that contained the *mrp* gene, compared with the copy number of the vector plasmid alone. A possible explanation for this is that high-level expression of *mrp* is deleterious to *E. coli*. This fact might also explain why we were unable to clone the entire *mrp* gene using a lambda vector.

The DNA sequence of the *mrp* gene revealed an open reading frame coding for a polypeptide of 1,256 amino acids with a calculated MW of 135,794. The sequence had all the features expected for a cell surface protein of a gram-positive bacterium. It contained a sequence of 47 amino acids at the N terminus that showed all the characteristics of a signal peptide (33). At the C terminus was a domain that is probably responsible for the anchoring of the protein to the cell envelope. As in several other cell envelope-associated proteins of various gram-positive bacteria (4, 5, 7, 8, 10, 12, 22, 23, 34), this region contained a stretch of hydrophobic amino acids followed by a region of highly charged amino acids. In addition to the putative membrane anchor domain, the C terminus of MRP contained another stretch of amino acids that may be responsible for binding of MRP to the cell envelope. This domain preceded the putative membrane anchor and consisted of the amino acids LP-X-TGE. Such domains have also been observed in the cell envelope-associated proteins of various gram-positive bacteria (4, 5, 7, 8, 10, 12, 22, 23, 34). Pancholi and Fischetti (19) proposed that the domain is involved in the attachment of these proteins to the peptidoglycan moiety. The MRP sequence also showed a proline-rich region, a region with repeated units, and a region of 824 amino acids, part of which showed some similarity with the *S. aureus* protein that binds fibronectin.

A number of other streptococcal proteins contain proline-rich domains. Along with the conserved LP-X-TGE sequences, these domains are involved in the attachment of the protein to the peptidoglycan moiety of the cell (19). The proline-rich domain immediately precedes the conserved LP-X-TGE domain in these proteins; however, in MRP, the proline-rich domain is separated from the conserved LP-X-TGE sequence by a region of 239 amino acids which contains the repeated units. Therefore, it is not clear whether the proline-rich domain in MRP functions in the binding of MRP to the cell wall.

Many pathogenic bacteria produce cell envelope-associated proteins that contain repeated units of amino acids. It has been proposed that the repeats play a role in the binding of the bacterium to eucaryotic components that are specific for the host, such as factor H and fibrinogen (protein M [6]), albumin and immunoglobulins (protein G [9, 24]), and fibronectin (23). Whether the repeats in MRP likewise affect the binding of the bacterium to eucaryotic host components is unknown.

A subsequence of MRP (residues 619 to 985) has some similarity to the fibronectin-binding protein of *S. aureus*. The homologous region in this protein includes the sequence required for the binding of fibronectin, a unit of 38 amino acids, that is repeated three and a half times (23). Although this result suggests that MRP is involved in the binding to fibronectin, such binding could not be detected. Therefore, the function of the MRP remained unclear.

The cloned *mrp* gene can now be used to develop DNA probes for diagnosing *S. suis* infections in pigs. Moreover, now that the gene has been cloned, we can try to delete the *mrp* gene from virulent strains to determine whether these strains remain virulent.

ACKNOWLEDGMENTS

We thank Henk Wisselink for helpful comments and advice and we thank Victoria Thatcher for editing the manuscript.

REFERENCES

1. **Abou-Zeid, C., T. Garbe, R. Lathigra, H. G. Wiker, M. Harboe, G. A. W. Rook, and D. B. Young.** 1991. Genetic and immunological analysis of *Mycobacterium tuberculosis* fibronectin-binding proteins. Infect. Immun. **59:**2712–2718.
2. **Arends, J. P., and H. C. Zanen.** 1988. Meningitis caused by *Streptococcus suis* in humans. Rev. Infect. Dis. **10:**131–137.
3. **Clifton-Hadley, F. A.** 1983. *Streptococcus suis* type 2 infections. Br. Vet. J. **139:**1–5.
4. **Fahnestock, S. R., P. Alexander, J. Nagle, and D. Filpula.** 1987. Gene for an immunoglobulin-binding protein from a group G *Streptococcus*. J. Bacteriol. **167:**870–880.
5. **Ferretti, J. J., R. R. B. Russell, and M. L. Dao.** 1989. Sequence analysis of the wall-associated protein precursor of *Streptococcus mutans* antigen A. Mol. Microbiol. **3:**469–478.
6. **Fischetti, V. A.** 1989. Streptococcal M protein: molecular design and biological behavior. Clin. Microbiol. Rev. **2:**285–314.
7. **Fischetti, V. A., V. Pancholi, and O. Schneewind.** 1990. Conservation of a hexapeptide sequence in the anchor region of surface proteins from Gram-positive cocci. Mol. Microbiol. **4:**1603–1605.
8. **Frithz, E., L.-O. Hedén, and G. Lindahl.** 1989. Extended sequence homology between IgA receptor and M proteins in *Streptococcus pyogenes*. Mol. Microbiol. **3:**1111–1119.
9. **Guss, B., M. Eliasson, A. Olsson, M. Uhlén, A.-K. Frej, H. Jörnvall, J.-I. Flock, and M. Lindberg.** 1986. Structure of the IgG-binding regions of streptococci protein G. EMBO J. **5:**1567–1575.
10. **Guss, B., M. Uhlén, B. Nilsson, M. Lindberg, J. Sjöquist, and J. Sjödahl.** 1984. Region X, the cell-wall-attachment part of staphylococcal protein A. Eur. J. Biochem. **138:**413–420.
11. **Hager, P. W., and J. C. Rabinowitz.** 1985. Translational specificity in *Bacillus subtilis*, p. 1–32. *In* D. A. Dubnau (ed.), The molecular biology of the bacilli. Academic Press, Inc., New York.
12. **Hollingshead, S. K., V. A. Fischetti, and J. R. Scott.** 1987. The complete nucleotide sequence of type 6M protein of the group A *Streptococcus*: repetitive structure and membrane anchor. J. Biol. Chem. **261:**1677–1686.
13. **Konings, R. N. H., E. J. M. Verhoeven, and B. P. H. Peeters.** 1987. pKUN vectors for the separate production of both DNA strands of recombinant plasmids. Methods Enzymol. **153:**12–34.
14. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
15. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

16. **Messing, J.** 1979. A multipurpose cloning system based on the single-stranded DNA bacteriophage M13. Recombinant DNA technical bulletin. NIH publication no. 79-99, 2, no. 2, p. 43–48. National Institutes of Health, Bethesda, Md.
17. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Murray, N. E., W. J. Brammer, and K. Murray.** 1977. Lamboid phages that simplify the recovery of *in vitro* recombinants. Mol. Gen. Genet. **150:**53–58.
19. **Pancholi, V., and V. A. Fischetti.** 1988. Isolation and characterization of the cell-associated region of group A streptococcal M6 protein. J. Bacteriol. **170:**2618–2624.
20. **Platt, T.** 1986. Transcription termination and the regulation of gene expression. Annu. Rev. Biochem. **55:**339–372.
21. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
22. **Schneewind, O., K. F. Jones, and V. A. Fischetti.** 1990. Sequence and structural characteristics of the trypsin-resistant T6 surface protein of group A streptococci. J. Bacteriol. **172:**3310–3317.
23. **Signäs, C., G. Raucci, K. Jönsson, P.-E. Lindgren, G. M. Anantharamaiah, M. Höök, and M. Lindberg.** 1989. Nucleotide sequence of the gene for a fibronectin-binding protein from *Staphylococcus aureus*: use of this peptide sequence in the synthesis of biologically active peptides. Proc. Natl. Acad. Sci. USA **86:**699–703.
24. **Sjobring, U., C. Falkenberg, E. Nielson, B. Åkerström, and L. Björck.** 1988. Isolation and characterization of a 14-kDa albumin-binding fragment of streptococcal protein G. J. Immunol. **140:**1595–1599.
25. **Smith, H. E., et al.** Unpublished data.
26. **Talay, S. R., E. Ehrenfeld, G. S. Chhatwal, and K. N. Timmis.** 1991. Expression of the fibronectin-binding components of *Streptococcus pyogenes* in *Escherichia coli* demonstrates that they are proteins. Mol. Microbiol. **5:**1727–1734.
27. **Tinoco, I., Jr., P. N. Borer, B. Dengler, M. D. Devine, O. C. Uhlenbeck, D. M. Crothers, and J. Gralla.** 1973. Improved estimation of secondary structure in ribonucleic acids. Nature (London) New Biol. **246:**40–41.
28. **Vecht, U., J. P. Arends, E. J. van der Molen, and L. A. M. G. van Leengoed.** 1989. Differences in virulence between two strains of *Streptococcus suis* type 2 after experimentally induced infection of newborn germfree pigs. Am. J. Vet. Res. **50:**1037–1043.
29. **Vecht, U., L. A. M. G. van Leengoed, and E. R. M. Verheyen.** 1985. *Streptococcus suis* infections in pigs in The Netherlands (part one). Vet. Q. **7:**315–321.
30. **Vecht, U., H. J. Wisselink, J. Annakotta, and H. E. Smith.** Submitted for publication.
31. **Vecht, U., H. J. Wisselink, M. L. Jellema, and H. E. Smith.** 1991. Identification of two proteins associated with virulence of *Streptococcus suis* type 2. Infect. Immun. **59:**3156–3162.
32. **Vecht, U., H. J. Wisselink, J. E. van Dijk, and H. E. Smith.** 1992. Virulence of *Streptococcus suis* type 2 strains in newborn germfree pigs depends on phenotype. Infect. Immun. **60:**550–556.
33. **Von Heijne, G.** 1986. A new method for predicting signal sequence cleavage sites. Nucleic Acids Res. **14:**4683–4690.
34. **Vos, P., G. Simons, R. J. Siezen, and W. M. de Vos.** 1989. Primary structure and organization of the gene for a procaryotic cell envelope-located serine proteinase. J. Biol. Chem. **264:**13579–13585.