Research Note

AVRXa10 Protein is in the Cytoplasm of *Xanthomonas oryzae* pv. *oryzae*

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AVRXa10 from *Xanthomonas oryzae* pv. *oryzae* was tagged with a unique hydrophilic octapeptide (FLAG) to permit antibody-mediated identification and purification of the gene product. *X. o.* pv. *oryzae* that produced tagged AVRXa10 elicited a hypersensitive response (HR) on rice cultivars containing the resistance gene *Xa-10*, but not on cultivars lacking *Xa-10*. The tagged AVRXa10 protein purified from *Escherichia coli* or *X. o.* pv. *oryzae* did not elicit a hypersensitive response in rice with the *Xa-10* resistance gene. Anti-FLAG monoclonal antibodies reacted with a 119-kDa protein in both *E. coli* and *X. o.* pv. *oryzae* cells expressing the tagged *avrXa10* gene. Polyclonal antibodies raised against purified AVRXa10 protein reacted with the 119-kDa protein and several additional proteins from *X. o.* pv. *oryzae*, which probably are the products of genes related to *avrXa10*. Biochemical fractionation and immunoelectronmicroscopy analysis was used to demonstrate that AVRXa10 was located in the cytoplasm of *X. o.* pv. *oryzae* cells when grown *in planta* or in culture medium.

Additional keywords: gene tagging, immunocytochemistry, electron microscopy, bacterial resistance.

Xanthomonas oryzae pv. *oryzae* (Ishiyama) Dye (Swings *et al.* 1990), the causal agent of bacterial blight of rice, interacts in a race-specific manner with rice cultivars containing different resistance genes (Mew 1987). The mechanisms by which the host and pathogen interact and result in the elicitation of disease resistance in a race-specific manner have yet to be elucidated. The products of avirulence genes cloned from both fungi and bacteria function either directly as race-specific elicitors or indirectly in governing the synthesis of race-specific elicitors. The product of the *avr9* gene of *Cladosporium fulvum*, a 28-amino-acid peptide after posttranslational processing, directly elicits a hypersensitive response (HR), which is associated with disease resistance in tomato cultivars carrying the Cf9 locus (Schottens-Toma and de Wit

1988, van Kan *et al.* 1991). The *avrD* gene product of *Pseudomonas syringae* pv. *tomato* directs the synthesis of syringolide compounds, which elicit the HR in soybean cultivars containing the Rpg4 resistance gene (Keen *et al.* 1990).

A family of avirulence genes identified from *X. o.* pv. *oryzae* hybridized with *avrBs3*, an avirulence gene from *Xanthomonas campestris* pv. *vesicatoria* (Bonas *et al.* 1989, Hopkins *et al.* 1992). Two of these genes (*avrXa7* and *avrXa10*) are structurally similar, each containing an internal domain with several copies of a 102-bp directly repeated sequence (Hopkins *et al.* 1992). The nucleotide sequence of *avrXa10* consisted of a 3,306-bp open reading frame, which would code for a predicted 119-kDa protein. The number of functional *avrBs3*-related genes in the genome of *X. o.* pv. *oryzae* is not known. As many as 12 *avrBs3* homologs are predicted in *X. o.* pv. *oryzae* strain PXO86 on the basis of restriction fragment analysis (Hopkins *et al.* 1992). Antiserum raised against the AvrBs3 protein was shown to cross-react with six to seven proteins in extracts of strain PXO86, indicating that several genes from the family may be expressed (Knoop *et al.* 1991).

Knowledge of the location of avirulence gene products in bacterial-plant interactions could provide insight regarding AVR protein function in the elicitation of resistance. Analysis of the nucleotide sequence of *avrXa10* and *avrBs3* revealed no apparent membrane spanning domains in the predicted protein products. Knoop and co-workers (1991) used an antiserum directed against AvrBs3 to demonstrate that most of the AvrBs3 protein was located in the cytoplasm. However, 20–30% was associated with the insoluble protein fraction of cell extracts. In a subsequent study using immunocytochemistry, the *avrBs3* gene product was shown to be located in the cytoplasm of *X. c.* pv. *vesicatoria in planta* and in bacterial cultures (Brown *et al.* 1993). The cellular location of the AvrBs3 product suggests that the protein is not recognized directly by plant cells. In this study, we investigated the location of the AVRXa10 protein from *X. o.* pv. *oryzae* both in inoculated plant tissues and bacterial cultures.

Characterization of AVRXa10.

The bacterial strains, plasmids, and phage used and their sources are listed in Table 1. The *avrXa10* gene was subcloned from the cosmid clone pXO5-15 (Hopkins *et al.* 1992) into pBluescript KS+. A portion of the downstream sequence, a 0.6-kb *Sma*I fragment, and the promoter-distal *Pst*I site were removed by digesting the clone with *Sma*I and

religating. The promoter and 5′ portion of the coding region were inserted as a 1.2-kb *Pst*I fragment. A 1.0-kb fragment upstream of the promoter was removed by digestion with *Xho*I, treated with mung bean nuclease, and ligated to *Eco*RI linkers (Promega G1061). The resulting construct, pBavrXa10, was used for further manipulations.

The *avrXa10* gene is one of approximately 12 related genes in the *X. o.* pv. *oryzae* genome. Therefore, an immunological tag (FLAG) was introduced into the C-terminal coding domain to detect the *avrXa10* gene product specifically (Fig.

1A). Single-stranded DNA of pBavrXa10 was isolated (Crouse *et al.* 1983) from *E. coli* and subjected to site-directed mutagenesis (Kunkel *et al.* 1987) with the oligonucleotide 5′GTTGAATGCCGGGAATTCTTTATCATCGT-CATCCTTGTAATCCGCAGCCCCAGC3′, which anneals to *avrXa10* at nucleotides 3376–3409 (Hopkins *et al.* 1992), to construct pBavrXa10.F1 (Fig. 1A). Seven additional codons were inserted in frame between 3390–3391 that encode the FLAG peptide DYKDDDDK (in bold). The T to A change at nucleotide 3393 created an *Eco*RI restriction site

Table 1. Bacterial strains, plasmids, and phage used in this study

| Designation	Relevant characteristics	Source
Xanthomonas oryzae pv. *oryzae*		
PXO99ᴬ	Race 6; virulent to rice with *Xa-10* resistance gene and grows on 200 µM 5-azacytidine	Hopkins *et al.* 1992
PXO86	Race 2; avirulent to rice with *Xa-10* resistance gene	T. Mew, IRRI, Los Baños, Philippines
Escherichia coli		
DH5α MCR	F⁻, *mcrA, recA, 80dlacZ, M15*	Gibco BRL, Grand Island, NY
BL21(DE3)	F⁻, lacUV5, ompT⁻, m⁻, Rifˢ	Novagen, Inc., Madison, WI
CJ236	*dut1, ung1, thi1, relA1*/pCJ105	Kunkel *et al.* 1987
Plasmids		
pUFR027	Kmʳ, *lacZ⁺*, Mob⁺, *mob*(P), Par⁺	De Feyter *et al.* 1990
pBluescript KS+	Apʳ, M13; *lacZ⁺*	Stratagene Inc., La Jolla, CA
pXO5-15	pHMI clone from *X. o.* pv. *oryzae* that contains *avrXa10*	Hopkins *et al.* 1992
pBavrXa10	pBluescript clone with a 4.6-kb *Eco*RI-*Sma*I fragment that contains *avrXa10*	This study
pBUavrXa10	pBavrXa10 clone cut with *Xba*I and cloned into pUFRO27	This study
pBUavrXa10-2	pBavrXa10 clone cut with *Kpn*I and cloned into pUFRO27	This study
pBavrXa10.F1	pBavrXa10 clone containing *avrXa10* tagged with the FLAG epitope sequences	This study
pBUavrXa10.F1	pBavrXa10.F1 clone cut with *Xba*I and cloned into pUFRO27	This study
pBUavrXa10.F2	pBavrXa10.F1 clone cut with *Kpn*I and cloned into pUFRO27	This study
pBU	pBluescript cloned into the *Eco*RI site of pUFRO27	This study
pET-22b+	Apʳ, T7*lac, pelB, lacI*	Novagen, Inc., Madison, WI
pET-avrXa10.F1	pET-22b+ clone with a 4.6-kb *Bam*HI fragment that contains the tagged *avrXa10*	This study
Phage		
M13K07		Vieira and Messing 1987
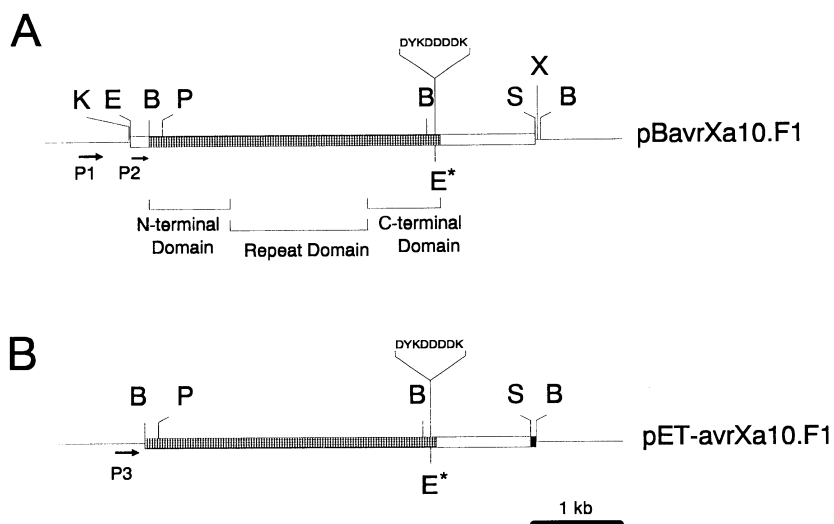
Fig. 1. Subclones containing *avrXa10*. The hatched boxes represent the *avrXa10* open reading frame. Arrows indicate promoters and the orientation of transcription. In pBavrXa10.F1, *avrXa10* is under the influence of its native promoter (P2) and the pBluescript *lacZ* promoter (P1). pBavrXa10.F1 carries a 21-nucleotide insertion that, with three nucleotides from the original clone, encodes for the FLAG peptide (DYKDDDDK). In addition, pBavrXa10.F1 carries an engineered *Eco*RI site indicated by the "*." The pET-avrXa10.F1 clone is under the influence of the pET-22b+ *T7lac* promoter (P3) and lacks the *avrXa10* promoter. Restriction enzyme cleavage sites are indicated: B, *Bam*HI; E, *Eco*RI; K, *Kpn*I; P, *Pst*I; S, *Sma*I; X, *Xba*I.

spanning nucleotides 3391 to 3396. Mutant clones of pBavrXa10.F1 were identified by cleaving with EcoRI. To test for avirulence activity, both pBavrXa10 and pBavrXa10.F1 were introduced into the broad-host-range plasmid pUFRO27. The plasmids were cleaved with either XbaI or KpnI, to orient the avrXa10 and avrXa10.F1 gene with respect to the pBluescript lacZ promoter, and ligated to pUFRO27 to generate pBUavrXa10.F1, pBUavrXa10.F2, pBUavrXa10, and pBUavrXa10-2. The endogenous avrXa10 promoter by itself (pBUavrXa10.F2 and pBUavrXa10-2) did not allow for levels of expression of the AVRXa10 protein that were sufficient for detection by immunocytochemistry in E. coli (data not presented).

Both clones pBUavrXa10 and pBUavrXa10.F1 were introduced to the X. o. pv. oryzae strain PXO99^A by electroporation as described (Choi and Leach 1994). The transformants were inoculated to rice cultivars IR24 and IR-BB10 as previously described (Reimers and Leach 1991) to test for the ability to elicit an HR. Rice cultivar IR-BB10 is near-isogenic to IR24 and contains the Xa-10 gene for bacterial blight resistance (Ogawa and Khush 1989; Ogawa and Yamamoto 1987). IR24 lacks the Xa-10 gene and is susceptible to all strains of X. o. pv. oryzae used in this study. In interactions involving avrXa10 with and without the immunological tag, and the resistance gene Xa-10 (IR-BB10), a dark brown color was observed throughout the infiltrated site within 24–48 hr, whereas in the interactions without Xa-10 (IR24) the infiltration site remained water soaked. The immunological tag (FLAG) was introduced into three other locations in the N-terminal, repeat, and C-terminal domains; however, the avirulence activity was lost in these mutants (data not presented).

To detect AVRXa10.F1, total protein extracts from X. o. pv. oryzae strain PXO99^A containing pBUavrXa10.F1 were analyzed in Western blots. Xanthomonas strains were grown in Terrific Broth (TB; Tartof and Hobbs 1987) supplemented with carbenicillin (100 μg/ml) and kanamycin (100 μg/ml), on a rotary shaker (250 rpm) at 28° C. Proteins were extracted and separated on sodium dodecyl-sulfate (SDS)-polyacrylamide gels (10% PAGE) as described (Laemmli 1970)

and either stained with Coomassie Blue R-250 or transferred electrophoretically to nitrocellulose membranes (Schleicher & Schuell, Keene, NH) using Towbin buffer (Towbin et al. 1979). Anti-FLAG IgG M2 monoclonal antibody (IBI/Kodak, New Haven, CT), which detects the FLAG peptide, specifically recognized a protein of approximately 119 kDa from cell extracts of X. o. pv. oryzae strain PXO99^A harboring the tagged avrXa10 gene (Fig. 2A, lane 3). A PXO99^A transformant harboring the untagged avrXa10 gene did not contain protein that reacted with the anti-FLAG antibody (Fig 2A, lane 2).

To prepare AVRXa10 polyvalent antiserum, plasmid pBavrXa10.F1 was digested partially with BamHI to isolate a 4.6-kb fragment. The 5′ terminus of the fragment was at the BamHI site in the coding region for the N-terminus of the peptide and included the portions coding for the FLAG sequence and carboxy-terminus. The 4.6-kb BamHI fragment was inserted into the expression vector pET-22b(+) (Novagen, Inc. Madison, WI) to generate pET-avrXa10.F1 (Fig. 1B). For the production of protein, E. coli BL21(DE3) containing pET-avrXa10.F1 was induced with 1 mM isopropyl-β-thiogalactopyanoside (IPTG). The protein was extracted by sonication and purified by chromatography on anti-FLAG M2 affinity gel, as described by the manufacturer (IBI/Kodak). For immunization of female white New Zealand rabbits, the protein (250 μg) was excised from SDS-polyacrylamide gels and emulsified with Freund's incomplete adjuvant (1:1). Antibodies for Western analysis were purified from the serum by protein A column chromatography (Sigma, St. Louis, MO) and multiple cross adsorption to BL21(DE3) acetone powder as described (Harlow and Lane 1988). The antibodies were used at a dilution of up to 1:5,000 relative to the original antiserum.

Polyclonal antibodies to AVRXa10 reacted only with the 119-kDa AVRXa10 protein in E. coli harboring the avrXa10 gene (Fig. 2B; lane 2). However, the antibodies also reacted with several protein bands in addition to the 119-kDa band in extracts from both PXO99^A and PXO86 (Fig. 2B; lanes 4–7). Antiserum raised to AvrBs3 cross-reacted with several proteins in extracts from X. o. pv. oryzae strain PXO86 and also
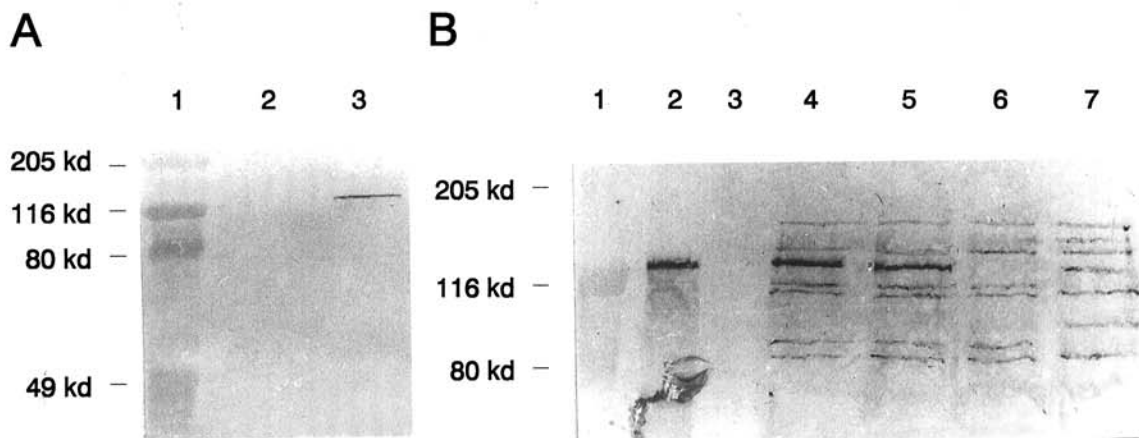

Fig. 2. Western blot analysis of total protein extracts from Xanthomonas oryzae pv. oryzae and E. coli expressing the AVRXa10 protein. Proteins were separated on a denaturing 10% polyacrylamide gel and transferred electrophoretically to nitrocellulose. **A,** X. o. pv. oryzae strain PXO99^A with untagged (lane 2) and tagged (lane 3) AVRXa10 protein, probed with anti-FLAG antibody. **B,** Total protein extracts from E. coli (DH5α) strains: DH5α (pBUavrXa10) (lane 2); DH5α(pBU) (lane 3); and X. o. pv. oryzae strains PXO99^A(pBUavrXa10) (lane 4); PXO99^A(pBUavrXa10.F1) (lane 5); PXO99^A (lane 6); PXO86 (lane 7). The blot was incubated with anti-AVRXa10 polyclonal antibody. High molecular weight markers (Bio-Rad Laboratories) are in lane 1.

in other pathovars of *X. campestris* (Knoop *et al.* 1991). Because several avirulence genes (*avrb6, avrBs3, avrBs3-2, avrBsP,* and *avrXa10*) from the *avrBs3*-related family of *Xanthomonas* show a high degree of sequence identity (> 90%, Bonas *et al.* 1989; Canteros *et al.* 1991; Bonas *et al.* 1993; De Feyter *et al.* 1993; Hopkins *et al.* 1992), cross-reactivity with AVRXa10 antiserum is likely due to similarity of epitopes in the protein products of the *avrBs3*-family. Preimmune serum did not react with any of these proteins (data not shown).

E. coli with pET-avrXa10.F1 that expressed the AVRXa10.F1 protein did not elicit the HR on IR-BB10 (data not presented). Affinity purified AVRXa10.F1 from *E. coli* also did not elicit an HR on IR-BB10. Similarly, purified AVRXa10.F1 protein from *X. o.* pv. *oryzae* did not elicit the HR when infiltrated into rice cultivars IR-BB10 or IR24 (at a maximum concentration of 1 mg/ml). These data indicate that the AVRXa10 protein is not directly involved in the elicitation of resistance. However, we cannot rule out the possibility that AVRXa10 may be modified to an active state before being exported from the bacterium, as a result of interacting with the plant.

Localization of AVRXa10 protein.

The location of AVRXa10 in cells of *X. o.* pv. *oryzae* expressing tagged AVRXa10 was determined by biochemical fractionation and immunoelectronmicroscopy. For biochemical fractionation, *X. o.* pv. *oryzae* cells were grown in TB overnight and harvested by centrifugation. Cells were washed and resuspended to an OD_{600} of 0.7 in cold (4° C) fractionation buffer (10 mM Tris HCl [pH 8.0], 200 mM NaCl, 0.5 mM EDTA, 2 mM 2-mercaptoethanol) containing RNase A (10 µg/ml), DNase I (10 µg/ml), phenylmethlysulfonyl fluoride (PMSF) (100 µg/ml), Leupeptin (1 µg/ml), and Pepstatin A (1 µg/ml). Bacterial cells were compressed and passed through a prechilled French pressure cell (16,000 lbs/in²) three times and centrifuged at 235,000 × *g* for 4 hr at 4 C to separate soluble and insoluble fractions. To obtain total membranes, the insoluble fraction was resuspended in fractionation buffer, layered on a 20–60% sucrose density gradient, centrifuged at 55,000 × *g* for 1.5 hr at 4° C, and collected from the sucrose gradient.

For extraction of extracellular proteins, *X. o.* pv. *oryzae* cells were grown in 100 ml each of TB and a medium re-
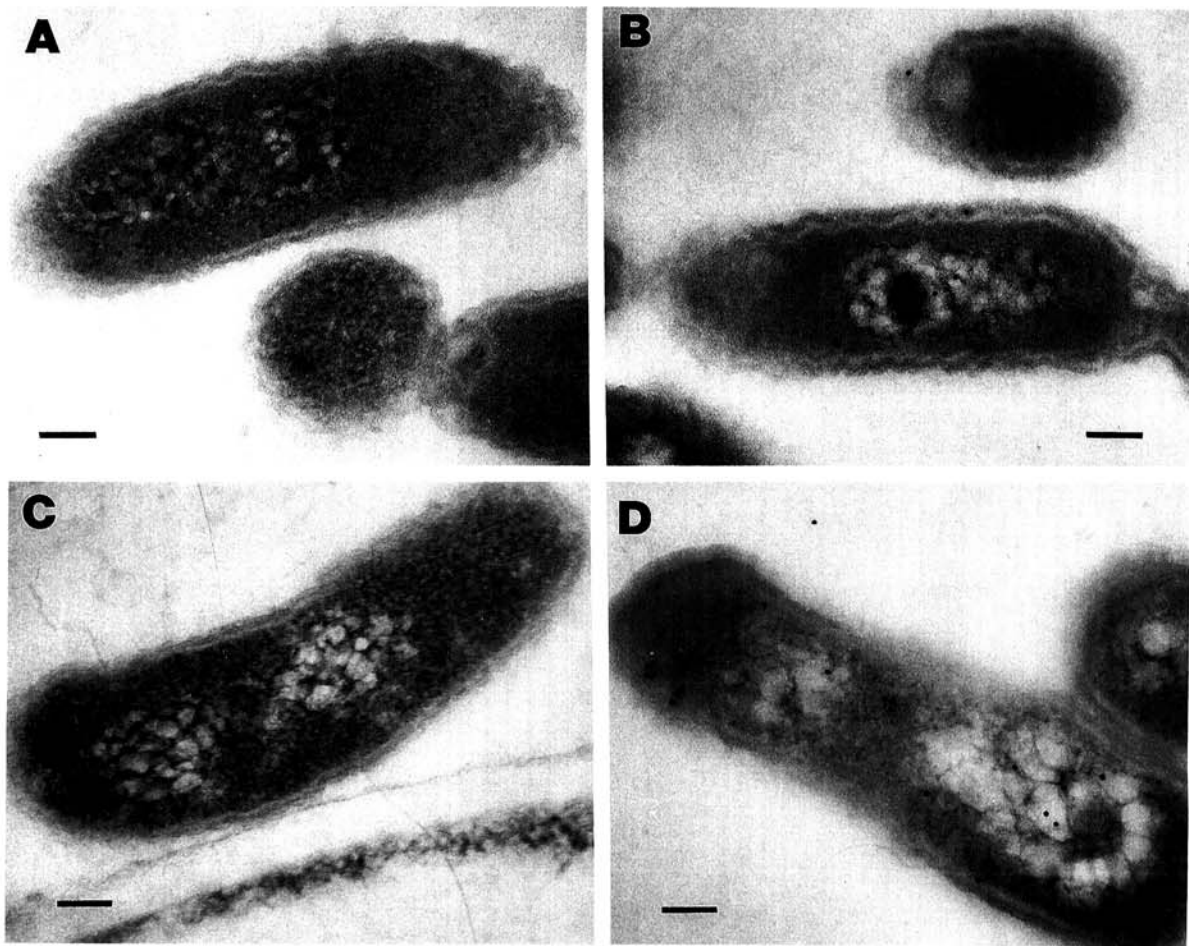

Fig. 3. Immunogold localization of tagged AVRXa10 protein. **A, B,** Cells of *X. o.* pv. *oryzae* PXO99^A (pBUavrXa10) and *X. o.* pv. oryzae PXO99^A (pBUavrXa10.F1), respectively, grown in liquid cultures. **C, D,** Cells of *X. o.* pv. *oryzae* PXO99^A (pBUavrXa10) and *X. o.* pv. *oryzae* PXO99^A(pBUavrXa10.F1), respectively, in the vessels of leaves of IR-BB10 at 24 hr after inoculation. Sections were labelled with anti-FLAG antibody. Bar = 0.5 µm.

ported to induce *hrp* genes in *X. c.* pv. *vesicatoria*, MM1 (Schulte and Bonas 1992), overnight. Cultures were treated either in the presence or absence of 500 µM PMSF, 5 µM Pepstatin A, and 5 µM Leupeptin for 1 hr before cells were pelleted. In addition, leaf tissues from IR24 and IR-BB10 were macerated and extracted in 10 mM Tris buffer (pH 8.0), and 10-ml aliquots were added to the MM1 medium. Extracellular proteins were precipitated by addition of ammonium sulfate to a concentration of 95% (w/v). The precipitate was collected by centrifugation, dissolved in TBS buffer (50 mM Tris-HCL [pH 7.4], 150 mM NaCl) with 0.1% bovine serum albumin, and dialyzed overnight at 4° C in TBS. All fractions were compared densitometrically after analysis in Western and dot blots with both AVRXa10 polyvalent antisera and anti-FLAG M2 antibody.

AVRXa10 was detected primarily in the cytoplasmic (soluble) fraction, with less than 12% present in the membrane (insoluble) fraction. Similar results were reported for the related AvrBs3 protein from *X. c.* pv. *vesicatoria* (Knoop *et al.* 1991). However, no AVRXa10 protein was detected in concentrated culture supernatants of *X. o.* pv. *oryzae* cells or from intercellular fluids of rice leaves from IR-BB10 and IR24 after infiltration with *X. o.* pv. *oryzae* PXO99^A (pBUavrXa10.F1). These data indicate that the AVRXa10 protein is not located outside the bacterial cells, although the amount or forms of AVRXa10 present may have limited detection of the protein outside the bacterium.

The location of AVRXa10 was determined in inoculated leaves and in bacteria grown overnight in TB by using both AVRXa10 antibody and anti-FLAG antibody in immunoelectronmicroscopy. One site per leaf of 10-day-old seedlings was inoculated with *X. o.* pv. *oryzae* PXO99^A (pBUavrXa10) and PXO99^A (pBUavrXa10.F1) as previously described (Reimers and Leach 1991). For each treatment, two leaves were combined from each of four different pots, and the inoculated sites were sampled at 3, 6, 12, and 24 hr after inoculation. Leaf tissues and bacterial cell pellets were treated with 2.5% glutaraldehyde in CAB at 4° C overnight and 30 min, respectively. Fixed leaf tissues and bacterial cells were dehydrated

and infiltrated with LR White as described by the manufacturer (Data Sheet #305A, Polysciences, Inc.).

Thin sections, placed onto parlodion-covered nickel grids, were immunogold-labeled and stained as described (Lutkenhaus and Bi 1991). The sections were examined with an electron microscope (Hitachi H-300, Hitachi Instruments Inc., San Jose, CA) at magnifications of 30,000× to 40,000×. The gold particles in and around the bacteria were counted in 40 fields per treatment, nonspecific labeling was subtracted, and mean numbers ± standard error of the mean (SEM) of gold particles were determined. Each treatment consisted of two to four replicates.

Gold particles were distributed randomly within the cytoplasm of *X. o.* pv. *oryzae* PXO99^A (pBUavrXa10.F1) cells both from cultures and in rice cultivars IR-BB10 (Fig. 3B and D). A few gold particles were found in the rice tissue (cell walls, vacuoles, cytoplasm, and intercellular spaces), but the number of particles bound to host tissue was similar to the nonspecific labeling observed on sections that were not treated with the anti-FLAG antibody. Specific gold-labeling when the anti-FLAG antibodies was used was absent in PXO99^A cells containing an untagged *avrXa10* gene (Fig. 3A and C). The number and distribution of gold particles at 3, 6, and 24 hr after inoculation were the same in cells of *X. o.* pv. *oryzae* PXO99^A (pBUavrXa10.F1) grown either in IR24 or IR-BB10 rice leaves (Fig. 4). The mean number of gold particles associated with cells of *X. o.* pv. *oryzae* PXO99^A (pBUavrXa10.F1) grown in culture was 4.6 ± 0.46. The majority of the gold particles were in the cytoplasm of the cultured bacteria, with less than 5% of the particles associated with the membrane. In addition, the results suggest that *avrXa10* is expressed constitutively and is not induced to higher levels *in planta*. Previously, *avrBs3*, *pthA*, and *avrb6* were shown to be expressed constitutively (Knoop *et al.* 1991; Swarup *et al.* 1992; De Feyter *et al.* 1993). Similar experiments were performed using the anti-AVRXa10 antibody; the mean numbers of gold particles observed on *X. o.* pv. *oryzae* PXO99^A (pBUavrXa10.F1) grown in IR24 and IR-BB10 for 24 hr after infiltration were 14.5 ± 1.2 and 14.1 ±
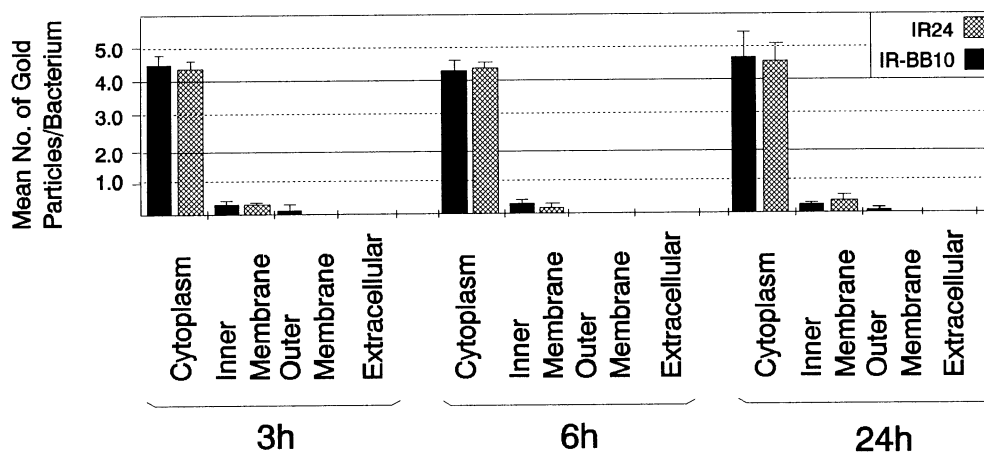

Fig. 4. Quantitative analysis of the distribution of immunogold particles within cells of *X. o.* pv. *oryzae* PXO99^A(pBUavrXa10.F1) 3, 6, and 24 hr after inoculation into rice leaves of IR24 (hatched bars) and IR-BB10 (solid bars). Location of gold particles indicated underneath bars (cytoplasm, inner membrane, outer membrane, and extracellular). Sections were labeled with anti-FLAG antibody. Mean numbers of gold particles recorded at each site in 200 separate bacteria are given (bars = SEM).

0.87, respectively. The three- to four-fold increase in the number of bound gold particles with the polyclonal antiserum probably was due both to the increase in available antigenic sites and to the presence of products from genes related to avrXa10.

The detection of AVRXa10 protein in the cytoplasm by immunocytochemistry is consistent with the results obtained for AvrBs3 protein in X. c. pv. vesicatoria (Brown et al. 1993). By using antiserum that reacts specifically to a known extracellular plant peroxidase in immunogold labeling experiments, we detected the peroxidase in the intercellular spaces and vessels of the same tissues used for detection of AVRXa10 (Young and Leach, unpublished results). Thus, extracellular proteins were not washed from these spaces during fixation processes. We cannot rule out the possibilities that, if AVRXa10 was exported, the amounts outside the cell may have been too low to detect immunologically or that the protein may have been modified such that it was elicitor-active but not recognized by the antiserum.

Since AVRXa10 and AvrBs3 (Brown et al. 1993) are located in the cytoplasm and were not detected outside the bacterial cells, it is unlikely that these avr gene products interact directly with plant cells. Thus, the mechanism by which disease resistance is elicited in a race-specific manner in these plant-bacterial interactions remains unclear. Studies on the function of AVRXa10 and related proteins in the bacterium will offer valuable insight into the role of this family of proteins in plant-pathogen interactions.

ACKNOWLEDGMENTS

LITERATURE CITED

Bonas, U., Stall, R. E., and Staskawicz, B. J. 1989. Genetic and structural characterization of the avirulence gene avrBs3 from Xanthomonas campestris pv. vesicatoria. Mol. Gen. Genet. 218:127-136.

Bonas, U., Conrads-Strauch, J., and Balbo, I. 1993. Resistance in tomato to Xanthomonas campestris pv. vesicatoria is determined by alleles of the pepper-specific avirulence gene avrBs3. Mol. Gen. Genet. 238:261-269.

Brown, I., Mansfield, J., Irlam, I., Conrads-Strauch, J., and Bonas, U. 1993. Ultrastructure of interactions between Xanthomonas campestris pv. vesicatoria and pepper, including immunocytochemical localization of extracellular polysaccharides and the AvrBs3 protein. Mol. Plant-Microbe Interact. 6:376-386.

Canteros, B., Minsavage, G., Bonas, U., Pring, D., and Stall, R. 1991. A gene from Xanthomonas campestris pv. vesicatoria that determines avirulence in tomato is related to avrBs3. Mol. Plant-Microbe Interact. 4:459-465.

Choi, S. H., and Leach, J. E. 1994. Identification of the XorII methyltransferase gene and a vsr-homolog from Xanthomonas oryzae pv. oryzae. Mol. Gen. Genet.244:383-390.

Crouse, G. F., Frischauf, A., and Lehrach, H. 1983. An integrated and simplified approach to cloning into plasmids and single-stranded phages. Methods Enzymol. 101:78-89.

De Feyter, R., Yang, Y., and Gabriel, D. W. 1993. Gene-for-gene interactions between cotton R genes and Xanthomonas campestris pv. malvacearum avr genes. Mol. Plant-Microbe Interact. 6:225-237.

Harlow, E., and Lane, D. 1988. Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Hopkins, C. M., White, F. F., Choi, S.-H., Guo, A., and Leach, J. E. 1992. Identification of a family of avirulence genes from Xanthomonas oryzae pv. oryzae. Mol. Plant-Microbe Interact. 5:451-459.

Keen, N. T., Tamaki, S., Kobayashi, D., Gerhold, D., Stayton, M., Shen, H., Gold, S., Lorang, J., Thordal-Christensen, H., Dahlbeck, D., and Staskawicz, B. 1990. Bacteria expressing avirulence gene D produce a specific elicitor of the soybean hypersensitive reaction. Mol. Plant-Microbe Interact. 3:112-121.

Knoop, V., Staskawicz, B., and Bonas, U. 1991. Expression of the avirulence gene avrBs3 from Xanthomonas campestris pv. vesicatoria is not under the control of hrp genes and is independent of plant factors. J. Bacteriol. 173:7142-7150.

Kunkel, T. A., Roberts, J. D., and Zakour, R. A. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol. 154:367-382.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680-685.

Lutkenhaus, J., and Bi, E. 1991. FtsZ ring structure associated with division in Escherichia coli. Nature 354:161-164.

Mew, T. W. 1987. Current status and future prospects of research on bacterial blight of rice. Annu. Rev. Phytopathol. 25:359-382.

Ogawa, T., and Khush, G. S. 1989. Major genes for resistance to bacterial blight in rice. Pages 177-192 in: Bacterial Blight of Rice. IRRI, Manila, Philippines.

Ogawa, T., and Yamamoto, T. 1987. Selection of recurrent parents to develop near-isogenic lines resistant to bacterial leaf blight of rice. Jpn. Agric. Res. Q. 21:65-69.

Reimers, P. J., and Leach, J. E. 1991. Race-specific resistance to Xanthomonas oryzae pv. oryzae conferred by bacterial blight resistance gene Xa-10 in rice (Oryza sativa) involves accumulation of a lignin-like substance in host tissues. Physiol. Mol. Plant Pathol. 38:39-55.

Schottens-Toma, I. M. J., and de Wit, P. J. M. 1988. Purification and primary structure of a necrosis-inducing peptide from the apoplastic fluids of tomato infected with Cladosporium fulvum (syn. Fulvia fulva). Physiol. Mol. Plant Pathol. 33:59-67.

Schulte, R., and Bonas, U. 1992. A Xanthomonas pathogenicity locus is induced by sucrose and sulfur-containing amino acids. Plant Cell. 4:79-86.

Swarup, S., Yang, Y., Kingsley, M. T., and Gabriel, D. W. 1992. A Xanthomonas citri pathogenicity gene, pthA, pleiotropically encodes gratuitous avirulence on non-hosts. Mol. Plant-Microbe Interact. 5:204-213.

Swings, J., Van den Mooter, M., Vauterin, L., Hoste, B., Gillis, M., Mew, T. W., and Kersters, K. 1990. Reclassification of the causal agents of bacterial blight (Xanthomonas campestris pv. oryzae) and bacterial leaf streak (Xanthomonas campestris pv. oryzicola) of rice as pathovars of Xanthomonas oryzae (ex Ishiyama 1922) sp. nov., nom. rev. Int. J. Syst. Bacteriol. 40:309-311.

Tartof, K. D., and Hobbs, C. A. 1987. Improved media for growing plasmid and cosmid clones. Bethesda Res. Lab. Focus 9:12.

Towbin, H., Staehelin, T., and Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. USA. 76:4350-4354.

Vieira, J., and Messing, J. 1987. Production of single-stranded plasmid DNA. Methods Enzymol. 153:3-11.

van Kan, J., van den Ackerveken, G., and de Wit, P. 1991. Cloning and characterization of cDNA of avirulence gene avr9 of the fungal pathogen Cladosporium fulvum, causal agent of tomato leaf mold. Mol. Plant-Microbe Interact. 4:52-59.