Nucleotide Sequence and Organization of Copper Resistance Genes from *Pseudomonas syringae* pv. *tomato*

MICHAEL A. MELLANO AND DONALD A. COOKSEY*

Department of Plant Pathology, University of California, Riverside, California 92521

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The nucleotide sequence of a 4.5-kilobase copper resistance determinant from *Pseudomonas syringae* pv. *tomato* revealed four open reading frames (ORFs) in the same orientation. Deletion and site-specific mutational analyses indicated that the first two ORFs were essential for copper resistance; the last two ORFs were required for full resistance, but low-level resistance could be conferred in their absence. Five highly conserved, direct 24-base repeats were found near the beginning of the second ORF, and a similar, but less conserved, repeated region was found in the middle of the first ORF.

Copper compounds have been widely used in agricultural systems to control undesirable microorganisms, and in recent years, copper-tolerant microbes have been isolated from such environments (19). Plasmid-encoded resistance to copper has been demonstrated for several bacterial species, including Escherichia coli (18), Mycobacterium scrofulaceum (8), and the plant pathogens Xanthomonas campestris pv. vesicatoria and Pseudomonas syringae pv. tomato (1, 17). Previous work on P. syringae pv. tomato demonstrated the involvement of a 35-kilobase (kb) plasmid (pPT23D) in copper resistance (1, 2). This plasmid was highly conserved among copper-resistant strains of P. syringae pv. tomato isolated in southern California (5). Copper resistance determinants were isolated on a 4.5-kb PstI-PstI fragment cloned from pPT23D. Tn5 insertions indicated that at least 3.9 kb of this fragment was involved in copper resistance (2). To further understand the basis of copper resistance in the P. syringae pv. tomato system, the DNA sequence of the 4.5-kb fragment was determined. This paper presents the structure, organization, and relative contribution to copper resistance of four genes contained within this fragment.

DNA sequence analysis. The 4.5-kb *PstI-PstI* fragment was subcloned from pCOP2 (2) into pUC119 (20) in both orientations, and overlapping deletions differing by approximately 100 base pairs (bp) were made by the method of Henikoff (11) using exonuclease III and S1 nuclease. Single-stranded template DNA was prepared by coinfecting with the helper bacteriophage M13K07 (20). Both strands were sequenced by the Sanger dideoxy method (14) using ³⁵S-labeled dATP (3). Sequence data were analyzed by the computer programs of BIONET (Intelligenetics, Palo Alto, Calif.).

Four open reading frames (ORFs) of 1,827, 984, 378, and 930 bp occurred in the same orientation and were preceded by sequences resembling those of *E. coli* consensus ribosome-binding sites (15). These ORFs were designated A, B, C, and D, respectively (Fig. 1). Several additional, widely dispersed ORFs with possible ribosome-binding sites occurred in the opposite orientation with sizes of 234, 243, 291, 510, 225, 342, and 417 bp. However, Tn_5 insertions affecting copper resistance (2) and the deletions and frameshift mutations described later in this paper were not consistent with the involvement of these ORFs in copper resistance.

The sequence had an overall G+C content of 60%, similar to that of genomic DNA of P. syringae (13). Codon usage in all the ORFs showed that there was a strong preference (74.5%) for codons containing G or C at position 3. All four of the predicted protein products contained unusually high levels of methionine (7.1, 4.9, 5.6, and 4.9% in ORFs A, B, C, and D, respectively). The hydropathic nature of the four predicted proteins, determined by the algorithm of Kyte and Doolittle (12), indicated that ORFs A, B, and C lacked any significant hydrophobic regions with the exception of fairly large hydrophobic stretches at the beginning of each ORF (data not shown). The nature of these hydrophobic stretches is similar to that expected for signal peptides (16, 21, 22), each possessing a charged amino acid at the N terminus, a hydrophobic core region, and a polar C-terminal region. The predicted product from ORF D had several potential membrane-spanning hydrophobic regions and also possessed a leader region resembling that of a signal peptide.

Five direct repeats of 24 bases each were present near the beginning of ORF B (Fig. 1). This region would encode a highly conserved polypeptide of eight amino acids (Asp-His-Ser-Gln/Lys-Met-Gln-Gly-Met) directly repeated five times (Fig. 2). A similar methionine-rich repetitive region was found in ORF A (Fig. 1); it was not as highly conserved as the region in ORF B, nor were the repeats tandemly linked throughout the entire region. However, the general structure Asp-His-X-X-Met-X-X-Met was conserved between several of the ORF A repeats and those in ORF B (Fig. 2). The function of the repeated sequences is not yet known, but a computer search of protein data bases with the ORF A protein indicated similarities between a region of about 50 amino acids just preceding the repeated sequences and a region of the azurin proteins from Pseudomonas aeruginosa, Pseudomonas dentrificans, Bordetella bronchiseptica, and Alcaligenes denitrificans. In addition, a region of 18 amino acids with similarity to plastocyanin from Chlorella fusca was detected 10 amino acids upstream from the region with similarity to azurin. The plastocyanin and azurin blue copper proteins bind copper between the sulfur atoms of a cysteine and a methionine and between nitrogen atoms in the imidazole groups of two histidine residues (4). Although there are no cysteines near the repeated region of ORF A and only one in the entire protein, the region is rich in methionine and

^{*} Corresponding author.

Psti 10 20 30 40 50 60 Hindii 70 CTGCAGATAC TAAAAAAACT GAAAGCTCTA AGGCATGTTG CTAACCAACG CAGGTTTTCA AGCTTACAGA 80 90 100 110 120 130 140 AATGTAATCG CGCCGCTTAC GATGCTGTGA CATCGTCCAC TCCAGTACCT TAAACCCAGT ACACGGCTTA 150 160 170 ORFA 187 202 AATGCCGTCC TTGCCTACCT GGACCCGGCG GT ATG GAA TCA AGA ACT TCT CGA CGT ACT TTC MET GIU Ser Arg Thr Ser Arg Arg Thr T GTC AAA GGC CTC GCG GCT GGC GGC GTG GGT GGG CTA GGC TTG TGG GGC GTT TGG GGC GTT GGG CGT TGG GGC CTG TGG GGC CTG TGG GGC CTG GGC CT 262 277 292 307 AGC TGG GCG GCG TCC GGC TGG GCG GTG AGC GGT GTG AGC GGG GTG GGC GTC GAC Ser Trp Alagala ser Gly Ser Pro Ala Lau Ser Val Lau Ser Gly Thr Glu Phe Asp 382 <u>Apsi 197</u> 412 427 ATC MAT GGC GGG CTG CCC CTG CTG CGC TGG AAA GAG GGT GAC ACT GTC ACG Tie Aan Giv Giv Leu Pro Giv Pro Leu Lau Arg Trp Lys Giu Giv Asp Thr Val Thr $\begin{array}{c} 442\\ \text{CTC CGG GTA CGC AAC CGG CTC GAC GGT GCA ACC TCC ATA CAC TGG CAC GGC ATT ATC Law Arg Vai Arg Awn Arg Law App Ala Ala Thr Ser Ile His Trp His Gly lie lie \\ \end{array}$ CTG CCG CCG AAC ATG GAC GGC GTT CCA GGA CTG AGC TTC GCG GGC ATC GAG CCG GGT Law Pro Pro Asm MET Asp Giy Val Pro Giy Law Sar Phe Ala Giy Ila Giu Pro Giy 547 562 577 562 577 592 KpM GGC GTG TAC GTC CAG TTC AAG GTC CAA CAG AAC GGG ACG TAC TGG TAC CAC AGC GJy Val Tyr Val Tyr Gin Phe Lys Val Gin Gin Aen Gly Thr Tyr Trp Tyr His Ser 607 CAC TCC GGA TTT CAG GAG GGG GTG GGG GTG ATA GCCG GTC GTC ATC GAG GCG AAA His ser Gly Phe Gln Glu Gln Val Gly Val Tyr Gly Pro Leu Val Ile Glu Ala Lys 727 GAT GAA GAT CCC GTC TCG CTG ATG CGT ACC CTC AAA AAG CAG TCC GAT TAC TAC AAC Asp Glu Asp Pro Val Ser Leu HET Arg Thr Leu Lys Lys Gln Ser Aep Tyr Tyr Aen 787 TTC CAC AAG CGC ACA GTC GGT GAC TTC GTC AAC GAT GTG GCT GAT AAG GGC TGG GCC Phe His Lys Arg Thr Val Gly Asp Phe Val Asn Asp Val Ala Asp Lys Gly Trp Ala 832 GCA ACC GTC GCG GAT GCC AAG ATG TGG GCC GAG ATG AAG ATG AAC CCC ACG GAC CTT Ala Thr Val Ala Asp Arg Lys MET Trp Ala Glu MET Lys MET Asn Pro Thr Asp Lau 892 927 922 922 937 $^$ 952 AAC TGG ACC GGC TTG TTC CGT CCT GGC GAA AAG CTG CGC CTG CGG TTC ATC AAC GGC AAS TTP Thr Gly Leu Phe Arg Pro Gly Glu Lys Leu Arg Leu Arg Phe Ile Asn Gly 1012 1027 1042 1057 TGG GCT ATG ACG TAC TTC GAC ATC CGT ATT CCA GGC CTG AAA ATG ACC GTG GTA GT Ser Ala MET Thr Tyr Phe Asp Ile Arg Ile Pro Gly Leu Lys MET Thr Val Val Ala 1072 1087 1102 TCG GAT GGC CAG TTC GTG AAC CCG GTT GAG GTC GAT GAA TA GGC ATT GCC GTG GCC Ser Asp Gly Gin Phe Val Asen Fro Val Glu Val Ase Glu Law Arg Ile Ale Val Ale 1117 1162 GAA ACC TTC GAT GTG ATC GTT GAG CCC ACT GCC GAG GCG TAT ACG GTC TTT GCT CAA Glu Thr Phe Asp Val Ile Val Glu Pro Thr Ala Glu Ala Tyr Thr Val Phe Ala Glu 1177 1192 1207 1222 TCC ATG GAT CGC ACG GGC ACC GGC GGC GGC GGC GGG GAA GGC TTG GTA Ser MET Asp Arg Thr Gly Tyr Ala Arg Gly Thr Lau Ala Val Arg Glu Gly Lau Val 1237 GCC CAG GTC CCC CTT GAT CCT CGT CCG CTG GTC ACG ATG GAC GAT ATG GGC ATG Ala Gin Vai Pro Pro Lau Amp Pro Atg Pro Lau Vai Thr MET Amp Amp MET Giy MET 1227 1312 GGT GGT ATG GAC CAT GGC AGG ATG GAT GGC ATG GAC ATG GAT TGG GGT GGC GGC GGC ATG GAT TGG GGT GGC AGG GJY GLY MET Amp Him Gly Smr HET Amp GJY MET Smr GJY AHET Amp Smr GJy Ala Amp ALC GGC ATG AGC ATG AGC AGC ATG GGG GGC GAC TCC ATG CCC GCC ATG GAC CAT Asp Gly MET GIn The MET Ser Ser MET Gly Gly App Ser MET Pro Als MET Asp Mis 1417 AAA ATG TCT ACC ATG CAG GGT ATG GAC CAC GGC GCT ATG TCG GGC ATG GAC CAT Lys MET Ser Thr MET Gin Giy MET App His Giy Ala MET Ser Giy MET App His

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 GGT GCG ATG GGC GGC ATG GTG ATG CAG AGC CAC CCT GCC AGC GAG AAC GAC AAC CCG
 GIY Ala MET GIY GIY MET Val MET GIn Ser His Pro Ala Ser Glu Asn Asp Asn Pro

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 CTG GTG GAC ATG CAG GCC ATG AGC CCT ACC GCC AAC GAT CCT GGC GTG GGC

 Leu Val Asp MET Gln Ala MET Ser Pro Thr Ala Lys Leu Asn Asp Pro Gly Leu Gly

1582 CTG CGT AAT AAC GGG CGC AAG GTG CTC ACC TAT GCC GAC CTT AAA AGC ACC TTC GAA Leu Arg Aan Aan Gly Arg Lys Val Lau Thr Tyr Ala Aap Leu Lys Ser Thr Phe Glu

1687 1702 1717 1732 GAA AAA TTT GCA TGG TCG TTT GAC GGC ATC AAA TTC GCG GAC GCC CAA CCT CTG ATA Glu Lys Phe Ala Tcp Ser Phe Asp Gly Ile Lys Phe Ala Asp Ala Gln Pro Leu Ile 1747 1792 1777 A TCC AAA TAC GGC GAA GGG GTA AGG ATC GGC GGG GTA GAC AGG ATG ACC CAC Lau Lys Tyr Gly Glu Arg Val Arg 11e Val Lau Val Asn Asp Thr MET MET Thr Mis 1807 CCG ATC CAT CTG CAT GGG ATG TGG AGT GAC TTG GAG GAC GAA GAC GGA AAC TTT GGG Pro 11e His Law His Gly MET Trp Ser Asp Lew Glu Asp Glu Asp Gly Asm Phe Arg 1867 1982 1897 1912 GTG CGC AAG CAC ACC ATT GAT ATG CGG CCA GGC TCC AAG CGC AGC TAC CGT GTC ACC Val Arg Lys His Thr Ile Asp MET Pro Pro Gly Ser Lys Arg Ser Tyr Arg Val Thr 1927 1942 1957 GCT GAT GCC TG GGC GGC TGG GCC TAT CAC TGT CAC CTG GTC TAC CAC ATG GAG ATG Ala Asp Ala Lau Gly Arg Trp Ala Tyr His Cys His Lau Lau Tyr His HET Glu HET 1972 1987 ORFB 2022 GGT ATG TTC CGC GAA GTT CGG GTA GAG GAG T<u>GAGG</u>CCA ATG ACT TTG AAT AGA CTC Gly MET Phe Arg Glu Val Arg Val Glu Glu SD HET Thr Val Lau Asn Arg Lau His Val Cys Ser Leu Leu Ala Val Ser Ser Leu Giy HEI Leu FLO VEL C., 2007 2012 2027 2322 2367 CC AAT CGG GCT GCG GTC TAC CGA AGT GCC AAA GC CAC ACT GTC CAT GAC GAC AGT AGT AT GAC AAT GCC AAA GC CAC ACT GTC CAT GAC GAC ALL Asm Arg Sar Ala Lys Gly His Thr Val His Asp Glu Ala 2382 GCT AAT TAT TTC CTG CTC TTC GAT CAA CTC GAA TGG CAG GAC GCC GAC AAC GGC Ala Am Tyr Phe Leu Leu Phe Amp Gin Leu Giu Trp Gin Amp Ala Amp Am Giy 2442 Mgal 2457 2472 GTC CTT AAT TGG GAC GTT AAC GGC TGG GGT GGT GGT GAC CAC CGG CTC TGG ATT Val Lau Asn Trp Asp Val Asn Jy Trp Val Gly Gly Asp Ile Asp Arg Lau Trp Ile 2487 2502 2517 Eagl 2532 CGC TCC GAG GGC GAA CGT ACC AAC GGC AAG ACC GAA TCG GCC GAG CTG CAA GCG CTG Arg Ser Glu Gly Glu Arg Thr Asn Gly Lys Thr Glu Ser Ala Glu Leu Gin Ala Leu 2547 2562 2577 2592 TGG GGC CAT GCG ATC AGT CTT TGG TGG GAC CTG GTC GGC GGC GTC CGG CAG GAC TTC Trp Gly His Als Ile Ser Pro Trp Trp Asp Leu Val Gly Gly Val Arg Gin Asp Phe 2667 2682 2697 2712 AAC TTC GAA GCC GAA GCG ACT GCG TTT CTT GGT GAA GGC GGC CAA ACC GGG TTA AGG Aan Phe Glu Ala Glu Ala Thr Ala Phe Lau Gly Glu Gly Gly Gln Thr Gly Lau Arg 2727 2742 2757 CTG GAA GGC GAC TAC GAC ATT TTG CTG ACT AAC CGG CTG ATT TTA CAG CCC ACG GCT Leu Glu Gly Asp Tyr Asp Ile Leu Leu Thr Asn Arg Leu Ile Leu Gln Pro Thr Ala 2772 2787 2802 2817 GAG GTT AAT TTC TAC GGT CAG AGC GAT CCT CAG CGC GGC ATC GGC TCT GGC CTG TCT Glu Val Asn Phe Tyr Gly Gin Ser Asp Pro Gln Arg Gly Ile Gly Ser Gly Leu Ser 2832 2847 2862 2877 GAA ACC GAA GTC GGC GTA CGA CTG CGC TAC GAA ATC CGC CGC GAG TTT GCC CCG TAC Glu Thr Glu Val Gly Val Arg Leu Arg Tyr Glu Ile Arg Arg Glu Phe Ala Pro Tyr 2892 2937 2922 2937 ATT GGC GTC ACC TGG AAC CGC TCC TAC GGC AAT ACA GCC GAC TTT GCC CGC GAG GAA lie Giy Val Thr Trp Aan Arg Ser Tyr Giy Asn Thr Ala Amp Phe Ala Arg Giu Giu 2952 2967 2982 1001 GGC GAG GAC CGC GAG GAC CGC CGC TTA GTC CTG GGC GTG CGC ATG TGG TTC TGAGCCGTTC Gly Glu Amp Arg Ser Glu Ala Arg Lau Val Leu Gly Val Arg MET TTP Phe 3011 3021 3031 3041 3051 ORFC TAGTCTGAAA ATCTGATCCC CCACGAACGG CCTTTTTGGG CTGTAACGAG TTCG TTG TTG AAC SD HET Law Law Ann
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 CGC ACA AGT TTC GTC ACG CTC TTT GCC GCT GGG ATG CTG GTC AGC GCA TTG GCC CAA
 ATG TTG GCC CAC
 GCC CAC GCA TTG GTC TTT GCC GCA GA

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 GCC CAC CCC AAG CTG GTG TCT TCG ACT CCG GCT GAA GGT AGT GAA GGC GCG GCC CCT
 Ala His Pro Lys Lau Val Ser Ser Thr Pro Ala Glu Gly Ser Glu Gly Ala Ala Pro

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FIG. 1. Nucleotide sequence and predicted translation products of the copper resistance gene cluster from *P. syringae* pv. tomato PT23. Nucleotides are numbered 1 to 4478. Pertinent restriction sites are indicated as overstrikes. Potential Shine-Dalgarno (SD) ribosome-binding sites (15) are underlined. Repeating segments are underlined with arrows. Potential signal sequence cleavage sites are designated by vertical arrows.

histidine and might contain repeated copper-binding domains that could play a role in copper resistance.

Deletion analysis and site-specific mutations. Various exonuclease III deletion derivatives generated for sequencing and specific restriction fragments from the copper resistance gene cluster were subcloned from pUC119 into the broadhost-range vector pRK415 (7; N. T. Keen, personal communication) or pDSK519 (N. T. Keen, personal communication). Most of the deletion derivatives were cloned in both orientations with respect to the lac promoter in these vectors. These subclones were mobilized into P. syringae pv. syringae PS61 by using the helper plasmid pRK2013 (9) as described previously (2), and they were screened for their level of resistance to copper sulfate. Removal of a 60-bp PstI-HindIII fragment from the 5' end of the 4.5-kb fragment up to the HindIII site (pCOP6A and pCOP6B) had no significant effect on the level of copper resistance as compared with that of the intact fragment (Fig. 3). Deletion of 86 bp (pCOP7B) or 124 bp (pCOP8B) from the 5' end reduced the level of copper resistance, but resistance was elevated when these derivatives were cloned with the ORFs oriented behind the lac promoter (pCOP7A and pCOP8A). This result suggested that sequences essential for expression of copper

				1	(
A	1295	Asp	His	Gly	Ser	Met	Asp	Gly	Met
	1395	Asp	His	Ser	Lys	Met	Ser	Thr	Met
	1427	Asp	His	Gly	Ala	Met	Ser	Gly	Met
	1451	Asp	His	Gly	Ala	Met	Gly	Gly	Met
								,	
в	2113	Asp	His	Ser	Gln	Met	Gln	Gly	Met
	2137	Asp	His	Ser	Lys	Met	Gln	Gly	Met
	2161	Asp	His	Ser	Gĺn	Met	Gln	Gly	Met
	2185	Asp	His	Ser	Lys	Met	Gln	Gly	Met
	2200	Agn	Wie	Ser	GÌn	Mot	Gln	ดาง	Mot
	2209	L VSP	1119	Der	9111	1.00	U1	011	Mec

FIG. 2. Predicted amino acid sequences of homologous repeating units of ORFs A and B. The numbers at the left indicate the positions (in base pairs) of the first codon of each repeating unit. Conserved amino acids are boxed.

resistance were present well upstream from the ORF A start codon, although *E. coli* or *Pseudomonas* spp. consensus promoter regions (6, 10) were not observed in this region. Deletions from the 5' end into ORFs A, B, C, and D generally inactivated resistance to copper, regardless of orientation to the *lac* promoter. Deletions from the 3' end into ORFs D and C generally reduced resistance to intermediate levels. Further deletions from the 3' end into ORFs B and A inactivated resistance.

Frameshift mutations were generated in ORFs A and B by digesting the full-length clone with either KpnI (pCOP27), ApaI (pCOP31), or EagI (pCOP28), followed by deletion of the 3' overhangs or filling in of the 5' overhangs by using DNA polymerase I (Klenow fragment) and subsequent ligation. A second frameshift and deletion in ORF B (pCOP29) was generated by removal of the BsmI-HpaI fragment (pCOP29). A deletion and frameshift in ORF C (pCOP30) was generated by removal of the 94-bp BstEII-BstEII fragment. Each frameshift construct was subcloned into pRK415 or pDSK519 with the ORFs either behind the lac promoter (A) or in the opposite orientation (B) and conjugated into P. syringae pv. syringae PS61 to screen for expression of copper resistance. Frameshift mutations in ORF A or ORF B completely inactivated resistance regardless of orientation (Fig. 3). Resistance was also inactivated in the ORF C deletion derivative when cloned with the ORFs in the opposite orientation to that of the lac promoter (pCOP30B), but an intermediate resistance was observed when this derivative was cloned with the ORFs oriented behind the lac promoter (pCOP30A). Together with earlier observations of the inducibility of copper resistance in this system (D. A. Cooksey, Phytopathology 76:1076, 1986), these data suggest a possible role for ORF C in the positive regulation of copper resistance, especially since similarities were detected in computer searches of protein data bases between a region near the beginning of the ORF C protein and several DNAbinding proteins.

These data support the involvement of ORFs A, B, C, and D in copper resistance. Deletions and site-specific frameshift mutations in ORFs A and B suggest that these two ORFs are

		ORFA		ORFB	ORFC	ORFD	~						
Plasmid			-						MIG	C (mM	CuSO ₄	L)	
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PCOPSSA, B	1 1		1	<u> (i</u>	11		<u> </u>	12	÷	0.14	1 02	÷	0.10
DCOP7A B	Ĺ.I.		1		11		÷ ت	.73	+	0.23	0.47	÷	0.23
DCOPSA B	T I		1 I		ii.		ő	.72	+	0.16	0.60	Ŧ	0.06
DCOP9A B		I	1	11			ŏ	.50	÷	0.11	0.50	Ŧ	0.09
DCOP10A B					11	÷	ō	.40	÷	0.06	0.62	÷	0.04
DCOP11A B				11			_ o	.43	÷	0.08	0.33	Ŧ	0.05
pCOP12A.B					<u> </u>		ة تـــ	. 38	±	0.08	0.38	±	0.08
DCOP13A.B							ہ ب	.42	±	0.04	0.40	±	0.09
pCOP14B											0.47	±	ò.10
pCOP15A L	<u> </u>	l					- 0	.72	±	0.12			
pCOP16A LL_	<u> </u>						. 0	.68	±ι	0.17			
pCOP17A LL_		<u> </u>	î				0	.78	±	0.17			
pCOP18A, B		<u> </u>		L			0	.50	±	0.28	0.55	±	0.19
pCOP19A L	_ <u></u>					_	0	.63	±	0.19			
pCOP20A, B				_ Ų	<u>_</u>		0	.67	±	0.15	0.65	±	0.08
pCOP21A L	- ان ا		Ľ	<u> </u>	<u></u>		Ó	.78	±	0.31	•		
pCOP22A L	<u></u>						0	.47	±	0.08			
pCOP23A, B U							0	.48	±	0.13	0.42	±	0.12
pCOP24A L			1				0	. 38	±	0.13			
pCOP32A, B							0	.57	±	0.08	0.38	±	0.12
pCOP25A LL							0	.38	<u>+</u>	0.12			
pCOP26A U	<u></u>						0	.40	±	0.06			
pCOP31A, B							0	. 38	÷	0.08	0.45	÷	0.11
pCOP27A, B	<u> </u>					1.11		.38	÷	0.08	0.35	Ŧ	0.08
pCOP29A, B			<u>.</u>					.45	Ξ	0.11	0.37	Ξ	0.08
pCOP28A, B								. 52	Ξ	0.10	0.43	Ξ	0.08
pCOP30A, B							0	. 00	Ŧ	0.13	0.48	Ξ	0.18
pRK415							Ų		Ξ	0.00			

FIG. 3. MIC analysis of 5' deletions, 3' deletions, and frameshift mutations of a copper resistance gene cluster from *P. syringae* pv. tomato PT23. Derivatives were cloned in the broad-host-range vector pRK415 or pDSK519, with the ORFs oriented either behind the *lac* promoter (A) or in the opposite orientation (B). Horizontal lines to the right of each plasmid designation represent subcloned derivatives of the 4.5-kb *PstI-PstI* fragment. Vertical lines indicate important restriction sites. Missing restriction sites and gaps indicate positions of site-specific mutations and deleted DNA. Each MIC (tested at 0.1 mM CuSO₄ increments) was the mean \pm the standard deviation of two transconjugants replicated three times each. pRK415 was the negative control and represents the level of copper tolerance of the recipient *P. syringae* pv. *syringae* PS61 used to monitor expression of all mutant derivatives.

essential for resistance. Deletions and site-specific mutations in ORFs C and D suggest that they are required for full resistance, but low-level resistance can be conferred in their absence.

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