Identification of a Gene Required for Maturation of an Extracellular Lactococcal Serine Proteinase

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Directly upstream of the Lactococcus lactis subsp. cremoris Wg2 proteinase gene is an oppositely directed open reading frame (ORF). The complete nucleotide sequence of ORF1, encoding a 33-kilodalton protein, was determined. A protein of approximately 32 kilodaltons was synthesized when ORF1 was expressed in Escherichia coli by using a T7 RNA polymerase-specific promoter. L. lactis subsp. lactis MG1363 transformants carrying the proteinase gene but lacking ORF1 were phenotypically proteinase deficient, unlike transformants carrying both the proteinase gene and ORF1. Synthesis and secretion of proteinase antigen by L. lactis could be detected with proteinase-directed monoclonal antibodies regardless of whether ORF1 was present. The requirement of ORF1 for proteinase activation was reflected in a reduction in the molecular weight of the secreted proteinase. Furthermore, deletion of the 130 C-terminal amino acids of the Wg2 proteinase prevented attachment of the enzyme to lactococcal cells.

The widespread use of lactococci (formerly called lactic streptococci [22]) in food production and preservation is dependent on rapid growth and concomitant lactic acid production by these bacteria. To achieve this rapid growth in the production of cheese and other fermented milk products, the proteolytic enzymes of lactococci are essential. Because of their economic importance, the proteinases of lactococci are the subjects of extensive biochemical and genetic research (4, 14, 25). The complete proteinase gene of Lactococcus lactis subsp. cremoris Wg2 except for the 3'-terminal part (encompassing 130 coding triplets) was cloned and expressed in both L. lactis subsp. lactis and Bacillus subtilis (13). Notwithstanding the 3' deletion, the gene introduced in the proteinase-deficient strain L. lactis subsp. lactis MG1363 restored the ability to grow in milk to a high cell density. The Wg2 proteinase gene was sequenced completely and shown to encode a proteinase of 1,902 amino acids (200 kilodaltons [kDa]). The amino acid sequence of the proteinase showed remarkable homology with the sequences of subtilisins, the serine proteinases produced by several strains of bacilli (11). Although their casein specificities were quite different, both amino acid and nucleotide sequences of the proteinases from L. lactis subsp. cremoris SK11 and Wg2 appeared to be extremely homologous (14, 30, 32).

Sequence analysis of the cloned DNA fragment, containing the major 5' part of the Wg2 proteinase gene, has revealed the presence of an incomplete, oppositely directed open reading frame, ORF1, immediately upstream of the proteinase gene. Between the divergently transcribed open reading frames is a 320-base-pair noncoding DNA sequence containing the transcription and translation signals for both open reading frames (11). Interestingly, comparison of the restriction maps of the proteinase plasmids of L. lactis subsp. lactis 712 and L. lactis subsp. cremoris Wg2 and SK11 showed a total lack of similarity except for the proteinase gene locus and ORF1, which is contiguous with the proteinase gene in all three strains (4).

On the basis of the strongly conserved linkage and the highly conserved nature of both ORF1 and the proteinase gene, we hypothesized that ORF1 might be involved in expression of the proteinase gene or, possibly, with processing of the enzyme. This paper shows that ORF1 is required for the synthesis of active proteinase and that the ORF1 protein appears to be involved in activation of the enzyme. Furthermore, data showing that the C-terminal part of the proteinase is required for cell attachment are presented. Similar results, independently obtained with the L. lactis SK11 proteinase, are described by Vos et al. in the accompanying paper (33).

MATERIALS AND METHODS

Bacterial strains and media. Proteinase-producing (Prt+) and proteinase-deficient (Prt−) variants of L. lactis subsp. cremoris Wg2 (18) and L. lactis subsp. lactis MG1363 (6) were grown on MRS broth (3) modified as described elsewhere (8) and supplemented with 0.5% (wt/vol) glucose and 15 mM calcium chloride. For RNA isolation, cells were grown on 10% (wt/vol) reconstituted skim milk (Oxoid Ltd., London, England) containing 0.5% (wt/vol) glucose and 75 mM β-glycerolphosphate. Erythromycin was used at a concentration of 5 μg/ml. M17 agar containing 0.5% (wt/vol) glucose was used for plating.

Plasmids. Plasmids pGKV2, pGKV500, pGKV502, and pGKV507 have been described elsewhere (10, 12, 13). Plasmid pGKV500 was constructed by cloning the 6.4-kilobase (kb) ClaI-MluI fragment from Wg2 plasmid pWV05 (13) in pGK14, a pGK12 (12)-derived insertion inactivation vector, with unique sites for BamHI, BglII, ClaI, EcoRI, and MluI (J. Kok, unpublished results). The constructs are shown in Fig. 1.

L. lactis transformation. L. lactis subsp. lactis MG1363 was transformed by electroporation, using a Gene Pulser (Bio-Rad Laboratories, Richmond, Calif.), as described by van der Lelie et al. (28).

Molecular cloning and DNA sequence analysis. The 1.7-kb

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FIG. 1. Schematic representation of the protease gene region of the *L. lactis* subsp. *cremoris* Wg2 plasmid pWV05 (13) and parts of this region as they are present on pGKV500 (13), pGKV502 (10), pGKV507 (10), and pGKV550. Production of protease activity is indicated on the right. The gene products of ORF1 (prtM; segment 1) and the protease gene (prtP; segment 2) are shown. Restriction enzymes: B, BglII; C, ClaI; H, HindIII; M, MluI; N, NruI; S, SalI.

*Clal* fragment from Wg2 plasmid pWV05 (Fig. 1) was cloned in two orientations in bacteriophages M13mp18 and M13mp19 (34). This DNA fragment was sequenced on both strands by the dideoxy-chain termination method (21), using synthetic 17-mer DNA primers. A synthetic 33-mer (TGGACATTTTCAGAGGAGACCGAATCGATGGATC) was added. in which ORF1 was from grown molecular cloning by Maniatis promoter, specific amount filtered overnight determined by using Schuell, Inc., 1% (vol/vol) ml added. resuspended in pUC19 (34) that had been digested with *BamHI* and *SalI*, resulting in pSKH2. A 1.060-base-pair *Clal*-DraI DNA fragment from Wg2 plasmid pWV05, containing the complete ORF1, was inserted into pSKH2 that had been digested with *Clal* and *Smal*, resulting in pSKH1. Plasmid pSKH4, in which ORF1 is transcribed from the T7 RNA polymerase-specific promoter, was constructed by inserting the ORF1-containing 1.1-kb *EcoRI*-PstI DNA fragment from pSKH1 into pT712 (24) that had been digested with *EcoRI* and *PstI*. General molecular cloning techniques were carried out as described by Maniatis et al. (17).

Protease activity assay. Protease activity in milk-grown overnight cultures were determined by measuring the amount of hydrolyzed milk proteins, using the o-phthalaldehyde (OPA) spectrophotometric assay (2). To 1 ml of overnight culture, 2.2 ml 0.68 N trichloroacetic acid was added; the mixture was left for 10 min at room temperature and filtered through a 0.45-µm-pore-size filter (Schleicher & Schuell, Inc., Keene, N.H.). Two minutes after 50 µl of the filtrate was mixed with 1 ml of OPA reagent, the absorbance was determined by using a Philips PU8720 UV-visible light scanning spectrophotometer. OPA reagent consisted of 1 ml of OPA (40 mg/ml in methanol; Sigma Chemical Co., St. Louis, Mo.) added to 50 ml of 50 mM sodium tetraborate containing 1% (wt/vol) sodium dodecyl sulfate (SDS)-0.2% (vol/vol) 2-mercaptopethanol.

Enzyme-linked immunosorbent assay (ELISA). Cultures (20 ml) of *L. lactis*, grown overnight on modified MRS broth supplemented with glucose and calcium chloride, were centrifuged (10 min, 20°C, 2,500 × g). Cells were washed with 10 ml of coating buffer (50 mM sodium carbonate [pH 9.6], 15 mM calcium chloride) and finally resuspended in 2 ml of coating buffer. To 1 ml of the cell suspension, 9 ml of coating buffer and 2 ml of 7% perchloric acid were added. To 20 ml of the culture supernatant, 4 ml of 7% perchloric acid was added. Precipitates formed after the preparation stood on ice for 45 min were spun down (45 min, 0°C, 30,000 × g) and resuspended in 1 ml of coating buffer. After the pH of the suspensions was adjusted to pH 9.6 by addition of 0.5 N sodium hydroxide, 100-µl amounts of dilutions in coating buffer were applied to microdilution plates (Bioreba, Basel, Switzerland). ELISA was performed as described by Laan et al. (15), using monoclonal antibodies Wg2-1 and Wg2-10, raised against the purified *Wg2* protease (15), and peroxidase-conjugated rabbit antimouse antibodies (DAKO Immunoglobulins, Copenhagen, Denmark).

**Protease isolation.** *L. lactis* MG1363 carrying either pGKV500 or pGKV507 was grown overnight on modified MRS broth supplemented with glucose and calcium chloride. After removal of the cells by centrifugation (10 min, 4°C, 6300 × g), solid ammonium sulfate was added to the culture medium to 60% saturation at 0°C. The precipitate was spun down (45 min, 0°C, 18,000 × g) and dissolved in 5 ml of 15 mM calcium chloride. After dialysis overnight against several changes of 15 mM calcium chloride at 0°C, samples of the protease solutions were subjected to SDS-polyacrylamide gel electrophoresis as described by Laemmli (16).

Western blotting (immunoblotting) and antigen detection. Western blotting onto BA85 nitrocellulose (Schleicher & Schuell) was performed as described by Towbin et al. (26), using a Bio-Rad mini trans-blot apparatus. Antigens were detected by using monoclonal antibodies Wg2-1 and Wg2-10 as described by Laan et al. (15).

**T7 RNA polymerase-specific promoter-directed expression of prtM in Escherichia coli.** *E. coli* BL21 (DE3) (23) containing plasmid pT712 (24) or plasmid pSKH4 was grown to an optical density of 660 nm of 0.4. T7 RNA polymerase was induced by adding IPTG (isopropyl-β-D-thiogalactopyranoside) to the culture medium to a final concentration of 0.4 mM. After 1 h of incubation at 37°C, rifampin was added to a final concentration of 200 µg/ml. After an additional 1-h incubation at 37°C, cells were harvested and suspended in sample buffer (60 mM Tris hydrochloride [pH 6.8], 2% [wt/vol] SDS, 5% [vol/vol] glycerol, 0.01% [wt/vol] bromophenol blue, 2% [vol/vol] β-mercaptoethanol). Samples equivalent to 35 µl of cell culture were subjected to SDS-polyacrylamide gel electrophoresis as described by Laemmli (16).

**RESULTS**

Protease activity. In achieving rapid growth and lactic acid production when grown on milk, lactococci depend on proteolytic enzymes that degrade milk proteins. In many lactococci, this ability is plasmid encoded (14). By introducing plasmid pGKV500 (Fig. 1) into the plasmid-cured, protease-deficient strain MG1363 (6), the ability to grow to high cell density in milk was restored (13). Plasmid pGKV500 contains a 6.5-kb *HindIII* fragment of the *L. lactis* subsp. *cremoris* Wg2 protease plasmid pWV05. Nucleotide sequence analysis revealed the presence of a large N-terminal portion of a protease gene and an additional incomplete open reading frame, ORF1, on this DNA fragment (11). On plasmid pGKV550, the complete protease gene is present but ORF1 is absent (Fig. 1). Deletion of the major part of ORF1 from pGKV500 resulted in plasmid pGKV507 (Fig. 1) (10). Unlike *L. lactis* MG1363(pGKV500), neither MG1363(pGKV507) nor MG1363(pGKV550) showed production of lactic acid when grown on milk-based agar plates supplemented with bromocresol purple and glucose, which indicated a Prt phenotype (13; data not shown).

To quantify the protease activity of the lactococcal strains carrying the various plasmids, we assayed milk-
TABLE 1. Proteolysis of milk proteins and cell densities in milk-grown cultures of lactococcal strains carrying various plasmids

<table>
<thead>
<tr>
<th>Strain (plasmid)</th>
<th>Proteolysis (ΔA450)</th>
<th>Density (CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk blank</td>
<td>0.154</td>
<td></td>
</tr>
<tr>
<td>MG1363 (Prt-)</td>
<td>0.126</td>
<td>5.2 × 10⁸</td>
</tr>
<tr>
<td>MG1363(pGKV500)</td>
<td>0.780</td>
<td>4.0 × 10⁹</td>
</tr>
<tr>
<td>MG1363(pGKV502)</td>
<td>0.544</td>
<td>4.5 × 10⁹</td>
</tr>
<tr>
<td>MG1363(pGKV507)</td>
<td>0.117</td>
<td>6.2 × 10⁸</td>
</tr>
<tr>
<td>MG1363(pGKV550)</td>
<td>0.188</td>
<td>4.1 × 10⁸</td>
</tr>
<tr>
<td>Wg2 (Prt-)</td>
<td>0.608</td>
<td>3.3 × 10⁷</td>
</tr>
<tr>
<td>Wg2 (Prt+)</td>
<td>0.143</td>
<td>3.9 × 10⁸</td>
</tr>
</tbody>
</table>

* Measured by the OPA spectrophotometric assay (2) as described in Materials and Methods.

† Determined in cultures grown overnight at 30°C in 10% (wt/vol) reconstituted skim milk.

Table 2. Presence of Wg2 protease antigen as assayed by ELISA, using monoclonal antibodies against the Wg2 protease

<table>
<thead>
<tr>
<th>Strain (plasmid)</th>
<th>Protease antigen in:</th>
<th>Culture medium</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG1363 (Prt-)</td>
<td>0.97</td>
<td>0.044</td>
<td></td>
</tr>
<tr>
<td>MG1363(pGKV500)</td>
<td>0.77</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>MG1363(pGKV502)</td>
<td>0.83</td>
<td>0.050</td>
<td></td>
</tr>
<tr>
<td>MG1363(pGKV507)</td>
<td>0.62</td>
<td>0.046</td>
<td></td>
</tr>
<tr>
<td>MG1363(pGKV550)</td>
<td>0.54</td>
<td>0.330</td>
<td></td>
</tr>
<tr>
<td>Wg2 (Prt-)</td>
<td>0.07</td>
<td>0.060</td>
<td></td>
</tr>
<tr>
<td>Wg2 (Prt+)</td>
<td>0.42</td>
<td>0.291</td>
<td></td>
</tr>
</tbody>
</table>

† Determined in 10 ml of cells and 20 ml of culture medium (MRS broth) of MG1363 carrying various plasmids. Data are presented as relative ΔA606 per optical density (600 nm) unit of culture density.

Grown overnight cultures for proteolysis of milk proteins by the OPA method (2). Active protease was produced by strains Wg2 (Prt+), MG1363(pGKV500), and MG1363 (pGKV502) (Table 1), allowing these strains to grow on milk to high cell densities. In contrast, no proteolysis of milk proteins could be detected in milk-grown cultures of MG1363 (pGKV507) and MG1363(pGKV550). Consequently, the latter cultures grew to a much lower cell density on milk than did the proteolytically active strains, since they were completely dependent on the limited amount of free amino acids present in the milk. These results indicate that ORF1 is required for protease activity.

ELISA. To determine whether the protease gene was transcribed in the absence of ORF1, RNA extracted from the various strains was analyzed by dot blot hybridization. Proteinase-specific mRNA was found both in the presence and in the absence of ORF1 (results not shown).

The presence of a transcript of the protease gene in the absence of ORF1 prompted us to investigate whether ORF1 affected translation of the transcript or secretion of the proteinase. To this purpose, we used monoclonal antibodies raised against the purified Wg2 proteinase in an ELISA (15). Both cells and culture medium were assayed for the presence of the proteinase. Irrespective of the presence or absence of ORF1, all plasmid-carrying strains produced proteinase antigen (Table 2). In addition, proteinase was cell associated in strains Wg2 (Prt+) and MG1363(pGKV550), the only strains containing the complete proteinase gene, which indicated that the C-terminal part of the proteinase is required for attachment to the cell. Since the proteinase produced by the strains lacking ORF1, MG1363(pGKV507) and MG1363(pGKV550) (Table 1), was not active, we conclude that the protein specified by ORF1 is involved in the production of active proteinase.

Proteinase isolation and characterization. Proteinases were isolated from the culture medium of MG1363(pGKV500) and MG1363(pGKV507) and subjected to SDS-polyacrylamide gel electrophoresis. In the supernatant of MG1363(pGKV500), two prominent protein bands, of approximately 160 and 165 kDa, and three protein bands, of approximately 140, 130, and 115 kDa, were present (Fig. 2A). Western blot analysis (Fig. 2B) showed that these protein bands reacted with proteinase-specific monoclonal antibodies, as did a number of protein bands of lower molecular weight not visible in the Coomassie-stained gel of Fig. 2A. As with MG1363(pGKV507) in the absence of ORF1, the culture medium contained a protein of approximately 185 kDa that reacted with the monoclonal antibodies, as did a number of protein bands of lower molecular weight. Apparently, the presence of ORF1 leads to a reduction in the molecular weight of the largest form of the proteinase.

MG1363(pGKV2), the negative control in lanes 3 of Fig. 2A and B, shows the specificity of the monoclonal antibodies used, since no proteins that reacted with the monoclonal antibodies could be detected. Moreover, this result indicates that all proteins detected in lanes 1 and 2 of Fig. 2B originated from the Wg2 proteinase.

Sequence analysis. Since the presence of both the proteinase gene and the incomplete ORF1 were found to be essential for proteinase activity, we decided to determine the nucleotide sequence of the missing part of ORF1 (11). To this purpose, both DNA strands of the overlapping 1.7-kb ClaI fragment from pWV05 (Fig. 1), cloned in phages M13mp18 and M13mp19, were sequenced. This sequence analysis revealed the presence of four additional coding triplets of ORF1, followed by a translational stop codon. This stop codon was directly followed by a DNA sequence containing stop codons in all possible reading frames (Fig. 3). The complete ORF1 could encode a protein of 299 amino acids and a molecular weight of 33,137. The amino acid sequence derived from the nucleotide sequence is shown.
in Fig. 3. As noted earlier (11), the amino-terminal sequence of this protein bears the characteristics of a typical procaryotic signal sequence (31). The amino acid sequence around Cys-24 shows good homology with the consensus sequence for signal peptidase cleavage sites of procaryotic pre-lipoproteins (20).

Expression of ORF1 in E. coli. By using a synthetic DNA linker containing a ClaI restriction site and the putative ribosome-binding site of ORF1 (11), a DNA fragment containing ORF1 was inserted in plasmid pT712 (24). In the resulting plasmid, pSKH4, the gene constituted by ORF1 is under direction of a T7 RNA polymerase-specific promoter. Upon induction of the T7 RNA polymerase gene, E. coli BL21 (DE3) carrying plasmid pSKH4 produced a protein of approximately 32 kDa that was not synthesized by cells carrying plasmid pT712 without ORF1 (Fig. 4). The size of the protein found, approximately 32 kDa, agrees well with the expected size of the ORF1-specified protein, 33 kDa, as determined by the nucleotide sequence.

![Figure 4](image-url)

**FIG. 4.** Expression of the **prtM** gene (ORF1) in *E. coli* BL21 (DE3). Samples of *E. coli* BL21 (DE3) carrying either pT712 (lane 1) or pSKH4 (lane 2) were subjected to SDS-polyacrylamide (12.5%) gel electrophoresis. Standard molecular weight markers: phosphorylase b (97,400); bovine serum albumin (66,200); ovalbumin (42,699); carbonic anhydrase (31,000); trypsin inhibitor (21,500); and lysozyme (14,400). Molecular sizes (in kilodaltons) are shown on the left.

DISCUSSION

The results presented above clearly indicate that in addition to the structural gene for proteinase, **prtP**, a second gene, designated **prtM**, is required for production of enzymatically active proteinase. The observation that the **prtP** and **prtM** genes are jointly required for production of active proteinase appears to be reflected in the genetic organization of the two genes: both are present on the proteinase plasmid pWW05 of *L. lactis* subsp. *cremoris* Wg2 and are divergently transcribed from regulatory sequences contained within the same 345-base-pair ClaI fragment (Fig. 1) (11). The **prtM** gene was expressed in *E. coli* under direction of a T7 RNA polymerase-specific promoter, giving rise to the synthesis of a protein of approximately 32 kDa (Fig. 4). On the basis of the nucleotide sequence, the size of this protein is in good agreement with the expected size of the PrtM protein, 33 kDa. The fact that the **prtM** gene present on plasmids pGKV500 and pGKV502 (Fig. 1) is truncated and lacks four coding triplets does not seem to affect the activity of the PrtM protein.
With respect to the function of the \( \text{prtM} \) gene, we can exclude direct involvement in transcription of the proteinase gene because of the presence of a transcript of \( \text{prtP} \) in both the presence and the absence of \( \text{prtM} \) (data not shown). Moreover, involvement in translation and membrane translocation can be ruled out because monoclonal antibodies directed against the proteinase reacted with proteins present in the culture medium of strains containing \( \text{prtP} \) but lacking \( \text{prtM} \). Since a proteolytically inactive enzyme is secreted into the culture medium in the absence of \( \text{prtM} \), we propose that conversion of the inactive form to the active proteinase in the presence of \( \text{prtM} \) takes place during or after translocation of the enzyme across the membrane.

The largest protein specified by the 3'-truncated \( \text{prtP} \) gene in the presence of \( \text{prtM} \) (on pGKV500) is approximately 165 kDa. A 185-kDa inactive protein is formed from the same 3'-truncated \( \text{prtP} \) gene in the absence of \( \text{prtM} \) (in pGKV507). Although gel mobility of a protein does not necessarily correlate with actual size, the presence of the Pm protein seems to reduce the size of the proteinase to about 20 kDa. It is conceivable, though by no means certain, that this reduction in molecular weight is caused by removal of the prosequence from the proteinase precursor. The Wg2 proteinase is probably (11) and the homologous SK11 proteinase is certainly synthesized as a pro-enzyme. The SK11 precursor contains a pre-pro sequence of 187 amino acids (33). Since the Wg2 and the SK11 proteinases are extremely homologous (14), we assume that the Wg2 proteinase precursor is processed at the same site. The signal peptide is thought to contain 33 amino acids (11); therefore, the pro sequence would contain 153 amino acids, which is in the range of the molecular weight reduction of the precursor observed. Conclusive evidence for this hypothesis ultimately depends on the N-terminal amino acid sequences of both the active and inactive proteinases, which may be difficult to obtain since the inactive form seems to be blocked (33). Two possibilities as to the way in which Pm converts the inactive precursor to the active mature proteinase may be entertained. The first is that the Pm protein, or a protein induced by the \( \text{prtM} \) gene, removes the pro sequence from the precursor. Alternatively, the protein may activate the proproteinase to carry out a specific autocatalytic cleavage, resulting in removal of the pro sequence of the enzyme. In this regard, it is relevant that the cleavage of the pro region from prosubtilisin of \( \text{B. amylophiliquefaciens} \) is most likely an autocatalytic process that may be activated by an as yet unidentified activator (19). In addition, the proarcyptic protein streptokinase induces conversion of the eucaryotic zymogen plasminogen into active plasmin by inducing the plasminogen to carry out specific self-digestion (11).

The presence of the lower-molecular-weight forms of the proteinase in both MG1363(pGKV507) and MG1363(pGKV500) supernatants may be explained in several ways. The first is the presence or copurification of an additional proteinase activity. Second, these forms may result from autoproteolytic degradation of the enzyme. Even in the case of the inactive proteinase encoded by pGKV507, we cannot exclude the possibility that a low residual activity, insufficient to support growth on milk, causes limited self-digestion.

One further point warranting attention concerns the interesting observation that only in the cases in which the complete \( \text{prtP} \) gene was present [in strains Wg2 and MG1363(pGKV550)] was a substantial amount of the proteinase associated with the cells. Irrespective of the production of active or inactive proteinase, deletion of 130 amino acids from the C terminus of the protein, as in the case of MG1363 carrying plasmid pGKV500 or pGKV507, resulted in complete secretion into the culture medium. This observation indicates that at least (part of) this C-terminal stretch of 130 amino acids is required for attachment of the proteinase to the cell. In earlier experiments, proteolytic activity could be detected in the washing fluid after washing of MG1363(pGKV500) in a calcium-free buffer (10). We can reconcile these earlier data and those presented here by assuming that small amounts of the Wg2 proteinase were still attached to the cell envelope. The presence of residual amounts of proteinase in the cell envelope may also explain the results of Hugenholtz et al. (9), who, from immunogold-labeling experiments using polyclonal antibodies, concluded that the proteinase produced by MG1363(pGKV500) was localized on the outside of the cell wall. Inspection of the C-terminal amino acids of the proteinase shows the presence of a stretch of hydrophobic amino acids flanked by charged residues (Arg-1876 and Lys-1895) (11). This structure shows remarkable similarity to membrane anchor sequences found in other proteins, such as \( \text{Staphylococcus aureus} \) protein A (27) and type M protein of \( \text{Streptococcus pyogenes} \) (7), and may therefore be involved in attachment of the proteinase to the cell. Furthermore, the tentative membrane anchor of the Wg2 proteinase is preceded by a protein region with a high content of prolines and glycines (11). A similar region of the \( \text{S. pyogenes} \) type M protein, preceding the membrane anchor, is located in the streptococcal cell wall (5).

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**LITERATURE CITED**


