MOLECULAR PLANT-MICROBE INTERACTIONS
Vol. 3, No. 2, pp. 78-85, 1990

Cloning and Characterization of a Disease Resistance Response Gene in Pea Inducible by *Fusarium solani*

Chin C. Chiang and Lee A. Hadwiger

Program in Genetics and Cell Biology and Department of Plant Pathology, Washington State University, Pullman 99164-6430 U.S.A.
Received 10 July 1989. Accepted 23 October 1989.

Disease resistance response genes (DRRG) of peas are expressed as the tissue is expressing race-specific or nonhost resistance. A pea genomic clone DRRG49-c encompassing one DRRG structural gene, the expression of which is correlated with the expression of disease resistance, was sequenced and characterized. The 2.3-kb genomic segment sequenced encompassed 986 bp 5′ to the major transcriptional initiation site, a 474-bp open-reading frame interrupted by one 88-bp AT-rich intron and an additional 574-bp segment 3′ from the stop codon. Southern blot analysis indicated that the DRRG structural gene is one of a multigenic family, and an estimated five copies exist within the pea genome. Primer extension analysis of the 5′ terminus of the corresponding RNA suggested the presence of one major transcript with possibly two minor transcripts. The major transcript, located 65 bp from the translational initiation site, was expressed when challenged with *Fusarium solani* f. sp. *phaseoli* but not with water. The structural gene sequence corresponding to the genomic clone DRRG49-c is not identical with the structural gene of the cDNA clone DRRG49-a used as a probe for northern blot analysis, and thus, a possibility remains that it is not expressed in peas; however, the DRRG49-c promoter was able to express the chloramphenicol acetyltransferase reporter gene in tobacco protoplasts. Western blot analysis using antiserum prepared from a β-galactosidase-DRRG49-a fusion protein identified the DRRG49 gene product as a major protein accumulating during the host-pathogen interactions.

Additional keywords: gene structure, *Pisum sativum*, transient expression.

Fusarium solani (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) Snyder & Hans macroconidia or the chitosan released from its cell wall effectively elicits a broad range of host defense responses. These responses include the production of phytoalexins (Hadwiger and Beckman 1980). Levels of two hydrolytic enzymes, chitinase and β-1,3-glucanase (Mauch *et al.* 1984; Mauch *et al.* 1988), and at least 20 proteins (Loschke *et al.* 1983) are also enhanced. After a 6-hr lag period, the enhanced activation of chitinase and β-1,3-glucanase occurs simultaneously and equally in both susceptible and resistant reactions. However, the temporal activities of some genes can be distinguished in resistant and susceptible interactions (Daniels *et al.* 1987; Fristensky *et al.* 1985). These genes are induced in both the compatible and incompatible interactions; however, the host is unable to attain high levels or maintain large accumulations of their mRNAs and proteins (Hadwiger and Wagoner 1983) in the compatible reaction. The clones of genes whose induction correlates with the expression of disease resistance have been termed disease resistance response genes (DRRG).

The accumulation of DRRG mRNA in pea endocarp tissue occurs 2–4 hr after treatment with chitosan or inoculation with the incompatible pathogen *F. s.* f. sp. *phaseoli* (Fristensky *et al.* 1985); at this time, cytologically observed resistance is occurring. Conversely, there is a sharp reduction in DRRG mRNA accumulation at 20–24 hr when cytologically observed susceptibility occurs in the compatible reaction with *F. s.* f. sp. *pisi* (Lindford) Snyder & Hans.

To understand the molecular mechanism of induction and host-regulated responses, pea genomic clones of DRRG49-c were obtained using the homologous pI49 (hereafter called DRRG49-a) cDNA as a probe. A transient expression system was used to test the functionality of the DRRG49-c promoter. DNA sequence analysis identified several potential regulatory regions and a cluster of consensus sequences for topoisomerase II cleavage (Sander and Hsieh 1985). The implication of such sequences in gene regulation will be discussed. In this study, we also report the construction of a β-galactosidase-DRRG49-a fusion protein. The antiserum prepared from this hybrid protein was used to study the gene product of DRRG49 during the host-pathogen interactions.

MATERIALS AND METHODS

Bacterial strains. *Escherichia coli* strains MH1000 and TK1046 (Weinstock *et al.* 1983) were used for expression of the fusion protein.

RNA and DNA isolation. Total RNA was isolated from *Pisum sativum* L. cv. Alaska endocarp tissue 6 hr after water or *F. s.* f. sp. *phaseoli* treatments as described previously (Wagoner *et al.* 1982). Poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography (Maniatis

Address reprint requests to L. A. Hadwiger.

Nucleotide and/or amino acid sequence data is to be submitted to GenBank as accession number JO3680.

et al. 1982). Plasmid DNA was isolated by the small-scale alkaline lysis method (Birnboim and Doly 1979). Single-stranded DNA for sequencing analysis was isolated from a derivative of the Bluescript vectors using the K408 helper phage as described by the supplier (Stratagene, La Jolla, CA).

Screening of a genomic library. A pea genomic library in lambda vector Charon 32 was a gift from Mike Murray (Agrigenetics, Madison, WI). *E. coli* strain CES200 (Stratagene) was used as the host for lambda phage propagation. Approximately 10^6 recombinant plaques were screened using a random-primed DRRG49-a insert as a probe. Filter hybridization was performed essentially according to standard procedure (Maniatis *et al.* 1982). Positive plaques were selected and purified. Amplified plaques were further screened three times. The inserted DNA was purified (Maniatis *et al.* 1982) and analyzed using restriction enzymes. Restriction mapping was conducted by single and double enzyme digestion followed by Southern hybridization using the isolated DRRG49-a insert as a probe.

Southern hybridization. Total pea DNA was isolated from immature pea pods according to Polans *et al.* (1985). Ten micrograms of pea DNA was digested with the appropriate restriction enzymes and separated on a 0.8% agarose gel. Southern transfer and hybridization were done as described by Polans *et al.* (1985), except that formamide was omitted and hybridization was conducted at 65° C. The DRRG49-a cDNA insert was purified and ^{32}P-labeled by random oligonucleotide priming (Boehringer Mannheim Biochemicals, Indianapolis, IN). The washing was done in 2× SSC and 0.1% sodium dodecyl sulfate (SDS) at 65° C.

DNA sequencing. DNA sequencing was performed on clones derived either by subcloning restricted DNA fragments or with *Bal* 31 deletion treatment in Bluescript vectors. Sequencing reactions were conducted as recommended by the supplier of the Sequenase kit (U.S. Biochemical Corp., Cleveland, OH).

Primer extension analysis. A 20 nucleotide synthetic oligonucleotide (5′-AGTAAGAATGTCAGCATCTG-3′) complementary to position 139 to 158 from the transcriptional initiation site of DRRG49-c was synthesized (Stratagene) and purified on a 24% polyacrylamide-8 M urea sequencing gel. One hundred nanograms of oligonucleotide was end-labeled with 10 units of T4 polynucleotide kinase and 150 μCi γ-[^{32}P]ATP in a total of 20 μl of T4 polynucleotide kinase buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM dithiothreitol, pH 7.6). The mixture was incubated at 37° C for 45 min, and the reaction was then terminated by heating at 75° C for 10 min. After adding 1 μl of a 10 mg/ml stock solution of carrier yeast tRNA, 26 μl of 4 M ammonium acetate, and 125 μl of ice-cold 95% ethanol, the mixture was held at −80° C for 15 min and centrifuged at 4° C for 30 min. The pellet was washed with cold 95% ethanol and dried briefly. Approximately 20 ng of labeled primer was added in 5 μl of 4× extension buffer (400 mM Tris-HCl, 40 mM $MgCl_2$, and 560 mM KCl, pH 8.3) containing 40 units of RNasin (Promega Biotech, Madison), 0.75 mM dNTP, 20 mM β-mercaptoethanol, 10 units of AMV reverse transcriptase (International Biotechnologies, New Haven, CT), 5 μg of poly(A)$^+$ RNA, or 50 μg of total RNA from fungal-treated tissue or from the H_2O control. The resulting mixture was incubated at 42° C for 90 min. The primer extension products were analyzed on a 6% polyacrylamide-8 M urea sequencing gel along with the M13 dideoxy sequencing products.

Transient expression. Tobacco protoplasts were isolated from an exponentially growing tobacco suspension culture. The isolation of protoplasts and electroporation procedures were conducted as described by Fromm *et al.* (1985). CsCl-purified plasmid DNA was used in all the experiments. Protoplasts were allowed to incubate in the dark at 28° C for 20 hr before harvesting for the chloramphenicol acetyltransferase (CAT) assay.

Construction of a fusion protein and preparation of an antiserum. A 281-base pair (bp) gel-purified *Alu*I fragment from DRRG49-a cDNA was ligated in frame with the pORF1 expression vector (Weinstock *et al.* 1983). The resulting plasmid was used to transform *E. coli* strain MH1000, and the transformants were screened for β-galactosidase activities. Plasmids of the positive clones were used to transform TK1046 cells. The induction of β-galactosidase-DRRG49-a fusion proteins was as described previously (Weinstock *et al.* 1983). The large-scale protein preparation was fractionated by 10% SDS-PAGE. The distinct fusion protein band was excised and ground in 5 ml of complete adjuvant. The resulting mixture was subcutaneously injected into a New Zealand white rabbit at 1-wk intervals for 4 wk.

Western blot analysis. Total proteins from both fungal-inoculated and water-treated pea pods were isolated as described previously (Mauch *et al.* 1988). After electrophoresis, the proteins were electrotransferred onto a nitrocellulose sheet. Western blotting was conducted essentially as described by Knecht and Dimond (1984) using an alkaline phosphatase-conjugated second antibody.

Computer analysis. All computer manipulation was done using the University of Wisconsin Genetics Computer Group (UWGCG), Madison, package provided by the Visualization, Analysis and Design in the Molecular Science (VADMS) Laboratory at Washington State University.

RESULTS

Isolation and characterization of DRRG49-c. A pea genomic library constructed in lambda vector Charon 32 was used to isolate pea genomic clones. After screening approximately 10^6 recombinant plaques using a homologous cDNA, eight independent hybridization clones were identified and subsequently purified. Surprisingly, these eight clones have identical restriction patterns (data not shown). One of the genomic clones, designated DRRG49-c, was further characterized by Southern hybridization using a random-primed DRRG49-a cDNA insert. The region homologous with the DRRG49-a cDNA clone was mapped within a 2.3-kilobase (kb) *Sal*I-*Bam*HI fragment (Fig. 1). This fragment was subcloned into pUC18 or a Bluescript vector for further analysis.

Structural analysis of DRRG49-c. The genomic nucleotide sequence of the 2.3-kb *Sal*I-*Bam*HI fragment is shown in Figure 2. This sequence is organized into two exons, 181 bp and 293 bp, interrupted by an intron of 88 bp. The intron contains typical GT/AG splicing borders and has an A+T content of 75%. The deduced amino acid sequence of DRRG49-c contains 158 amino acids with a predicted molecular weight of 16,800 and a pI of 4.4.

Southern blot hybridization of *Eco*RI- and *Xba*I-digested pea DNA, using homologous DRRG49-a cDNA as a probe, reveals five bands of different intensities (Fig. 3, lanes 1 and 3). This indicates that the pea genome has five related DRRG49 genes which share different degrees of homology. A 10.5-kb *Eco*RI fragment and a 5.0-kb *Hin*dIII fragment (Fig. 3, lanes 1 and 2, respectively) correspond to the genomic map of DRRG49-c (Fig. 1A). A *Hin*dIII fragment of 11 kb shows a band of much darker intensity when compared with the others, which suggests more than one gene shares a similar 11-kb *Hin*dIII fragment. The presence of multigenic DRRG49 is also indicated by direct sequence comparisons.

The predicted amino acid sequences of DRRG49-c show 85% homology with two previously sequenced homologous cDNAs (Fristensky *et al.* 1988) (Fig. 4B). When comparisons were based on functionally conserved amino acids, a 93% sequence homology was observed. The 5′ noncoding regions of the two cDNAs share extensive DNA sequence homology with DRRG49-c, except for a deleted region in DRRG49-c (Fig. 4A). The two cDNAs represent nearly full-length mRNA copies when compared to the primer extension product of DRRG49-c. The 3′ noncoding region of DRRG49-c shares less homology with the two cDNAs, being only 66% overall (data not shown).

5′ transcript analysis of DRRG49-c. The transcription initiation site of DRRG49-c was determined by primer extension analysis. A 20 nucleotide synthetic oligonucleotide that is complementary to the first exon (position 139 to 158 in Fig. 2) was used to hybridize to different sources of RNA. This primer differs in 3 bp and 1 bp near the central region of DRRG49-a and DRRG49-b (previously designated pI49 and pI176), respectively. The primer extension products indicated one major transcript and two possible minor transcripts (Fig. 5). The major transcript is initiated at a position 65 bp from the translational initiation site, and its expression can be detected when treated with *F. s.* f. sp. *phaseoli* (Fig. 5, lanes 2 and 7). Two minor transcripts, initiated at positions which are 33 and 34 bp from the translational initiation site, are present in both the water control pods and pods treated with *F. s.* f. sp. *phaseoli* (Fig. 5, lanes 1, 2, and 7). There was no significant difference between the total RNA or poly(A)$^+$ RNA substrate in the primer extension analysis.

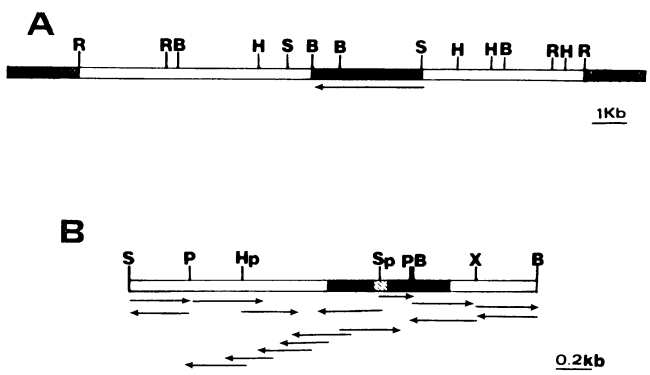

Fig. 1. Restriction map and sequencing strategy of DRRG49-c. **A**, The size of the genomic clone is 12.8 kilobases (kb). The left and right arms of lambda vector Charon 32 are shown as cross-hatched blocks. The restriction fragments hybridizing to the cDNA probe are shown as a black block, and the subsequently derived direction of transcription is indicated by an arrow. **B**, A restriction map of the *Sal*I-*Bam*HI fragment. The two exons are indicated by black blocks, and the intron is shown by a hatched box. The arrows indicate the direction and progression of the sequence. R, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; S, *Sal*I; P, *Pst*I; Hp, *Hpa*I; Sp, *Spe*I; and X, *Xba*I.

Transient expression of DRRG49-c in tobacco protoplasts. A chimeric gene composed of a DRRG49-c promoter and a CAT reporter gene was constructed. A *Bal*31 deletion end of the DRRG49-c promoter fragment spanning positions −1087 to +32 was transcriptionally fused to the CAT reporter gene and was followed by the insertion of the nopaline synthase terminator signal (Fig. 6B). Thus the chimera retains the major transcription initiation site of DRRG49-c. As shown in Figure 6A, lane 3, the DRRG49-c-CAT chimeric gene was expressed in the tobacco protoplasts. The 35S promoter driven CAT expression was used as a positive control (Fig. 6A, lane 2). No detectable CAT expression was obtained when the control Bluescript vector was used (Fig. 6A, lane 1).

5′ and 3′ flanking sequences of DRRG49-c. Sequence analysis of the 5′ flanking region of DRRG49-c reveals two putative consensus TATA boxes that are located −19 bp and −51 bp from the major transcriptional initiation site (boxed in Fig. 2). Two 7-bp direct repeats (ACCAAAT) homologous to the consensus "CCAAT" box are located at positions −99 and −140 (boxes with arrows in Fig. 2). The putative polyadenylation sequence of DRRG49-c is located at position 799 (underlined in Fig. 2). In general, the flanking sequences of DRRG49-c are AT-rich. The A+T content of the 5′ flanking sequence is 70%, and the 3′ flanking sequence has an A+T content of 76%. There are several putative regulatory elements found upstream of DRRG49-c. These include purine-rich direct repeats, inverted repeats, dyad symmetry, consensus enhancers, and topoisomerase II cleavage sequences. The significance of these sequences to gene regulation will be discussed below.

Construction and expression of a fusion protein in *E. coli*. To analyze DRRG49 gene products in pea, a DRRG49-a-β-galactosidase fusion protein was constructed in vector pORF1. This vector contains an *ompF* promoter, a regulator, a 33-bp structural region, and a synthetic linker followed by a *lacZ* gene. The *lacZ* gene is not in frame with the upstream linker region, which blocks *lacZ* expression. A 281-bp *Alu*I fragment that covers part of codon 22 and through codon 115 of DRRG49-a was inserted in the *Sma*I site of the linker region which resulted in a translational in-frame fusion with the β-galactosidase N-terminal sequence (Fig. 7). The hybrid protein was overexpressed in *E. coli* strain TK1046, which contains an *ompR* constitutive mutation.

Analysis of the DRRG49 gene product. Total pea proteins isolated from different periods in the pea-*F. s.* f. sp. *phaseoli* interaction were separated on 10–16% SDS-PAGE gradient gels. In the gel stained with Coomassie blue, a gradual increase in the 17-kDa protein was clearly visible at each inoculation time in the fungal-treated samples (Fig. 8A, lanes 1 to 6). On the other hand, only a low level of the 17-kDa protein band was present in extracts of water-treated pea pods. This 17-kDa protein closely matches the predicted molecular mass of the DRRG49 product. To confirm that the 17-kDa protein is the gene product of DRRG49, western blots were conducted with antiserum prepared from the β-galactosidase-DRRG49-a fusion protein. As shown in Figure 8B, a distinct 17-kDa protein band was visible. No detectable protein of DRRG49 was observed for the zero hour treatment (Fig. 8B, lane 1). The gene product of DRRG49 was detectable at 6 hr after treatment with *F. s.* f. sp. *phaseoli* and increased to the maximum as the incubation time increased to 46 hr (Fig. 8B, lanes 1 to 6). In contrast, no detectable DRRG49

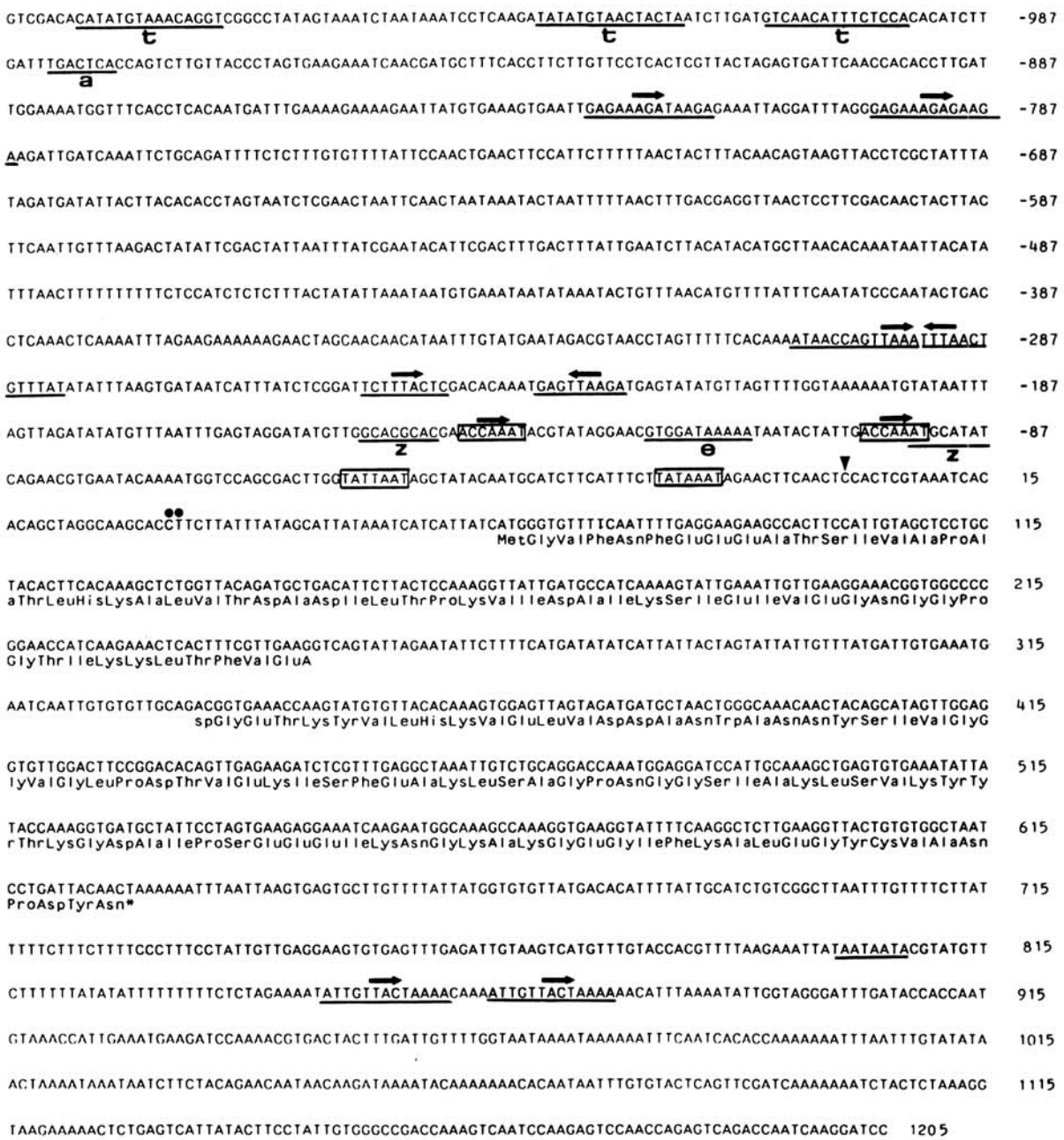

Fig. 2. Nucleotide sequence and deduced amino acid sequence of DRRG49-c. The putative consensus sequences TATA and CCAAT are boxed. The putative polyadenylation signal is underlined. The major transcription site is shown by an arrowhead. Two possible minor transcription initiation sites are indicated by dots. Repeats are underlined, and the direction of the repeats is indicated by arrows. All putative regulatory elements are underlined and designated with the following symbols: e, SV40 core enhancer; t, consensus topoisomerase II cleavage sequence; a, consensus nuclear factor AP-1 binding site; and z, potential Z-DNA-forming regions.

protein was observed until 16 hr after treatment with water, and the amount of protein remained at this basal level through hour 46 (Fig. 8B, lanes 7 to 11). It is apparent that the gene products of DRRG49 constitute a major pea protein fraction during fungal challenge.

DISCUSSION

A genomic clone, which is homologous with a cDNA clone induced in pea by an incompatible pathogen, *F. s.* f. sp. *phaseoli*, has been isolated and characterized. Both the coding region and a 5′ noncoding region of DRRG49-c share a high degree of homology with two DRRG49 cDNAs as indicated by direct sequence comparison. Primer extension analysis indicates that one major transcript is initiated 65 bp from the translational initiation site, primarily in fungal-challenged tissue. Since this primer differs from DRRG49-b by only one base pair (near the center), it is not certain whether the transcripts represent DRRG49-c. It is possible that the transcripts include both DRRG49-b and DRRG49-c. Due to the multigenic characteristics of DRRG49, it is difficult to address the expression of DRRG49-c alone in pea tissue. However, the transient expression of the CAT gene driven by a DRRG49-c promoter indicates that the DRRG49-c promoter is functional in tobacco protoplasts. Further studies, such as using sequence specific oligonucleotides, should be able to address the expression of individual members of the DRRG49 family in pea.

There are also two minor extension products located about 33 bp from the start codon. These products appear both in water control and fungal-induced RNA samples as shown in Figure 5 (lanes 1, 2, and 7). Since there are two putative TATA-like boxes located upstream of DRRG49-c, it is possible that the minor products represent the authentic constitutive transcripts. Although there are no strong secondary structures observed from computer analysis, the two minor products could alternately represent either preferential degradation products from the major transcript or the product(s) of premature transcripts.

The 5′ flanking region of DRRG49-c contains sequence characteristics typical of those in some expressed eucaryotic genes. In addition to putative TATA and CAAT boxes observed upstream of DRRG49-c, a sequence homologous to the SV40 core enhancer (Weiher *et al.* 1983) is present at position −125 (symbol e in Fig. 2). Furthermore, this sequence is flanked by two 7-bp direct "CCAAT-box-like" repeats and two blocks of potential Z-DNA-forming sequences (symbol z in Fig. 2). The potential Z DNA regions in cereal storage protein genes as well as in the *Arabidopsis*

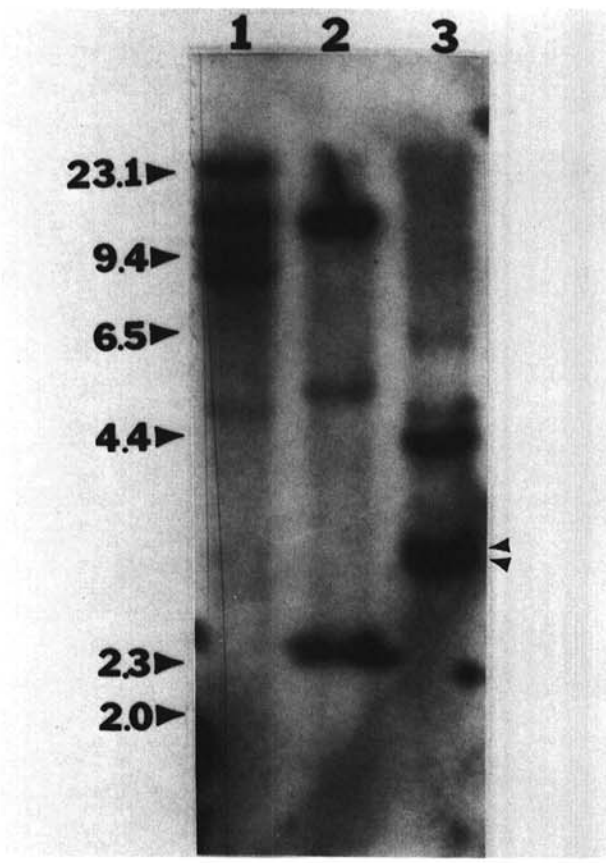

Fig. 3. Southern analysis of disease resistance response genes (DRRG) in pea. Total DNA was isolated from immature pea pods. Lanes 1, 2, and 3 show pea genomic DNA digested with *Eco*RI, *Hin*dIII, and *Xba*I, respectively. The blot was hybridized to the ^{32}P-labeled cDNA clone of DRRG49-a.

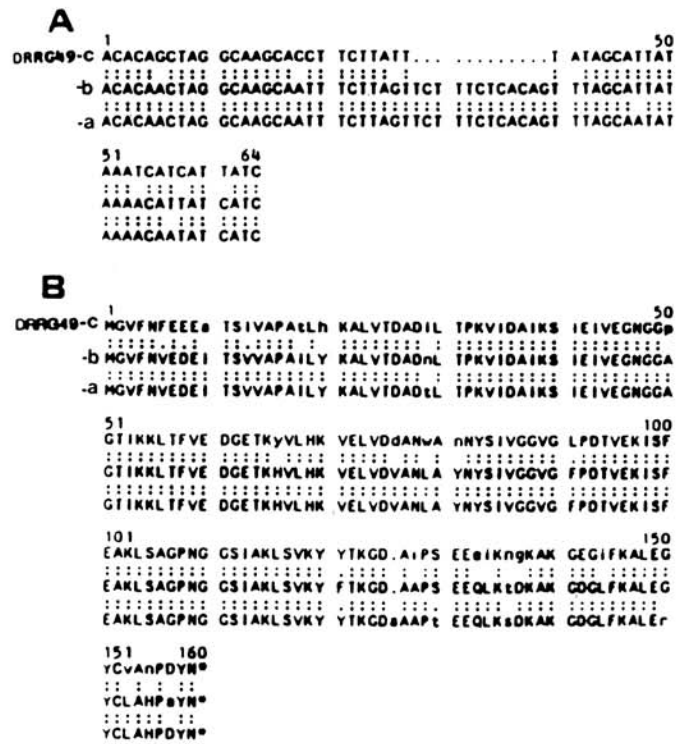

Fig. 4. Comparison of nucleotide and amino acid sequences of disease resistance response genes (DRRG). **A**, The 5′ noncoding regions of DRRG49-c are compared with two homologous cDNAs. The dotted lines within the nucleotide sequences are introduced during sequence alignment. **B**, The coding region of DRRG49-c is compared with two other cDNA protein sequences. DRRG49-a was formerly called pI49, and DRRG49-b was formerly referred to as pI176 (Fristensky *et al.* 1988). Capital letters indicate both identical and conserved amino acids. The lowercase letters indicate different amino acids. A single dot between amino acids indicates functional homology.

cab1 gene have been shown to be important in gene regulation (Maier *et al.* 1987; Ha and An 1988).

Another potentially important sequence shows a 26-bp dyad symmetry with a 1-bp mismatch located at 305 bp from the major transcriptional initiation site. In eucaryotic systems, such long dyad symmetry has been shown to be important for the binding of proteins and regulation of gene expression (Bram *et al.* 1986; Beier *et al.* 1985). The sequence TGACTCA, which is identical to the consensus binding site of the nuclear transcription factor AP-1 (Sassone-Corsi *et al.* 1988), is located at position −982. AP-1 binding sites appear to be highly conserved. Recently, it has been shown that a yeast nuclear factor also binds to specific AP-1 sequences and activates transcription in yeast (Jones *et al.* 1988).

Potentially important segments found 1 kb upstream of DRRG49-c are the clusters of consensus topoisomerase II cleavage sites (Figs. 2 and 9). Three consensus sites are located about 1 kb upstream from the major transcriptional initiation site (symbol t in Fig. 2). These sequences are clustered in a 5′ region of less than 100 bp. Topoisomerase II, which mediates some topological changes of DNA, has been shown to be associated with the nuclear matrix or scaffold (Berrios *et al.* 1985; Gasser *et al.* 1986). The nuclear scaffold attachment region (SAR) contains clusters of

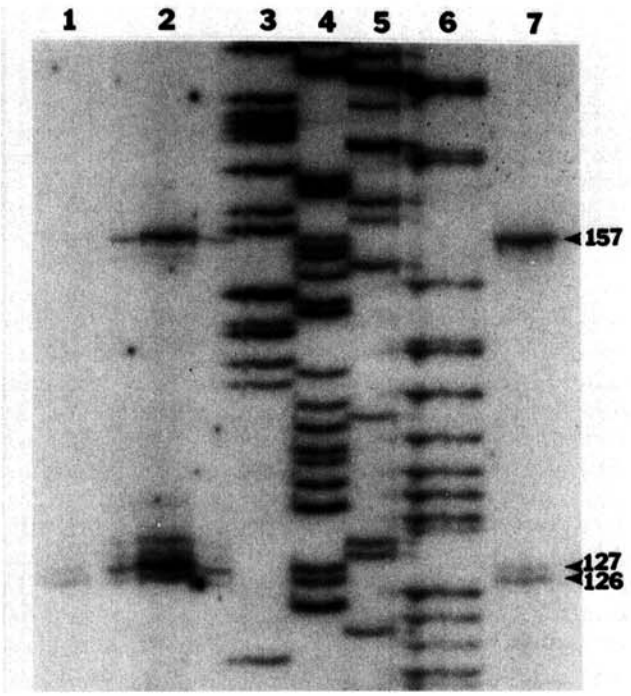

Fig. 5. Primer extension analysis of DRRG49-c. A 20 base pair synthetic oligonucleotide complementary to the first exon (at position 139 to 158 in Fig. 2) was used as a primer. Lane 1, total RNA extracted from pea pods treated with water; lane 2, poly(A)$^+$ RNA isolated from pea pods 6 hr after treatment with *Fusarium solani* f. sp. *phaseoli*; lanes 3–6, sequence ladder of G, A, T, C of M13 DNA, respectively; and lane 7, total RNA isolated from pea pods 6 hr after treatment with *F. s.* f. sp. *phaseoli*.

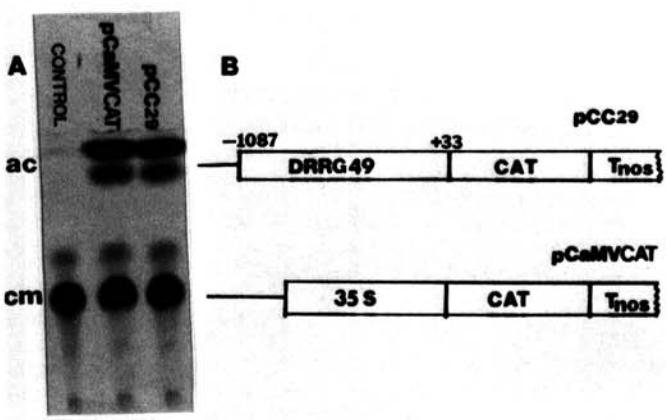

Fig. 6. Transient expression of a chimeric DRRG49-c-CAT gene in tobacco protoplasts. **A**, The expression of chloramphenicol acetyltransferase (CAT) with different constructs. Electroporation was done with 30 μg of constructs and 50 μg of carrier plasmid. For the control, 80 μg of carrier plasmid was used. **B**, Constructs of plasmids used for electroporation. cm, ^{14}C-labeled chloramphenicol; ac, acetylated ^{14}C-labeled chloramphenicol.

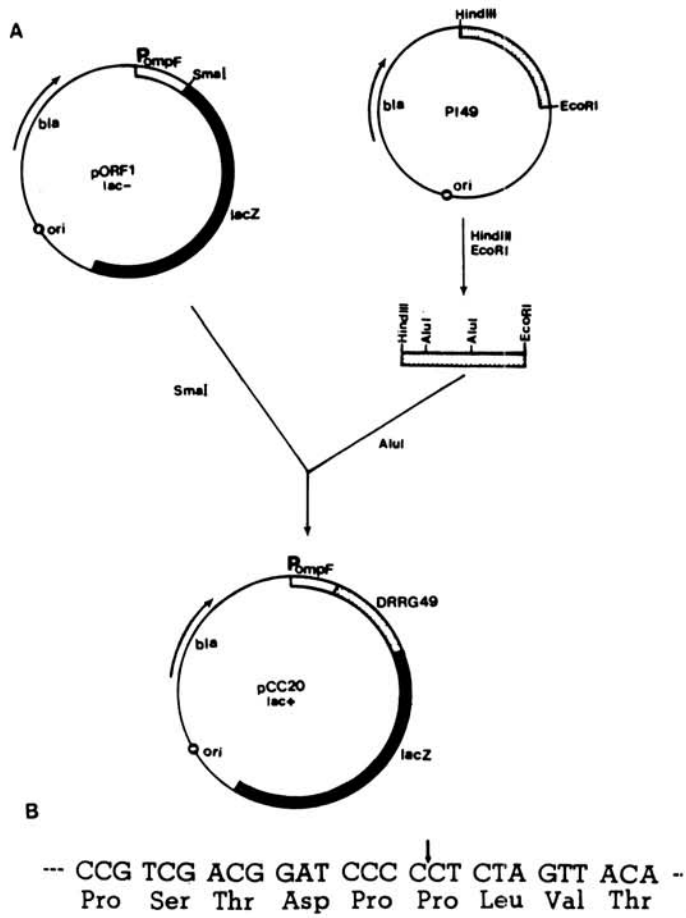

Fig. 7. Strategy for construction of a *lacZ*-DRRG49-a expression plasmid. **A**, Plasmid pI49 containing the DRRG49-a coding region (stippled block) was excised with restriction enzymes *Eco*RI and *Hin*dIII. The purified *Eco*RI-*Hin*dIII fragment was further digested with *Alu*I. A 281-base pair fragment was subsequently purified and ligated with the *Sma*I-digested pORF1 expression vector. The *lacZ*$^+$ clones were selected. The open block represents the promoter regions and 5′ coding sequence of the *ompF* gene. The darkened block indicates the *lacZ* structural gene that is missing the first eight amino acids. **B**, Fusion junction of *lacZ* and DRRG49-a. The vertical arrow indicates the fusion junction.

consensus topoisomerase II cleavage sequences (Gasser and Laemmli 1986a) and has been shown to be closely associated with *cis*-acting or enhancer elements in many eucaryotic systems (Gasser and Laemmli 1986b; Cockerill and Garrard 1986; Phi-Van and Strätling 1988; Jarman and Higgs 1988). Furthermore, when attached to the transforming vector, the boundary regions of the SAR appear to eliminate the position effect in transgenic systems (Gasser and Laemmli 1987).

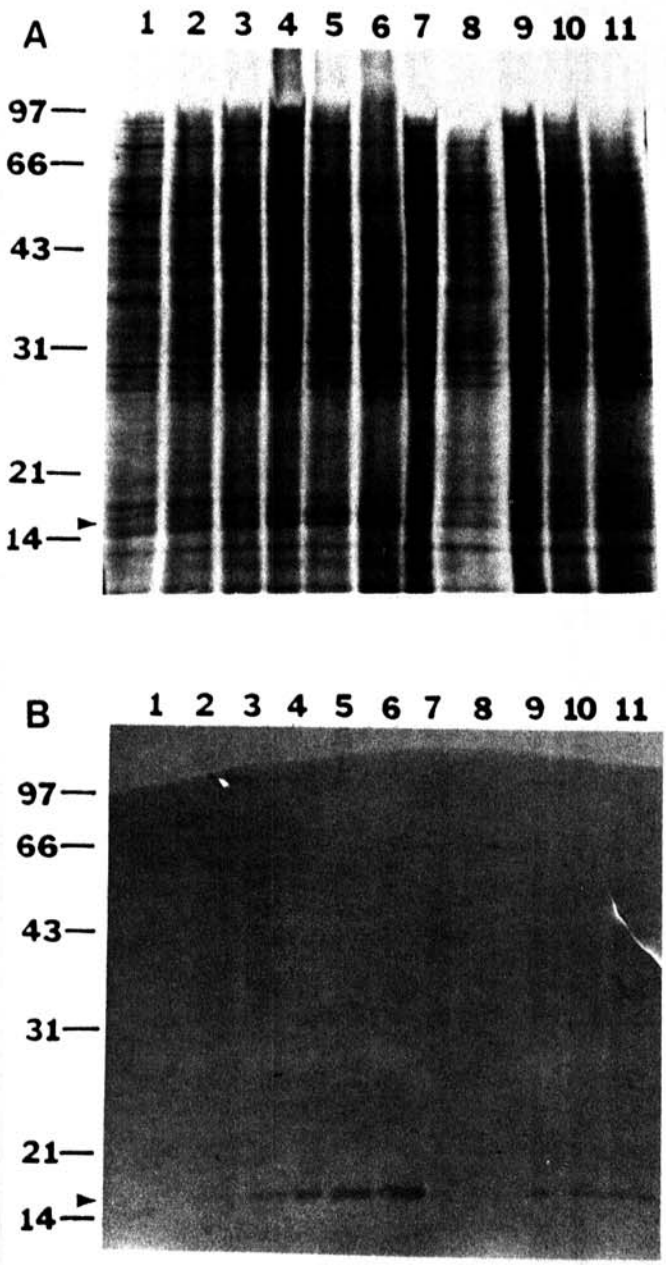

Fig. 8. Analysis of the DRRG49 protein in pea. **A**, Profile of total pea proteins following water or *Fusarium solani* f. sp. *phaseoli* treatments. Lanes 1 to 6, pea pods treated with fungus at 0, 6, 12, 16, 24, and 46 hr, respectively. Lanes 7 to 11, pea pods treated with water at 6, 12, 16, 24, and 46 hr, respectively. Forty micrograms of total pea proteins was loaded in each lane. **B**, Immunoblot of the DRRG49 protein following water or fungal treatments. Lane designations are the same as in **A**.

Topoisomerase II consensus sequence	GTN(A/T)(A/C)ATTNATNN(G/A)	
PEA DRRG49		
-1009	GTCAACATTTcTCCA	14/15
-1032 (opp. strand)	aTATACATTgATGAT	13/15
-1079 (opp. strand)	GTATACATTTgTCCA	14/15

Fig. 9. Consensus topoisomerase II cleavage sequence in DRRG49-c. The left-hand column shows the location of the topoisomerase II consensus sequence. The center and right-hand columns indicate the sequence and match to the topoisomerase II consensus sequences, respectively. The compared consensus sequence was taken from Sander and Hsieh (1985).

One of the important findings in this report is that the DRRG49 gene products constitute a major protein fraction following fungal challenge. In soybean suspension culture, a major 17-kDa protein was observed following elicitor treatment (Leguay *et al.* 1988). This protein has been shown to constitute about 20% of the total protein following treatment with fungal elicitors. An N-terminal protein sequence revealed that it shares extensive amino acid homology with DRRG49 (J. Leguay, Sanofielf Bio Recherches Labege Innopole, personal communication). Although the function of DRRG49 remains to be determined, we feel that because of its substantial accumulation in fungal-challenged pea tissue, it is likely to play an important role during host-pathogen interactions.

ACKNOWLEDGMENTS

We are grateful to Ann Scott Ettinger for screening the pea genomic library. We thank C. H. Daniels for critical reading of the manuscript. We also thank L. Thomashow for helpful advice on the fusion protein work. This work, PPNS 0032, College of Agriculture and Home Economics, Washington State University, Pullman, was supported by Agricultural Research Center Project 1844 and in part by the University of Washington Sea Grant program grant RX-13 and the Washington Technology Center.

LITERATURE CITED

Beier, D. R., Sledziewski, A., and Young, E. T. 1985. Deletion analysis identifies a region, upstream of the AdH2 gene of *Saccharomyces cerevisiae*, which is required for ADR1-mediated derepression. Mol. Cell. Biol. 5:1743-1749.

Berrios, M., Ucheroff, N., and Fisher, P. 1985. In situ localization of DNA topoisomerase II, a major polypeptide of the *Drosophila* nuclear matrix. Proc. Natl. Acad. Sci. USA 8:4142-4146.

Birnboim, H. C., and Doly, J. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7:1513-1523.

Bram, R. J., Lue, N. F., and Kornberg, R. D. 1986. A GAL family of upstream activating sequences in yeast: Roles in both induction and repression of transcription. EMBO J. 5:603-608.

Cockerill, P. N., and Garrard, W. T. 1986. Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II. Cell 44:273-282.

Daniels, C. H., Fristensky, B., Wagoner, W., and Hadwiger, L. A. 1987. Pea genes associated with non-host disease resistance to *Fusarium* are also active in race-specific disease resistance to *Pseudomonas*. Plant Mol. Biol. 8:309-316.

Fristensky, B. W., Hadwiger, L. A., Riggleman, R. C., and Wagoner,

W. 1985. Gene expression in susceptible and disease resistant interactions of peas induced with *Fusarium solani* pathogens and chitosan. Physiol. Plant Pathol. 27:15-28.

Fristensky, B. W., Hadwiger, L. A., and Horovitz, D. 1988. cDNA sequences for pea disease resistance response genes. Plant Mol. Biol. 11:713-715.

Fromm, M., Taylor, L. P., and Walbot, V. 1985. Expression of genes transferred into monocot and dicot plant cells by electroporation. Proc. Natl. Acad. Sci. USA 82:5824-5828.

Gasser, S. M., and Laemmli, U. K. 1986a. The organization of chromatin loops: Characterization of a scaffold attachment site. EMBO J. 5:511-518.

Gasser, S. M., and Laemmli, U. K. 1986b. Cohabitation of scaffold binding regions with upstream/enhancer elements of three developmentally regulated genes of *D. melanogaster*. Cell 46:521-530.

Gasser, S. M., and Laemmli, U. K. 1987. A glimpse at chromosomal order. Trends Genet. 3:16-22.

Gasser, S. M., Laroche, T., Falquet, J., Boy de la Tour, E., and Laemmli, U. K. 1986. Metaphase chromosome structure and involvement of topoisomerase II. J. Mol. Biol. 188:613-629.

Ha, S.-B., and An, G. 1988. Identification of upstream regulatory elements involved in the developmental expression of the *Arabidopsis thaliana cab1* gene. Proc. Natl. Acad. Sci. USA 85:8017-8021.

Hadwiger, L. A., and Beckman, J. M. 1980. Chitosan as a component of pea-*Fusarium solani* interactions. Plant Physiol. 66:205-211.

Hadwiger, L. A., and Wagoner, W. 1983. Electrophoretic patterns of pea and *Fusarium solani* proteins synthesized *in vitro* or *in vivo* which characterize the compatible and incompatible interactions. Physiol. Plant Pathol. 23:153-162.

Jarman, A. P., and Higgs, D. R. 1988. Nuclear scaffold attachment sites in the human globin gene complexes. EMBO J. 7:3337-3344.

Jones, R. H., Moreno, S., Nurse, P., and Jones, N. C. 1988. Expression of the SV40 promoter in fission yeast: Identification and characterization of an AP-1-like factor. Cell 53:659-667.

Knecht, D. A., and Dimond, R. L. 1984. Visualization of antigenic proteins on western blots. Anal. Biochem. 136:180-184.

Leguay, J. J., Piecoup, M., Puckett, J., and Jouanneau, J. P. 1988. Common responses of cultured soybean cells to 2,4-D starvation and fungal elicitor treatment. Plant Cell Rep. 7:19-22.

Loschke, D. C., Hadwiger, L. A., and Wagoner, W. 1983. Comparison of mRNA populations coding for phenylalanine ammonia-lyase and other peptides from pea tissue treated with biotic and abiotic phytoalexin inducers. Physiol. Plant Pathol. 23:163-173.

Maier, U.-G., Brown, J. W. S., Toloczyki, C., and Feix, G. 1987. Binding of a nuclear factor to a consensus sequence in the 5′ flanking region of zein genes from maize. EMBO J. 6:17-22.

Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Mauch, F., Hadwiger, L. A., and Boller, T. 1984. Ethylene: Symptom not signal for the induction of chitinase and β-1,3-glucanase in pea pods by pathogens and elicitors. Plant Physiol. 76:607-611.

Mauch, F., Hadwiger, L. A., and Boller, T. 1988. Antifungal hydrolases in pea tissue. I. Purification and characterization of two chitinases and two β-1,3-glucanases differentially regulated during development and in response to fungal infection. Plant Physiol. 87:325-333.

Phi-Van, L., and Strätling, W. H. 1988. The matrix attachment regions of the chicken lysozyme gene co-map with the boundaries of the chromatin domain. EMBO J. 7:655-664.

Polans, N. O., Weeden, N. F., and Thompson, W. F. 1985. The inheritance organization and mapping of *rbcS* and *cab* multigene families in pea. Proc. Natl. Acad. Sci. USA 82:5083-5087.

Sander, M., and Hsieh, T. 1985. *Drosophila* topoisomerase II double strand DNA cleavage site: Analysis of DNA sequence homology at the cleavage site. Nucleic Acids Res. 13:1057-1071.

Sassone-Corsi, P., Pamph, W. W., Kamps, M., and Verma, I. M. 1988. *fos*-associated cellular p39 is related to nuclear transcription factor AP-1. Cell 54:553-560.

Wagoner, W., Loschke, D. C., and Hadwiger, L. A. 1982. Two-dimensional electrophoretic analysis of *in vivo* and *in vitro* synthesis of proteins in peas inoculated with compatible and incompatible *Fusarium solani*. Physiol. Plant Pathol. 20:99-107.

Weiher, H., König, M., and Gruss, P. 1983. Multiple point mutations affecting the Simian Virus 40 enhancer. Science 219:626-631.

Weinstock, G. M., ap Rhys, C., Berman, M. L., Hampar, B., Jackson, D., Silhavy, T. J., Weisemann, J., and Zweig, M. 1983. Open reading frame expression vectors: A general method for antigen production in *Escherichia coli* using protein fusions to beta-galactosidase. Proc. Natl. Acad. Sci. USA 80:4432-4436.