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# Gene for Spiralin, the Major Membrane Protein of the Helical Mollicute *Spiroplasma citri*: Cloning and Expression in *Escherichia coli*

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**A library of cloned *Spiroplasma citri* genomic sequences was constructed by incorporating *Hin*dIII digestion fragments into the plasmid vector pBR328. Immunological screening allowed the identification of a recombinant plasmid containing the gene for spiralin, the major membrane protein of *S. citri*. The spiralin produced by the *Escherichia coli* transformant was characterized by immunological detection with monoclonal antibody after Western blotting of two-dimensional (isoelectric focusing and sodium dodecyl sulfate-polyacrylamide) electrophoresis gels and by partial proteolytic mapping. The gene for spiralin occurred within a 6.5-kilobase-pair cloned DNA fragment. Spiralin in *E. coli* was produced regardless of the orientation of the insert within the pBR328 vector. A spiroplasmal DNA sequence which acted as a promoter in *E. coli* was cloned along with the structural spiralin gene which is expressed in *E. coli* from that sequence.**

Mollicutes (mycoplasmas) are the smallest organisms capable of growing outside a host cell. They have been isolated from a wide variety of plant and animal sources (1, 8, 16, 23). Their structural and biochemical simplicity as well as the small size of their genome ($0.5 \times 10^9$ to $1 \times 10^9$ daltons [Da]) make them useful models for studying basic problems in cell biology, such as those concerning membrane structure and function. Among the mollicutes, the spiroplasmas are unique in that they are motile, have helical morphology, and are pathogenic for plants, insects, and vertebrates (1). *Spiroplasma citri* is the first such organism to have been cultured and characterized (1, 19). It is also the first mollicute isolated from plants. *S. citri* causes an economically important disease of citrus (stubborn disease) and is responsible for brittle root disease of horseradish (7, 18). Moreover, many other plants in nature have been found to be infected by *S. citri*, and a number of plants are susceptible to experimental infections (for a review, see references 1 and 2).

Proteins from the cell membrane of *S. citri* have been resolved into more than 29 bands by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), with apparent molecular sizes ranging from 12 to 170 kDa (28, 29). Spiralin, the most abundant protein in the *S. citri* membrane (30), is an amphiphilic polypeptide (27) with an apparent molecular size of 26 to 28 kDa, depending on polyacrylamide gel composition, and it can form homooligomers in situ. Immunotopographical experiments suggest that spiralin spans the spiroplasma membrane. In addition, spiralin is the main antigen of *S. citri*, and antibodies elicited against spiralin are largely specific for *S. citri* (14, 26).

This report describes the cloning and expression of the spiralin gene in *Escherichia coli*.

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## MATERIALS AND METHODS

**Chemical reagents, enzymes, and antisera.** Restriction endonucleases, T4 DNA ligase, and alkaline phosphatase were obtained from New England BioLabs, Inc. (Beverly, Mass.) and used according to the manufacturer's directives. Antibiotics, RNase A, proteinase K, protease type XVII from *Staphylococcus aureus* V8, agarose, and *para*-nitrophenylphosphate were purchased from Sigma Chemical Co. (St. Louis, Mo.). 4-Chloro-1-naphthol was from Merck & Co., Inc. (Rahway, N.J.). Nitrocellulose filters (BA85) were purchased from Schleicher & Schuell, Inc. (Keene, N.H.).

The conjugate between horseradish peroxidase and sheep immunoglobulin G (IgG) against either rabbit or mouse IgG was purchased from Institut Pasteur Productions (Marnes-la-Coquette, France). Polyspecific antibodies against whole *S. citri* cells were elicited in rabbits as described elsewhere (20). Mouse monoclonal antibody against spiralin was kindly provided by G. J. McGarrity (Department of Microbiology, Institute for Medical Research, Camden, N.J.). Its specificity for spiralin was demonstrated by immunoaffinity chromatography (13) and immunological detection on Western blots (this paper).

**Mollicutes and bacterial strains and culture conditions.** All mollicute strains were triply cloned. *S. citri* R8A2 (ATCC 27556) (19) was cultured aerobically at 32°C in BSR medium (3).

The bacterial strain used was *E. coli* HB101. *E. coli* was routinely cultured in L broth (10 g of tryptone, 5 g of yeast extract, 5 g of NaCl per liter). Solid medium consisted of 37 g of brain heart infusion (Bio-Merieux, Charbonnières-les-Bains, France) and 10 g of Bacto-Agar (Difco Laboratories, Detroit, Mich.) per liter. The concentrations of ampicillin, tetracycline, and chloramphenicol used for selecting transformants was 50, 15, and 25 μg/ml, respectively.

**DNA isolation.** Genomic DNA from *S. citri* R8A2 was isolated from 50-ml cultures at the end of the exponential phase of growth. Organisms were harvested by a 20-min centrifugation at 30,000 × *g* at 4°C. The pellet was sus-

pended in 100 mM Tris hydrochloride (pH 9.0)–150 mM NaCl–35 mM SDS–10 mM EDTA–50 mM β-mercaptoethanol. After the addition of 0.1 ml proteinase K solution at 10 mg/ml, the suspension was slowly stirred for 20 min at room temperature. The nucleic acids were submitted to a phenol treatment, followed by a chloroform-isoamyl alcohol (24:1, vol/vol) extraction, and were finally recovered by ethanol precipitation. They were then solubilized in 10 ml of 30 mM Tris hydrochloride (pH 8.0)–50 mM NaCl–5 mM EDTA and incubated with 0.5 mg of RNase for 30 min at 20°C. The DNA was finally deproteinized and recovered by phenol and chloroform extractions and ethanol precipitation.

Recombinant plasmids were purified from *E. coli* by the SDS-cleared lysate method and subsequent CsCl-ethidium bromide density gradient centrifugation (10).

**Preparation of an *S. citri* genomic library and immunological screening.** Genomic DNA (4 μg) purified from *S. citri* R8A2 was digested with 400 U of *Hin*dIII for 2 h at 37°C in 0.3 ml of a 100 mM Tris hydrochloride (pH 7.5)–50 mM NaCl–10 mM $MgCl_2$ buffer. The restriction fragments were purified by phenol and chloroform-isoamyl alcohol extractions followed by ethanol precipitation. The purified restriction fragments were ligated for 14 h at 14°C with 5 μg of alkaline phosphatase-treated, *Hin*dIII-cut pBR328 (21) in 0.1 ml of a reaction mixture containing 50 mM Tris hydrochloride (pH 7.4), 10 mM $MgCl_2$, 20 mM dithiothreitol, 1 mM ATP, and 20 U of T4 DNA ligase. The ligase reaction products were used to transform *E. coli* HB101 cells (24). *E. coli* transformants that expressed chloramphenicol resistance ($Cam^r$) were selected on solid medium and checked for their tetracycline sensitivity ($Tet^s$). Clones carrying a $Cam^r$ $Tet^s$ phenotype were further screened for their ability to express *S. citri* antigens by an enzyme-linked immunosorbent assay (ELISA) involving rabbit antibodies directed against whole *S. citri* cells (20). Each *E. coli* transformant was inoculated into 0.2 ml of chloramphenicol-containing L broth deposited in one of the wells of a sterile microtitration plate (Titertek; Flow Laboratories, Inc., McLean, Va.). Plates were then incubated overnight at 37°C. The next day, 0.02 ml of a lysozyme solution (5 mg/ml of 25 mM Tris hydrochloride [pH 8.0]) was added to each well. The plates were further incubated for 30 min at room temperature, submitted to three freeze (−80°C) and thaw cycles, and finally incubated for 10 min at 37°C. Each *E. coli* lysate was subsequently transferred to a new microtitration plate previously coated with rabbit immunoglobulin against *S. citri* R8A2 cells (20). The purified IgG was used at a concentration of 6 μg/ml. The plates were stored overnight at 4°C. The specific antigen-antibody complexes were then detected with a conjugate between alkaline phosphatase and rabbit anti-*S. citri* immunoglobulin; *para*-nitrophenylphosphate was the substrate (20). The conversion of the colorless substrate to a yellow product was monitored after a 30-min reaction at room temperature by measuring the $A_{405}$ in a Titertek Multiskan spectrophotometer (Flow Laboratories).

**Electrophoresis of proteins.** *S. citri* protein samples were prepared as described previously (15). For the preparation of *E. coli* protein samples, cells carrying various plasmids were grown overnight at 37°C in 20 ml of L broth medium with chloramphenicol. The cells were recovered by centrifugation at 10,000 × *g* for 10 min and suspended in 2 ml of electrophoresis sample buffer (9). The protein extracts were separated by one- or two-dimensional electrophoresis (isoelectric focusing before SDS-PAGE) on 15 to 20% polyacrylamide gradient slab gels, as previously described (15).

**Immunological detection of *S. citri* antigens on nitrocellulose blots.** Upon electrophoresis, the proteins were transferred to nitrocellulose sheets (4). After transfer, the blots (9.5 by 16.5 cm) were dried for 30 min at 32°C and then soaked for 30 min at 20°C in TS buffer (50 mM Tris hydrochloride [pH 7.4], 200 mM NaCl) containing 30 mg of bovine serum albumin per ml. For immunological detection, the blots were incubated for 4 h at 20°C in 10 ml of TS buffer containing 30 mg of bovine serum albumin per ml and 0.2 ml of rabbit antiserum raised against whole *S. citri* R8A2 cells (20) or monoclonal antibodies against spiralin (13). The blots were then washed for 30 min in three 100-ml changes of TS buffer containing 2 mg of SDS and 2 mg of Triton X-100 per ml and then twice in 100 ml of TS alone.

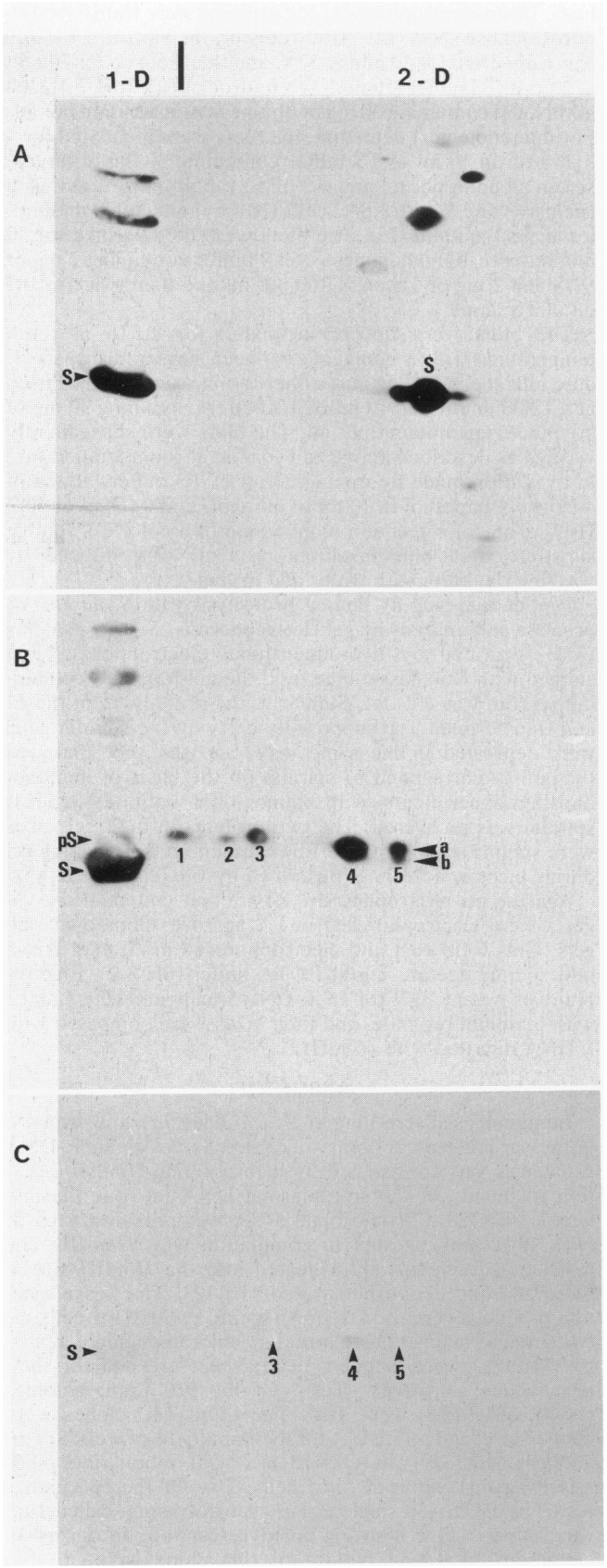
The blots were further incubated for 2.5 h at room temperature with a conjugate between horseradish peroxidase and sheep IgG against either rabbit or mouse IgG used at a 1:800 dilution in 10 ml of TS buffer containing 30 mg of bovine serum albumin per ml. The blots were subsequently washed as described above and soaked at room temperature in a solution made by mixing 50 ml of TS buffer, 30 mg of 4-chloro-1-naphthol in 10 ml of ethanol, and 0.02 ml of 30% $H_2O_2$. The color reaction (conversion of a colorless soluble substrate to a blue insoluble product) was stopped by washing the blots with water and drying.

**Peptide mapping by limited proteolysis with *S. aureus* V8 protease and analysis by gel electrophoresis.** *S. citri* proteins were separated by two-dimensional electrophoresis and stained with Coomassie blue, and the gel fragment containing spiralin was cut out. Similarly, the proteins from the *E. coli* transformant Tsp supposedly carrying the spiralin gene were separated in the same way, and the spot that was thought to correspond to spiralin on the basis of immunoblotting experiments with monoclonal antibody against spiralin was recovered. The two proteins in their gel slices were submitted to protease V8 treatment in the stacking gel during electrophoresis as described by Cleveland et al. (5).

**Agarose gel electrophoresis.** Restriction endonuclease digests were electrophoresed in 1% agarose submerged slab gels. Gels were cast and electrophoresed in 40 mM Tris–5 mM sodium acetate–1 mM EDTA buffer (pH 8.2). Electrophoresis was at 50 V for 16 h. DNA fragments were stained with ethidium bromide, and their sizes were compared with λ DNA digested with *Hin*dIII.

## RESULTS

**Immunological screening of *E. coli* transformants expressing *S. citri* antigens.** A library of cloned *S. citri* genomic DNA sequences was constructed by incorporating *Hin*dIII digestion fragments of the spiroplasmal DNA into the plasmid vector pBR328. Chromosomal DNA was prepared from *S. citri* R8A2 and digested to completion with *Hin*dIII. The restriction fragments were ligated into the *Hin*dIII site of linearized and dephosphorylated pBR328. The ligase reaction products were used to transform *E. coli* HB101 cells. *E. coli* transformants that expressed chloramphenicol resistance ($Cam^r$) were isolated and further screened for their tetracycline sensitivity ($Tet^s$). Of the 920 $Cam^r$ colonies tested, 565 (61%) were $Tet^s$. The $Cam^r$ $Tet^s$ clones were further screened by ELISA for their ability to express *S. citri* antigens. ELISA was carried out with rabbit antibodies raised against whole *S. citri* cells (20). Of the 565 clones tested by ELISA, a single *E. coli* transformants, called Tsp, was found to give a strong positive reaction. In our assay conditions, a 0.2-ml culture of this clone gave an $A_{405}$ superior to 2.0 units, a level obtained with about 0.3 μg of *S. citri* proteins. As shown below, the Tsp transformant har-




bored a recombinant plasmid of 11.4 kilobase pairs (kbp), called pES1. Fifteen other *E. coli* clones from the spiroplasmal DNA library gave a weaker ELISA reaction, corresponding to an absorbance of about 0.25 units per 0.2-ml culture, but were not further characterized. All the other $Cam^r$ $Tet^s$ clones tested had an $A_{405}$ lower than 0.1 unit, a value similar to that obtained in control assays with *E. coli* transformants carrying pBR328 alone. Finally, when the *E. coli* transformant Tsp was analyzed by ELISA with IgG from preimmune serum instead of anti-*S. citri* IgG, the assay was entirely negative.

**Expression of spiralin in transformant Tsp.** To identify the *S. citri* antigen expressed by Tsp, polypeptides from this *E. coli* transformant and from *E. coli* cells carrying pBR328 alone and from *S. citri* R8A2 were separated by isoelectric focusing in cylindrical gels which were then loaded onto slab gels. Samples of the *S. citri* polypeptides were deposited on the left side of each slab gel, so that after the electrophoresis step, both a one-dimensional and a two-dimensional protein analysis were obtained on the same slab gel. The proteins were transferred from the slab gels to nitrocellulose sheets by Western blotting (4). One blot was incubated with rabbit antibodies against whole *S. citri* cells while a similar one was probed with a mouse monoclonal antibody against spiralin. The antigen-antibody complexes were detected with a conjugate between horseradish peroxidase and sheep IgG against either rabbit or mouse IgG; 4-chloro-1-naphthol was the substrate for the peroxidase reaction.

The rabbit immunoglobulin against whole *S. citri* cells recognized several polypeptides from *S. citri* cells (Fig. 1A), including the 28-kDa spiralin (Fig. 1A, S).

Several proteins from the *E. coli* transformant Tsp were also detected by the rabbit IgG against *S. citri* (Fig. 1B, 2-D, proteins 1 to 5). These proteins had different isoelectric points but closely related molecular weights. In addition, protein spots 4 and 5 comprised two polypeptides each: 4a and b and 5a and b. Protein 4a was the major protein that was recognized by the anti-*S. citri* IgG; next came 5a; 1, 2, 3, 4b, and 5b were only minor proteins. Only protein 4 could be detected by Coomassie blue staining (Fig. 2, arrow) or autoradiography of proteins labeled in vivo with $^{14}C$-amino acids (data not shown). When monoclonal antibody against spiralin was used, not only could spiralin from *S. citri* be detected on one-dimensional (Fig. 1C, S) or two-dimensional (data not shown) gels but also three proteins from the *E. coli* transformant Tsp (Fig. 1C, 2-D, proteins 3, 4, and 5). These proteins were identical with those numbered 3, 4, and 5 in Fig. 1B, since blots B and C originated from the same slab gel. Finally, in control experiments, no protein was detected when IgG from preimmune serum was used for immunodetection of proteins from *S. citri* or *E. coli* transform-

FIG. 1. Western blotting analysis of proteins from *S. citri* and *E. coli* transformant Tsp, using rabbit antibodies against whole cells (A and B) or mouse monoclonal antibody against spiralin (C). Track 1D on the left side of panels A, B, and C represents the one-dimensional analysis of *S. citri* R8A2 proteins by SDS-PAGE. Two-dimensional analysis (isoelectric focusing and SDS-PAGE) of strain R8A2 proteins is shown in panel A, 2-D, and that of *E. coli* transformant Tsp is given in panels B, 2-D, and C, 2-D. Samples submitted to electrophoresis contained 200 μg of proteins. The immunocomplexes were detected by horseradish peroxidase-conjugated sheep IgG against either rabbit or mouse IgG. Blots B and C were prepared at the same time, blot B corresponding to one face of the slab gel and blot C to the other. Designations are discussed in the text.

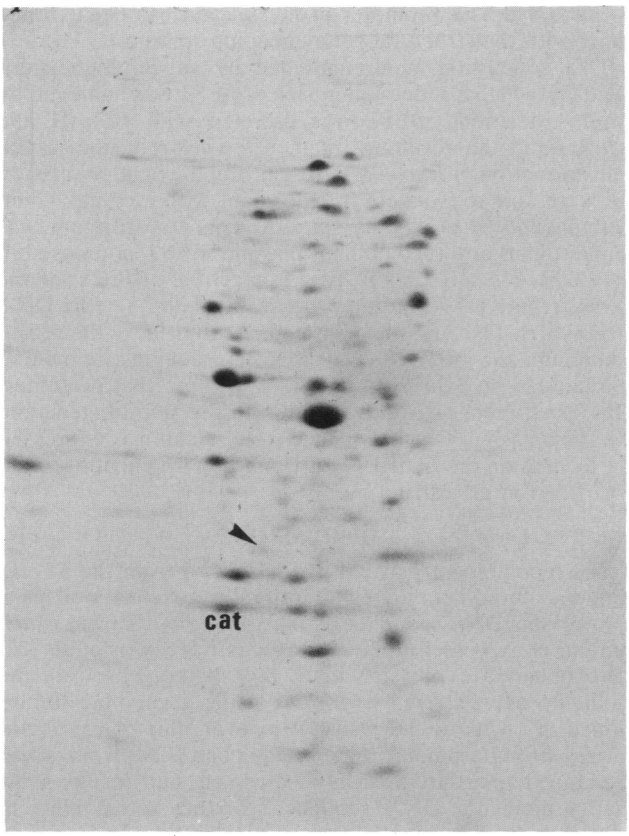



FIG. 2. Two-dimensional analysis of proteins from *E. coli* transformant Tsp. Proteins (200 μg) were submitted to analysis as described in Materials and Methods. Staining was with Coomassie blue. The arrow indicates protein 4 of Fig. 1B and 1C. The spot labeled CAT represents the chloramphenicol acetyl transferase coded by the pBR328 fragment of plasmid pES1.

ant Tsp after Western blotting. Also, *S. citri*-specific IgG as well as monoclonal antibody against spiralin gave no reaction with proteins of *E. coli* HB101 carrying plasmid pBR328 alone. On the basis of these results, proteins 3, 4, and 5 and very probably also 1 and 2 represent various forms of spiralin. To confirm the synthesis of spiralin by *E. coli* transformant Tsp, protein 4 from a two-dimensional gel stained with Coomassie blue and similar to that of Fig. 2 was cut out and then submitted in the gel slice to limited proteolysis with protease from *S. aureus* V8. The peptides generated by proteolysis were separated in a 15% acrylamide slab gel and stained by the silver nitrate method described by Oakley et al. (17). The resulting profile was compared with that given by spiralin recovered from a two-dimensional gel of *S. citri* proteins. Figure 3 shows that the two profiles are identical. This result is direct evidence that the immunologically stained protein 4 is closely related to spiralin on the basis of protein structure.

**Characterization of the *S. citri* DNA fragment coding for spiralin in *E. coli* transformant Tsp.** The recombinant plasmid (pES1) carried by *E. coli* transformant Tsp was purified upon amplification by spectinomycin. When *E. coli* HB101 cells were transformed with purified pES1, all the *E. coli* transformants with a Cam$^r$ phenotype gave a strong positive ELISA reaction, showing that the spiralin gene is indeed carried by pES1. Restriction mapping of pES1 revealed that a 6.5-kbp spiroplasmal DNA fragment was inserted into the *Hin*dIII site of the pBR328 vector (Fig. 4). The 6.5-kbp spiroplasmal DNA insert contained a unique *Pst*I and two *Eco*RI sites but had no *Bam*HI or internal *Hin*dIII sites. It should be noted that this cloned DNA fragment is much larger than the 0.8 kbp of DNA required to encode spiralin.

**Spiralin gene is expressed in *E. coli* from a spiroplasmal DNA sequence acting as a promoter.** It is well known that the pBR328 *Hin*dIII site lies in or very close to the promoter of the tetracycline resistance (Tet$^r$) gene (21). The spiralin gene on the 6.5-kbp DNA fragment from *S. citri* may thus be expressed from pES1 in two different ways: from the promoter of the Tet$^r$ gene or from a spiroplasmal DNA sequence carried by the *S. citri* DNA insert and recognized as a promoter by the bacterial RNA polymerase. We therefore constructed a recombinant plasmid, pES2, carrying the 6.5-kbp spiroplasmal DNA fragment inserted in an orientation opposite to that in pES1. The pES2 recombinant plasmid was obtained upon digestion of pES1 by *Hin*dIII and religation of the generated restriction fragments. The ligation products were used to transform *E. coli* HB101 cells. *E. coli*

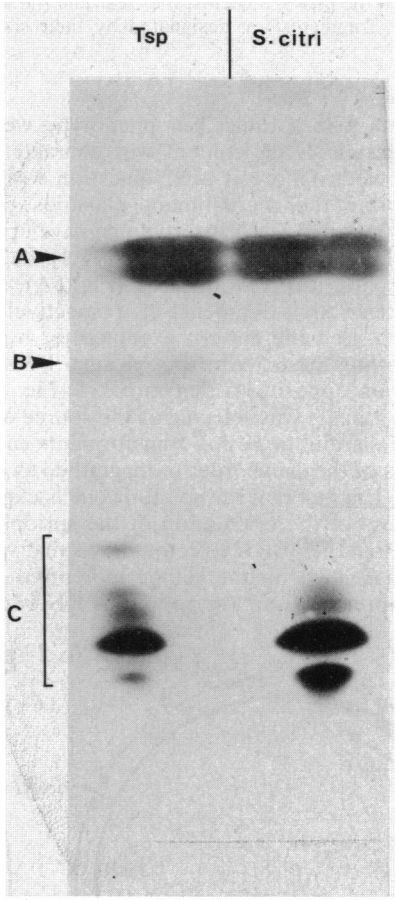



FIG. 3. Peptide maps of spiralin from *S. citri* and of protein 4 of *E. coli* transformant Tsp. The gel slices containing the proteins were applied to a second SDS gel (15% acrylamide) in the presence of 5 μg of *S. aureus* protease. The peptides generated by limited proteolysis were separated by SDS-PAGE in a 15% acrylamide gel and stained by the silver nitrate method described by Oakley et al. (17). (A) Protease from *S. aureus* V8; these were the only bands obtained when the protease was submitted alone to SDS-PAGE. (B) Uncleaved proteins: these were the only bands obtained in the absence of protease. (C) Peptides generated by the proteolytic treatment.

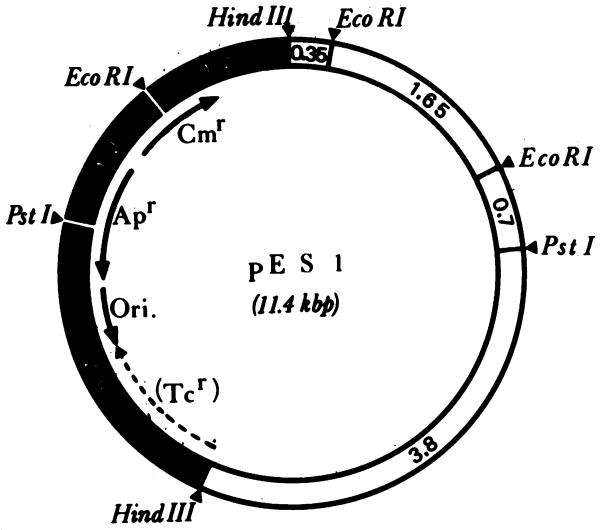



FIG. 4. Restriction cleavage map of plasmid pES1. The black line represents the pBR328 vector, the clear line the 6.5-kbp insert. The digestion fragments are designated by their size in kilobase pairs.

transformants with a Cam$^r$ Tet$^s$ phenotype were selected, and their plasmid DNA content was characterized by 1% agarose gel electrophoresis after digestion with *Hin*dIII or *Eco*RI. Among the recombinant plasmids carrying the 6.5-kbp spiroplasmal DNA insert, some gave upon digestion by *Eco*RI three fragments of, respectively, 8.2, 1.65, and 1.55 kbp, a pattern typical of pES1 (Fig. 5A), while others gave three restriction fragments of, respectively, 5.7, 4.05, and 1.65 kbp, i.e., the pattern expected for a recombinant plasmid carrying the 6.5-kbp spiroplasmal DNA inserted in the orientation opposite to that in pES1 (Fig. 5B). One of these transformants was selected as the source of pES2. The synthesis of spiralin in *E. coli* transformants carrying pES1 or pES2 was of the same order of magnitude as indicated by the ELISA. The fact that the spiralin gene is expressed in *E. coli* regardless of the orientation of the spiroplasmal DNA fragment within the pBR328 vector shows that spiralin is not expressed in *E. coli* from the Tet$^r$ promoter of the vector, but instead is expressed from a spiroplasmal DNA sequence that is functional as a promoter in *E. coli* and has been cloned along with the structural gene encoding spiralin.

This conclusion is strengthened by subcloning experiments in which a reduction in size of pES1 was achieved by various deletions. pES1 was digested with *Hin*dIII and *Eco*RI, and the resulting fragments were religated. Upon transformation of *E. coli* HB101 cells by the ligation mixture we were able to isolate a transformant with an Amp$^r$ Cam$^s$ Tet$^s$ phenotype producing spiralin. This transformant was found to harbor a recombinant plasmid, pES3, composed of the *Eco*RI-*Hin*dIII 3.7-kbp large fragment of pBR328 and the *Eco*RI-*Hin*dIII 4.5-kbp large fragment of the *S. citri* DNA insert carried by pES1 (Fig. 3C). pES3 thus lacks the region containing the pBR328 Tc$^r$ promoter, demonstrating that spiralin can be expressed in *E. coli* without that promoter. This experiment also shows that both the spiralin gene and the spiroplasmal DNA sequence from which it is expressed are located on the *Eco*RI-*Hin*dIII 4.5-kbp fragment of the *S. citri* DNA insert carried by pES1.

## DISCUSSION

The recombinant plasmid pES1 isolated from the *S. citri* genomic library contained the spiralin gene as well as a spiroplasma DNA sequence acting as a promoter from which spiralin is expressed in *E. coli*. Recently, Taylor et al. (22) have obtained expression in *E. coli* of antigens from the mollicute *Mycoplasma hyorhinis*, using a genomic library cloned in λ Charon 4A phage. However, this phage vector carries strong promoter regions (P*lac* and λ P4) from which the cloned genes are probably expressed. Our results show that a mollicute DNA sequence can act as an efficient promoter in *E. coli* and that a mollicute gene can be expressed from that sequence. This finding is interesting since mollicutes are thought to derive from bacteria by degenerative evolution (11).

Several forms of spiralin were produced in *E. coli* (Fig. 1B). They had different isoelectric points but quite closely related molecular sizes. Minor proteins 1, 2, and 3 and major proteins 4a and 5a had slightly higher molecular sizes (30 kDa) than genuine *S. citri* spiralin (28 kDa). Only proteins 4b and 5b had a molecular size close to that of *S. citri* spiralin. Interestingly, a minor form of spiralin (pS on Fig. 1B) with a molecular size of 30.5 kDa could sometimes be detected in *S. citri* by SDS-PAGE, especially when spiralin was purified by immunoaffinity chromatography before electrophoresis. The

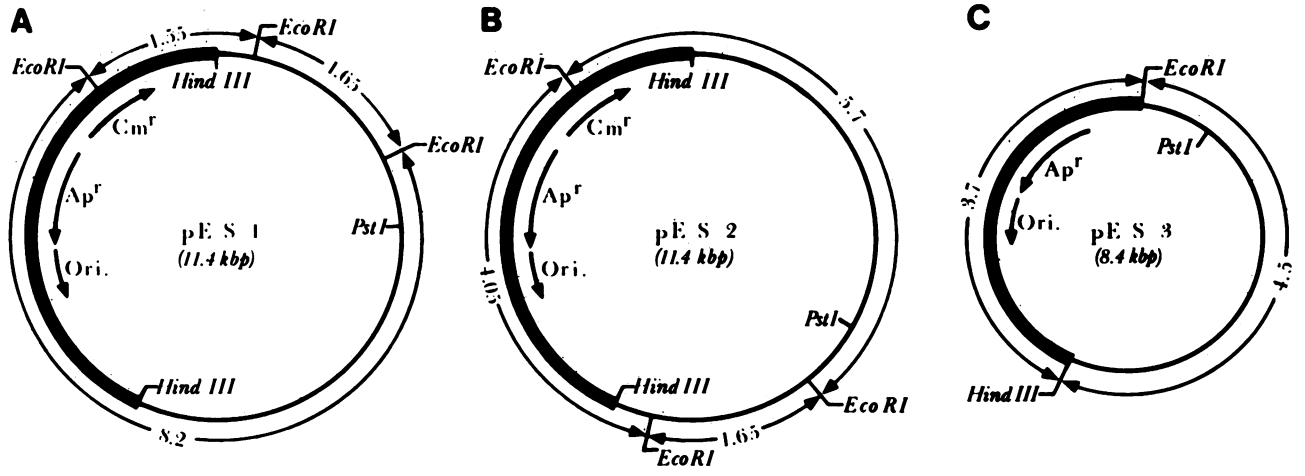



FIG. 5. Restriction maps of the three recombinant plasmids expressing spiralin in *E. coli*. The heavy lines represent the pBR328 vector-derived DNA. Ori, Origin of replication.

various forms of spiralin, ranging from 30.5 to 28.0 kDa, might represent a variety of postranslational modifications as has been suggested for the pectate lyase gene of *Erwinia chrysanthemi* cloned and expressed in *E. coli* (6). Knowing that spiralin is a membrane protein, it is tempting to envisage the minor spiralin form (30.5 kDa) in *S. citri* as a prespiralin containing a polypeptidic extension which may be a signal sequence equivalent to that demonstrated for bacterial and eucaryotic membrane proteins (25). The major form of spiralin in *S. citri* membrane (28 kDa) would lack the signal sequence. According to their molecular sizes proteins 1, 2, 3, 4a, and 5a in *E. coli* transformant Tsp would be intermediates between prespiralin and spiralin. An inefficient posttranslational processing may explain the accumulation of prespiralin-like protein in *E. coli*. However, an eventual signal sequence peptide could not be detected on the proteolytic digestion profile of protein 4. The signal peptide would be missed if it contained several protease V8 recognition sites.

Finally, the molecular cloning and expression of the *S. citri* spiralin gene in *E. coli* provide an experimental model for the study of membrane protein synthesis and processing in mollicutes. They also open the way to the large-scale production of mollicute antigens that can be useful for structural studies or for the development of vaccines against mollicutes pathogenic to animals. In this respect, the isolation and introduction in a cloning vector of the spiroplasmal promoter sequence carried by pES1 should prove very useful for the expression of other mollicute antigens in *E. coli*. Since we have also been able to clone a spiroplasma plasmid carrying a spiroplasmal replication origin (12), the construction of a shuttle vector able to replicate in *E. coli* as well as in *S. citri* should be possible soon.

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