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Similarity between Copper Resistance Genes from *Xanthomonas campestris* and *Pseudomonas syringae*

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Plasmid-borne copper resistance genes from copper-resistant strains of *Xanthomonas campestris* pv. vesicatoria from California, Florida, and Oklahoma shared structural similarities. A strain of *X. campestris* pv. campestris also contained plasmid-borne copper resistance genes similar to the resistance genes from *X. campestris* pv. vesicatoria. Furthermore, a region of the copper resistance genes from *X. campestris* pv. vesicatoria 07882 hybridized with *copA*, the first gene of the copper resistance operon (*cop*) of *Pseudomonas syringae* pv. tomato. A copper-inducible protein of similar size to CopA was detected by Western blot (immunoblot) analysis from the wild-type strain 07882 and from the cloned copper resistance genes of 07882 introduced into a copper-sensitive strain of *X. campestris* pv. vesicatoria. A low level of hybridization was observed with chromosomal DNA from other xanthomonads when the copper resistance genes from strain 07882 were used as probes.

Copper compounds have been used to control bacterial and fungal diseases of agricultural crops for more than 100 years (17, 23, 24, 39), but copper resistance has been reported for only a few plant-pathogenic bacteria, primarily in pathovars of *Pseudomonas syringae* and *Xanthomonas campestris* (1, 2, 4, 6, 11, 22, 35). In previous work, copper resistance genes cloned from *P. syringae* did not hybridize with copper resistance genes cloned from *X. campestris* in Southern blot hybridization experiments, suggesting that resistance may have evolved independently in these two plant-pathogenic species (5, 6).

Copper resistance was first reported for strains of *X. campestris* pv. vesicatoria, the causal agent of bacterial spot of tomato and pepper, from Florida in 1983 (22), but examination of older cultures suggested that resistance had been present in populations of the pathogen in Florida since 1968. Copper resistance in this pathogen is usually determined by large conjugative plasmids (designated pXvCu) of approximately 200 kb in strains from Florida and Oklahoma (6, 33). In different strains, these plasmids can vary considerably in restriction enzyme digest profiles and in size (6, 33). One such plasmid (pXV10A) was transmissible to several other pathovars of *X. campestris* but not to pseudomonads (6).

Plasmid-determined copper resistance in *P. syringae* pv. tomato was reported in 1986 from California (4), but resistance genes cloned from the nonconjugative 35-kb resistance plasmid found in copper-resistant strains of *P. syringae* pv. tomato (9) showed no similarity with copper resistance genes cloned from *X. campestris* in Southern blot hybridizations (5, 6). This apparent lack of similarity between copper resistance genes from *P. syringae* and *X. campestris*, and the highly conserved nature of the 35-kb plasmid in all strains examined (9), suggested that resistance may have evolved independently in the two species and more recently in *P. syringae* than in *X. campestris* (10, 12).

An apparently new type of copper-resistant strain of *X. campestris* pv. vesicatoria, in which copper resistance was encoded by a nonconjugative 100-kb plasmid, from California was recently reported (14). Copper resistance genes cloned from this plasmid hybridized with the copper resistance operon (*cop*) of *P. syringae* pv. tomato in Southern blot experiments with lowered stringency of posthybridization washes. After using similar hybridization conditions, we now report that copper resistance genes in *X. campestris* pv. vesicatoria from Florida, Oklahoma, and California are closely related and all have similarities to the *cop* operon from *P. syringae*.

MATERIALS AND METHODS

Bacterial strains, culture conditions, and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. Nutrient agar (Difco) was used to maintain xanthomonads, whereas pseudomonads were grown in mannitol-glutamate medium (18) supplemented with yeast extract at 0.25 g/liter at 28°C. Cultures of *Escherichia coli* were grown in Luria-Bertani (LB [27]) broth at 37°C. Antibiotics were used at the following concentrations: rifampin, 50 μg/ml; chloramphenicol, 20 μg/ml; ampicillin, 50 μg/ml; tetracycline, 10 μg/ml for both pseudomonads and xanthomonads and 25 μg/ml for *E. coli* strains.

General DNA manipulations. Large-scale chromosomal and plasmid isolations were carried out as described elsewhere (14). Two rounds of CsCl-ethidium bromide isopycnic gradient centrifugation were always performed for purification. Minipreparations of plasmid DNA were performed by the alkaline lysis method described by Maniatis et al. (21). Cloned DNA fragments containing copper resistance genes from different strains were isolated for Southern blot hybridizations by digestion with appropriate restriction enzymes and were gel purified with the Geneclean kit as suggested by the manufacturer (Bio 101, Inc., La Jolla, Calif.). The 6.8-kb insert of pCOP137 was isolated by digestion with *Sac*I and *Xba*I. The 6.4-kb insert of pCuR2 was isolated by digestion at polylinker sites (*Xba*I and *Eco*RI) adjacent to the *Bam*HI site in which the 6.4-kb *Bgl*II fragment was originally cloned. The 4.8-kb insert of pXVCul-16 was removed by digestion with *Hin*dIII and *Eco*RI, and *Pst*I was used to remove the 4.5-kb fragment of pCOP4 containing the *cop* operon.

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TABLE 1. Bacterial strains and plasmids and their characteristics

Strain or plasmid	Description[a]	Reference or source
Bacterial strains		
P. syringae pv. tomato PT23	Cu^r	4
X. campestris pv. vesicatoria		
07882	Cu^r	14
078518	Cu^s Rif^r	14
07882.1	Cu^s	This study
07882.2	Cu^s Rif^r	This study
078225	Cu^s	This study
10858	Cu^r	R. Stall[b]
03911	Cu^r	This study
XV8-79	Cu^s	P. Psallidas[c]
XV83-47	Cu^r	R. Gitaitis[d]
X. campestris pv. campestris		
04861	Cu^r	This study
11882	Cu^s	This study
X. campestris pv. juglandis XCJ5	Cu^r	M. Schroth[e]
E. coli		
HB101	F^- *hsdS20* (r^- m^-) *recA13 ara-14 proA2 lacY1 galK2 rpsL20* (Str^r) *xyl-5 mtl-1 supE44* λ^-	7
SM10	*thi thr leu tonA lacY supE recA* [RP4.2 Tc::Mu]; $Mu2^+$ Km^r Tra^+	32
S17-1	*thi pro hsdR hsdM recA* [RP4-2 (Tc::Mu) (Km::Tn7)]; Sm^r Tp^r Tra^+	32
Plasmids		
pUC119	Ap^r; cloning vector	37
pUC128	Ap^r; cloning vector	19
pRK415	Tc^r; Rk-2-derived cloning vector	19
pCOP2	Tc^r; 4.4-kb *Pst*I fragment containing the copper resistance genes from *P. syringae* pv. tomato PT23 cloned in pRK404	5
pCOP4	Ap^r; 4.4-kb *Pst*I fragment containing the copper resistance genes from *P. syringae* pv. tomato PT23 cloned in pUC119	25
pCOP100	Tc^r; pLAFR3 (34) cosmid clone containing the copper resistance genes from *X. campestris* pv. vesicatoria 07882	14
pCOP116	Tc^r; 10.5-kb *Sac*I fragment of pCOP100 cloned in pRK415	This study
pCOP115	Tc^r; 4.5-kb *Pst*I fragment of pCOP100 cloned in pRK415	This study
pCOP117	Ap^r; 10.5-kb *Sac*I fragment of pCOP100 cloned in pUC119	This study
pCOP120	Ap^r; 7.6-kb *Sac*I-*Sma*I fragment of pCOP117 cloned in pUC119	This study
pCOP121	Tc^r; 9.6-kb *Hin*dIII-*Sac*I fragment of pCOP117 cloned in pRK415	This study
pCOP124	Tc^r; 5.6-kb *Hin*dIII fragment of pCOP117 cloned in pRK415	This study
pCOP137	Ap^r; 6.8-kb *Xba*I-*Sac*I fragment of pCOP138 cloned in pUC119	This study
pCOP138	Tc^r; 6.8-kb *Xba*I-*Sac*I fragment of pCOP117 cloned in pRK415	This study
pCOP153A	Ap^r; 1.9-kb *Bam*HI fragment of pCOP116 cloned in pUC128	This study
pCOP155	Ap^r; 2.4-kb *Hin*dIII-*Bam*HI fragment of pCOP124 cloned in pUC119	This study
pCOP25	Ap^r; 3′ deletion derivative of the *cop* operon in pUC119	25
pCuR2	Tc^r; 6.4-kb *Bgl*II fragment of the copper resistance genes from *X. campestris* pv. vesicatoria XV10 cloned in the *Bam*HI site of pRK415	16
pXVCul-13	Tc^r; 4.8-kb *Hin*dIII-*Bgl*II fragment of the copper resistance genes from *X. campestris* pv. vesicatoria E3C5 cloned in pWB5A	B. Staskawicz[f]
pXVCul-16	Ap^r; 4.8-kb *Hin*dIII-*Bgl*II fragment of the copper resistance genes from *X. campestris* pv. vesicatoria E3C5 cloned in pUC19	B. Staskawicz
pRU868	pRU676::Tn*1737*Cm; Ap^r Cm^r; contains a promoterless *lacZ* reporter gene	36

[a] Cu^r, copper resistance; Cu^s, copper sensitivity; Rif^r, rifampin resistance; Km^r, kanamycin resistance; Sm^r, streptomycin resistance; Tp^r, trimethoprim resistance; Ap^r, ampicillin resistance; Tc^r, tetracycline resistance; Cm^r, chloramphenicol resistance; Tra^+, transfer function for mobilizable plasmids.
[b] R. Stall at the University of Florida, Gainesville.
[c] P. Psallidas at the Benaki Phytopathological Institute, Athens, Greece.
[d] R. Gitaitis at the Experimental Station, Tifton, Ga.
[e] M. Schroth at the University of California, Berkeley.
[f] B. Staskawicz at the University of California, Berkeley.

The 6.8-kb *Sac*I-*Xba*I fragment of pCOP137, the 2.4-kb *Hin*dIII-*Bam*HI fragment of pCOP155, the 1.9-kb *Bam*HI fragment of pCOP153A, and the 2.0-kb *Pst*I-*Bam*HI fragment of pCOP25 were gel purified and labeled by random primed labeling with digoxigenin-11-dUTP and the Genius DNA labeling and detection kit (Boehringer Mannheim) and were used as probes against the gel-purified copper resistance genes described above. Posthybridization washes were at relatively low stringency (0.5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate] at 50°C) for the different plasmid-borne copper resistance genes and at low stringency (0.5× SSC at room temperature) for detecting chromosomal homologs.

Introduction and removal of plasmid DNA from xanthomonad cells. The different fragments of the cosmid clone pCOP100 were subcloned in vector pRK415 (19). These

constructs were introduced into *X. campestris* pv. vesicatoria 078518 by electroporation as previously described (11), except that the bacteria were grown in nutrient broth and cells were washed with 0.5 M sucrose and incubated overnight in nutrient broth before being plated onto selective media. Electroporations were performed at 7.5 kV/cm with a single pulse by using a Gene Pulser apparatus (Bio-Rad Laboratories) with a 25-μF capacitor at 200 Ω. The resulting strains were tested for the ability to resist copper. A strain was designated copper resistant if the MIC for it (14) was higher than the MIC for recipient strain 078518.

To cure the copper resistance plasmid from strain 07882, bacterial cells were grown as mentioned above and the electroporated cells were plated on nutrient agar. Three hundred colonies were spotted on the same medium in a grid formation. The colonies then were tested for the loss of the copper resistance phenotype by being replica plated onto nutrient agar amended with 1.2 mM $CuSO_4$. Three colonies were copper sensitive, and their plasmid profiles were determined (15). A Southern blot with the 6.8-kb *Sac*I-*Xba*I fragment of pCOP137 as a probe was performed to verify the loss of the 100-kb plasmid. One such strain was designated as *X. campestris* pv. vesicatoria 07882.1. A spontaneous, rifampin-resistant mutant of 07882.1 was obtained and designated 07882.2.

Immunoblot analysis of proteins. Whole-cell proteins were isolated from *P. syringae* pv. tomato PT23 essentially as described previously (14) and used as a positive control. A small purification procedure was employed to concentrate proteins from xanthomonads. Proteins were concentrated essentially as described for purification of CopA from *P. syringae* pv. tomato (8). However, after the saturation and dialysis steps, samples were immediately quantified for their protein contents by the Lowry method (3) without any further purification steps. Proteins were electrophoresed on sodium dodecyl sulfate–10% polyacrylamide gels as described previously (3). For the immunodetection of CopA-related proteins, antisera raised against CopA of strain PT23 were used (8). The detection was performed as described previously (8).

Transposon mutagenesis. Saturation transposon mutagenesis with Tn*1737* (36), which contains a promoterless *lacZ* reporter gene, was performed with pCOP138. pCOP138 was cotransformed with pRU868 in *E. coli* S17-1, and colonies were selected in LB amended with tetracycline and ampicillin. Ten colonies were picked and grown in LB with tetracycline for a few generations to allow transposition to occur. The cultures then were mated on LB plates with *E. coli* SM10, and recombinant clones were selected in LB supplemented with kanamycin, tetracycline, and chloramphenicol. The transposon mutations were mapped by digesting the resulting recombinant plasmids with *Eco*RI, *Hin*dIII, and *Bam*HI. Twenty-five insertions were mapped on the 6.8-kb *Xba*I-*Sac*I fragment. The transposon mutations were introduced into the copper-sensitive strain 078518 by conjugation (33). To determine the orientation of transcription, the above-mentioned recombinant strains were tested for β-galactosidase activity (27) when grown in the absence or presence of $CuSO_4$ (0.1 mM).

RESULTS

Subcloning and characterization of copper resistance genes of *X. campestris* pv. vesicatoria 07882. Several subclones were made from the cosmid clone pCOP100 that carried copper resistance genes from strain 07882 (Fig. 1). The smallest fragment that conferred copper resistance when transferred to strain 078518 was a 6.8-kb *Xba*I-*Sac*I fragment (pCOP138) and was used in the rest of this study.

All transposon insertions that were mapped to the cloned DNA of pCOP138 caused copper sensitivity (Fig. 2). The positions of the insertions suggest that about 6.0 kb of DNA are required for copper resistance in strain 07882. In addition, the direction of transcription was determined from the β-galactosidase activities of the different Tn*1737* insertions. The insertion labeled 1 in Fig. 2 inactivated copper resistance but did not show any increased level of β-galactosidase activity. This suggests that the transposon lies within the promoter region.

Similarities among copper resistance genes. Specific internal probes of the four genes of the *cop* operon from strain PT23 (26) were used to detect similarities to the copper resistance genes of strain 07882. Only the specific probe for *copA* hybridized to the copper resistance genes of strain 07882 in Southern blot hybridizations with low-stringency posthybridization washes. The approximate area of similarity between *copA* and pCOP138 was mapped (Fig. 3).

Two subclones from pCOP138 were constructed for use in Southern blots to detect similarities with the cloned copper resistance genes from the other strains of *X. campestris* pv. vesicatoria. The first probe (pCOP155), from the 3′ half of the copper resistance genes from strain 07882, hybridized to the 3′ region of copper resistance genes in pCuR2 and pXVCul-13 from Oklahoma and Florida, respectively (Fig. 4B). The second probe (pCOP153A), from the 5′ half of the copper resistance genes of strain 07882, hybridized to the 5′ region of copper resistance genes in pCuR2 and pXVCul-13 (Fig. 4C). The 3′ and 5′ regions of pCuR2 and pXVCul-13 were designated after their physical maps were compared with the physical map of pCOP138 and with information on the transcriptional organization of the genes (Fig. 2). A lower level of hybridization was observed between these probes and the *cop* operon, primarily with the 1.9-kb *Bam*HI probe, which contains most of the *copA* similarity.

The physical maps of the three copper resistance gene clones from *X. campestris* pv. vesicatoria in California, Oklahoma, and Florida showed similarities (Fig. 3). In contrast, the physical maps of copper resistance genes of *X. campestris* pv. vesicatoria and the *cop* operon of *P. syringae* pv. tomato PT23 showed little similarity. Furthermore, pCuR2 conferred resistance to copper when introduced to strain 078518, whereas pXVCul-13 did not increase the resistance to copper at the same level as the other clones. By comparing the restriction maps, it can be seen that pXVCul-13 is lacking part of the 3′ region present in the other clones. Strain 078518(pRK415), which was used as a control, did not show an increase in copper resistance.

The cloned copper resistance genes of strain 07882 were used as probes in Southern blots to detect similarities among other xanthomonads at moderate stringency (Fig. 5). The copper-sensitive strains (078518, 078225, 07882.2, and XV8-79) did not show any homology to the copper resistance genes of strain 07882 with either chromosomal or plasmid DNA under these experimental conditions (Fig. 5A). However, when the stringency was lowered, the copper resistance genes hybridized weakly to DNA fragments in most copper-sensitive strains (Fig. 5B). In contrast, plasmid DNA of most copper-resistant strains showed strong hybridization with the copper resistance genes of strain 07882 (Fig. 5A). The plasmid DNA was compared with the total DNA for each isolate to determine whether additional DNA from the chromosome hybridized to the probe. The chromosomal

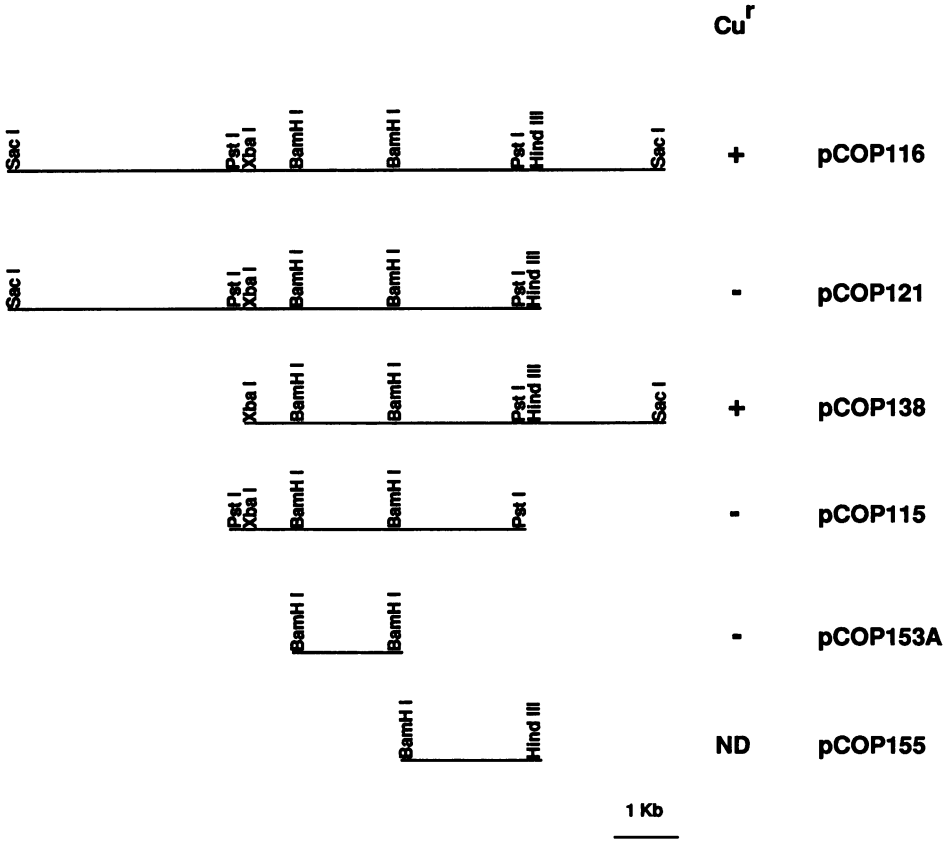

FIG. 1. Copper resistance phenotypes of different subclones of the cosmid clone pCOP100, which contains the copper resistance (Cu^r) genes of *X. campestris* pv. vesicatoria 07882. ND, not determined.

DNA did not contain any additional regions that hybridized to the copper resistance genes, except for copper-resistant strain XCJ5 of *X. campestris* pv. juglandis. XCJ5 contained no detectable plasmids but showed weak hybridization with the copper resistance genes of strain 07882 under low-stringency conditions (Fig. 5B).

Plasmid DNA from *X. campestris* pv. campestris 04861 hybridized strongly to the copper resistance genes of strain 07882 (Fig. 5A, lane 8P). When 04861 was conjugated with strain 078518, 75 to 100% of the recipient cells became copper resistant in several independent experiments. Transmission of copper resistance to 078518 was associated with a large plasmid of approximately 140 kb that hybridized strongly with the copper resistance genes cloned from 07882 (data not shown). Strain 04861 was originally isolated from cauliflower, and black rot symptoms were reproduced after

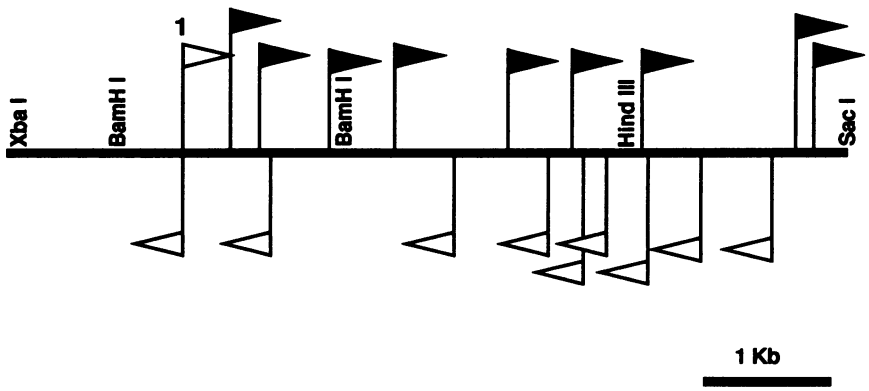

FIG. 2. Transcriptional orientation of copper resistance genes in the 6.8-kb *Xba*I-*Sac*I fragment of pCOP138 shown by Tn*1737* insertions. The direction of the arrows indicates the orientation of the promoterless *lacZ* gene in Tn*1737* insertions. Solid arrows indicate increased levels of β-galactosidase activity when bacteria were induced with 0.1 mM $CuSO_4$. Open arrows indicate that β-galactosidase activity was not affected when bacteria were grown in the presence of 0.1 mM $CuSO_4$. The insertion labeled 1 is discussed in the text.

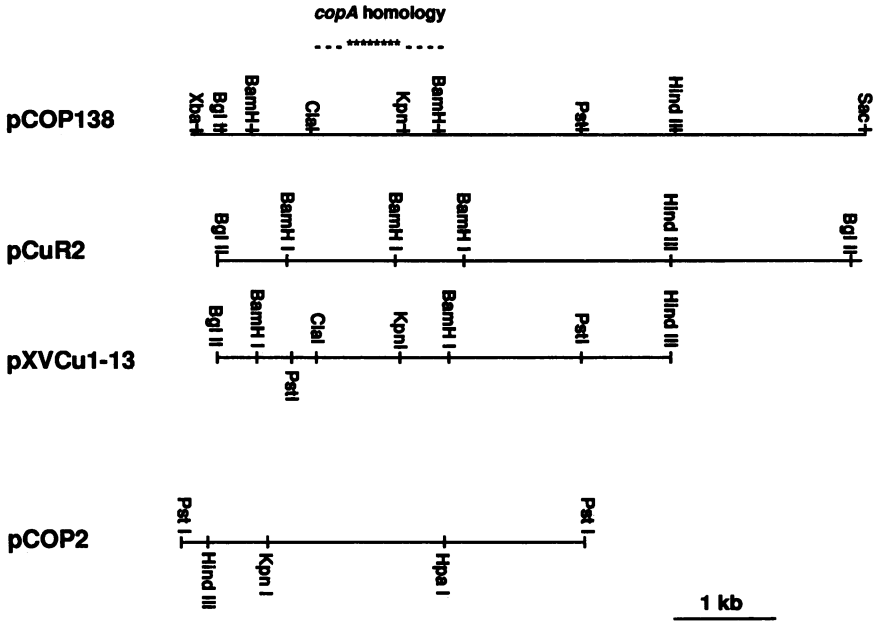

FIG. 3. Restriction endonuclease maps of cloned copper resistance genes from strains of *X. campestris* pv. vesicatoria (pCOP138, pCuR2, and pXVCul-13) and *P. syringae* pv. tomato (pCOP2). The *copA* homology is shown above the physical map of pCOP138. Asterisks represent positive hybridization, whereas the dashed lines indicate the approximate area that *copA* homology extends, on the basis of the size of the protein detected.

inoculation of cauliflower (cv. snowball) in greenhouse tests. The identity of this strain as *X. campestris* was confirmed by the Biolog carbon source utilization system (Biolog, Inc., Hayward, Calif.).

Immunoblot analysis of proteins. For immunoblot analysis of proteins, antisera raised to CopA were used to detect similar proteins from copper resistance genes of strain 07882. Strain 07882 produced a protein related to CopA (Fig. 6, lane 3). The estimated size of this protein was 67 kDa, compared with 72 kDa for CopA from strain PT23 of *P. syringae* pv. tomato (8). CopA was detected from strain 07882 only when bacteria were induced by copper. The strain cured of the 100-kb plasmid that contains the copper resistance genes did not produce a CopA-related protein (Fig. 6, lanes 8 and 9). Copper-sensitive strain 078518 did not produce a CopA-related protein (Fig. 6, lanes 4 and 5), but, when pCOP138 was introduced into strain 078518, CopA was detected in a copper-inducible manner (Fig. 6, lanes 6 and 7). In addition, the cloned copper resistance genes in pCuR2 and pXVCul-13 also produced a CopA-related protein of a size similar to that produced by pCOP138, also in a copper-inducible manner (data not shown).

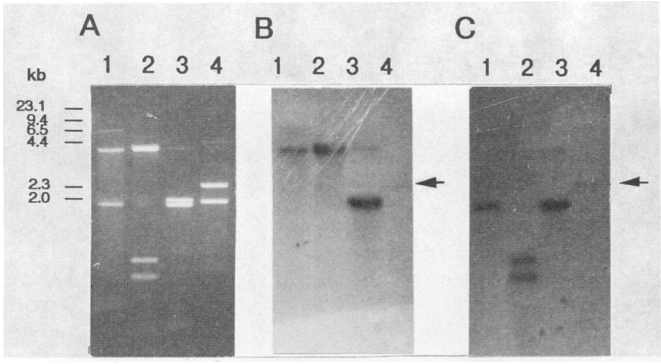

FIG. 4. Similarity among the copper resistance genes from different strains of *X. campestris* pv. vesicatoria probed with subclones of copper resistance genes of *X. campestris* pv. vesicatoria 07882. (A) Agarose gel electrophoresis of the gel-purified copper resistance genes from pCOP138 (lane 1), pCuR2 (lane 2), and pXVCul-13 (lane 3) digested with *Bam*HI and from pCOP4 (lane 4) digested with *Hpa*I. (B) Southern blot hybridization of the 2.4-kb *Hin*dIII-*Bam*HI fragment of the pCOP155 probe to DNA from panel A. (C) Southern blot hybridization of the 1.9-kb *Bam*HI fragment of the pCOP153A probe to DNA from panel A. Arrows at the right of panels B and C point to a band in lane 4 that hybridized faintly with both probes.

DISCUSSION

Copper resistance genes cloned from strains of *X. campestris* pv. vesicatoria isolated from California, Florida, and Oklahoma were closely related to each other, and all showed some similarity with the *cop* operon of *P. syringae* pv. tomato. Therefore, the hypothesis that copper resistance has evolved independently in these two plant pathogens should be reevaluated. *P. syringae* pv. tomato and many other species of *Pseudomonas* contain chromosomal DNA that hybridizes with the plasmid-borne *cop* operon under conditions of moderate to high stringency (14). This suggests the possibility that the *cop* operon may have evolved directly from these chromosomal homologs in pseudomonads. In contrast, chromosomal DNA of several *X. campestris* pathovars showed only limited hybridization under conditions of low stringency with cloned copper resistance genes from *X. campestris* pv. vesicatoria. Evolution of the plasmid-borne resistance genes in *X. campestris* directly from indigenous chromosomal genes in this species therefore seems unlikely. Exchange of plasmid DNA between pseudomonads and xanthomonads, or with other bacteria, is a more likely

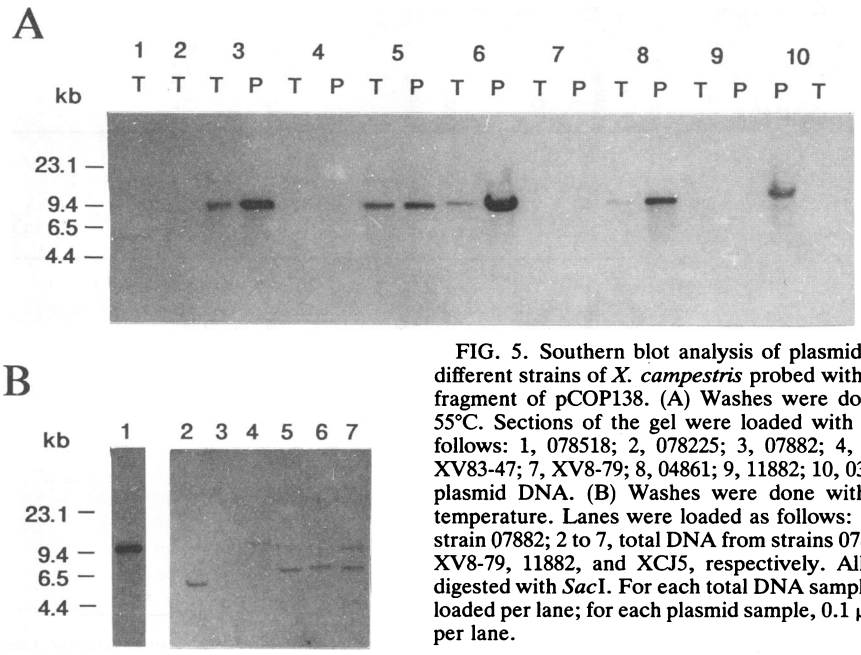

FIG. 5. Southern blot analysis of plasmid and total DNA from different strains of *X. campestris* probed with the 6.8-kb *Xba*I-*Sac*I fragment of pCOP138. (A) Washes were done with 0.5× SSC at 55°C. Sections of the gel were loaded with DNA from strains as follows: 1, 078518; 2, 078225; 3, 07882; 4, 07882.2; 5, 10858; 6, XV83-47; 7, XV8-79; 8, 04861; 9, 11882; 10, 03911. T, total DNA. P, plasmid DNA. (B) Washes were done with 0.5× SSC at room temperature. Lanes were loaded as follows: 1, plasmid DNA from strain 07882; 2 to 7, total DNA from strains 078518, 078225, 07882.2, XV8-79, 11882, and XCJ5, respectively. All DNA samples were digested with *Sac*I. For each total DNA sample, 0.5 μg of DNA was loaded per lane; for each plasmid sample, 0.1 μg of DNA was loaded per lane.

explanation for the observed similarities between *cop* and copper resistance genes in xanthomonads. The recent finding of sequence similarities between products of the *cop* operon and of the plasmid-borne *pco* copper resistance operon from *E. coli* (31) suggests that there may have been even broader exchanges of these genes among different families of bacteria.

Although only the first gene (*copA*) of the *cop* operon hybridized with the copper resistance genes from *X. campestris* in our experiments, it is possible that sequence analysis will reveal similarities outside of *copA*, just as sequencing revealed that the organization of the *pco* operon from *E. coli* is essentially the same as that of *cop* (31). The amount of similarity between *pco* and *cop* is not enough to expect them to hybridize or for their protein products to show strong antigenic similarities, except possibly for the PcoA and CopA proteins, which showed the highest degree of amino acid identity (79%). Our smallest cloned DNA fragment that gave full copper-inducible expression of resistance from xanthomonads was 6.8 kb, and transposon mutagenesis suggested that at least 6.0 kb of that fragment were required for resistance. Compared with the 4.5-kb sequence of the *cop* operon, it appears that the *Xanthomonas* copper resistance determinant is larger. However, recent work has shown that the 4.5-kb clone containing the *copABCD* genes is missing two additional genes (*copRS*) just downstream from the operon that are required for copper-inducible

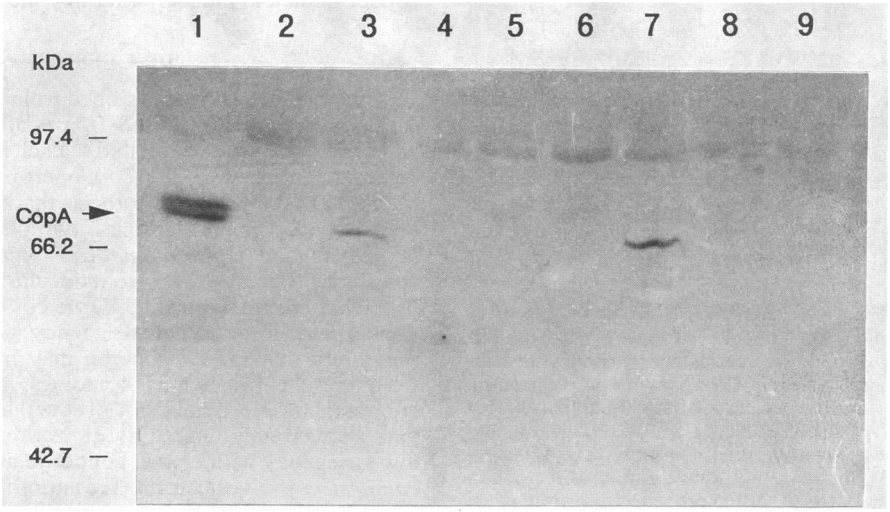

FIG. 6. Detection of CopA-related proteins from *X. campestris* pv. vesicatoria by immunoblot analysis. Total proteins from strain PT23 (lane 1) and partially purified proteins from strains 07882 (lanes 2 and 3), 078518 (lanes 4 and 5), 078518(pCOP138) (lanes 6 and 7), and 07882.2 (lanes 8 and 9) were used. Extracts in the odd-numbered lanes were prepared from cells grown in the presence of 0.1 mM $CuSO_4$. Equivalent amounts of total protein were loaded in each lane.

activation of the single *cop* promoter just in front of *copA* (28). This would make the full *cop* region about the same size as the *X. campestris* copper resistance gene cluster, the same size as the copper resistance genes from *X. campestris* pv. juglandis XCJ5 (20), and the same size as the *pco ABCDRS* resistance determinant from *E. coli* (31). However, the cloned *X. campestris* resistance genes are not expressed in pseudomonads or *E. coli*, and the cloned *cop* promoter does not function in xanthomonads or *E. coli* (28, 38). Therefore, although similarities exist among these different copper resistance genes, they have certainly diverged functionally, at least at the regulatory level.

Two different mechanisms of copper resistance have been proposed for the *cop* system in pseudomonads and for the *pco* system in *E. coli*. Products of the *cop* operon sequester large amounts of copper in the periplasm and outer membrane, suggesting a mechanism of copper sequestration that prevents toxic levels of free copper ions from entering the cytoplasm (8). In contrast, *E. coli* cells expressing *pco* genes accumulate less copper than wild-type strains, suggesting an efflux mechanism for resistance (29, 30). Although we have not measured copper accumulation of resistant strains of *X. campestris* pv. vesicatoria, these strains do not become blue when grown on media with high copper concentrations, as is observed for *cop*-containing *P. syringae* strains and several other copper-resistant, copper-accumulating species of *Pseudomonas* (13). The copper resistance mechanism for *X. campestris* may therefore be different than for the *cop* system, possibly involving an efflux system like the *pco* system of *E. coli*.

We have also identified a highly conjugative copper resistance plasmid in one strain of *X. campestris* pv. campestris from cauliflower, and this plasmid hybridized strongly with the cloned copper resistance genes from *X. campestris* pv. vesicatoria. This observation again suggests that copper resistance plasmids have been exchanged among pathovars of related plant pathogens, as was suggested for two pathovars of *P. syringae* which contained closely related plasmids carrying *cop* genes (11). Although copper resistance has been reported for only a few of the 166 or more pathovars of *X. campestris* and *P. syringae*, it is likely that resistance will become more widespread in this group in the coming years.

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