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DNA Hybridization Between Western Aster Yellows Mycoplasmalike Organism Plasmids and Extrachromosomal DNA from Other Plant Pathogenic Mycoplasmalike Organisms

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Native, ^{32}P-labeled plasmids isolated from the severe strain (SAY) of western aster yellows mycoplasmalike organism (MLO) or a cloned, 4.9-kb SAY plasmid fragment were used to probe Southern blots of undigested DNA isolated from plants infected with plant pathogenic MLOs from diverse geographic regions. SAY plasmid DNA hybridized with small extrachromosomal DNA molecules present in several virescence-inducing MLOs and the maize bushy stunt MLO (MBS), but not with DNA from decline-inducing MLOs or spiroplasmas. A cloned, 4.4-kb fragment of MBS extrachromosomal DNA hybridized with the plasmids present in six strains of western AY, and some, but not all of the virescence agents that hybridized with SAY probes. Sizes and numbers of extrachromosomal DNA molecules varied among MLO strains. No hybridization occurred with healthy plant or leafhopper DNA using either the SAY or MBS plasmid probes. The presence of homologous sequences between extrachromosomal DNAs of MLOs suggests they may contain similar genes or regulatory sequences.

Plant pathogenic mycoplasmalike organisms (MLOs) have been associated with disease in more than 300 plant species (McCoy *et al.* 1989). Because MLOs are morphologically indistinguishable in both their plant and insect hosts and have not been isolated in pure form or cultured *in vitro*, characterization of these plant pathogens has been difficult. Historically, classification of the MLOs has been based primarily on disease symptoms, plant host ranges, and insect vector relationships. These are characteristics that describe the interactions between MLOs and their hosts but do not completely characterize the pathogen itself. Plant pathogenic MLOs can be broadly grouped by the symptoms they induce in their host plants: those producing virescence (greening of floral tissue) and phyllody (leaflike petals and sepals) (virescence MLOs) and those producing general decline and death, but not virescence and phyllody (decline MLOs) (reviewed by Kirkpatrick, in press). However, this classification is somewhat subjective because members of both groups can cause similar symptoms, such as proliferation, chlorosis, vascular necrosis, and stunting. In addition, some virescence- and decline-inducing MLOs can be transmitted by the same insect vectors.

Three pathologically distinct strains of western aster yellows MLO (western AY) possess plasmid DNA (Kuske and Kirkpatrick 1990). Similarly, extrachromosomal DNAs have been described in the maize bushy stunt MLO (MBS, Davis *et al.* 1987) and a virescence MLO infecting *Oenothera* (OAY, Sears *et al.* 1989). Fragments of extrachromosomal DNA from these three MLOs have been cloned and used as diagnostic probes. In addition, an isolate of tomato big bud MLO appears to possess extrachromosomal DNA (Lee and Davis 1988) that contains sequences that hybridize with extrachromosomal DNAs of two other MLOs (Lee *et al.* 1990).

Extrachromosomal DNAs may facilitate genetic exchange among MLOs, and may carry traits that confer a selective advantage for survival or pathogenicity of these obligate parasites. The objectives of this study were to determine whether extrachromosomal DNAs present in the western AY and MBS shared homologous sequences with other MLOs and whether the presence of extrachromosomal elements correlated with the ability of MLOs to cause virescence and phyllody symptoms in plants.

MATERIALS AND METHODS

Sources of MLOs. Celery (*Apium graveolens* L.) and China aster (*Callistephus chinensis* Nees) plants, and *Macrosteles severini* Hamilton leafhoppers infected with the severe (SAY), dwarf (DAY), or Tulelake (TLAY) strains of western AY (Freitag 1964) were provided by A. H. Purcell, University of California, Berkeley. These strains have been continually maintained in plants by serial leafhopper transmission. Periwinkle (*Catharanthus roseus* L.) plants infected with SAY, DAY, TLAY, three California field isolates of AY (AY-HR, AY-SC, and AY-DAV), a Wisconsin isolate of eastern AY (WAY), western X-disease MLO (X), elm yellows MLO (EPN) (provided by W. A. Sinclair via R. E. Davis), or a decline-inducing MLO from pear (PD(X), Raju *et al.* 1983) were maintained in the greenhouse by graft transmission. A Florida isolate of MBS was maintained in maize by serial transmission with *Dal-*

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bulus maidis DeLong leafhoppers. MLOs causing phyllody in sesame (SESP), white leaf disease of bermudagrass (BG), and sugarcane (WL), were collected in their native host plants near Khon Kaen, Thailand, by B. C. Kirkpatrick.

Periwinkle infected with the beet leafhopper-transmitted virescence agent (BLTVA) was provided by D. A. Golino, USDA-ARS, University of California, Davis. A New Jersey isolate of eastern AY (EAY) in lettuce was provided by T. A. Chen, Cook College, Rutgers University, NJ. MLO-infected *Oenothera* (OAY) tissue was provided by B. Sears, Michigan State University. Periwinkle infected with a Canadian isolate (AY-27) of AY (CAY), clover proliferation (CP), or potato witches'-broom (PWB) MLOs were provided by C. Hiruki, University of Alberta, Canada.

DNA isolation. DNA from symptomatic tissues of the above plants was isolated from MLO-enriched fractions as reported previously (Kuske and Kirkpatrick 1990). DNA extracted from healthy celery fed on by healthy leafhoppers or other healthy plant species served as experimental controls.

Total DNA from plants infected with apple proliferation (AP), rape virescence (RV), stolbur (ST), safflower phyllody (SP), *Diplotaxis* virescence (DIV), *Catharanthus* virescence (CV), and European aster yellows (EURAY) MLOs was isolated as described by Kollar *et al.* (1990). These DNA preparations were further purified by DEAE cellulose chromatography (Elutip-d columns, Schleicher & Schuell, Keene, NH) and precipitated twice with ammonium acetate and ethanol before use (Maniatis *et al.* 1984). DNA isolated from *Veitchia* palm infected with lethal yellows MLO was provided by N. Harrison, University of Florida, Ft. Lauderdale. *Spiroplasma citri* Saglio and *S. kunkelii* Whitcomb isolates were cultured and the DNA isolated as described previously (Kuske and Kirkpatrick 1990).

Southern blots. Approximately 750 ng of undigested DNA from each of the above sources was treated with RNase A, electrophoresed in 1% agarose gels using Tris phosphate buffer (35 mM Tris, 33 mM NaH_2PO_4, 1 mM EDTA, pH 7.8) and transferred to nylon membranes (Nytran, Schleicher & Schuell) by using a modification of Southern's method (Maniatis *et al.* 1984).

Hybridization probes. Supercoiled SAY plasmids from SAY-MLO-infected celery were isolated by cesium chloride/ethidium bromide (CsCl/EtBr) gradient centrifugation (Kuske and Kirkpatrick 1990). A mixture of the four native SAY plasmids was labeled with ^{32}P-dATP by using random oligoprimers (Multiprime Kit, Amersham Corp., Arlington Heights, IL).

A cloned fragment of one SAY plasmid that was also used as a hybridization probe was obtained in the following manner. A mixture of the four native SAY plasmids was digested with *Eco*RI, ligated into *Eco*RI-digested pUC18, and transformed into competent *Escherichia coli* strain DH5α (Maniatis *et al.* 1984). Plasmid DNA from 85 recombinant clones was extracted and digested with *Eco*RI to determine the size of the cloned inserts. Southern blot hybridization of *Eco*RI-digested recombinant plasmids and native SAY plasmids was used to determine the origin of the cloned SAY plasmid fragments. The mixture of native SAY plasmids was used as the hybridization probe. One of the recombinant plasmids, pPSA45, contained a 4.9-kb fragment of SAY plasmid DNA. The cloned SAY plasmid fragment was separated from pUC18 DNA by electrophoresis of *Eco*RI-digested pPSA45 in a 1% agarose gel (Seakem GTG, FMC Bioproducts), followed by electroelution of the cloned SAY plasmid fragment from the gel using an Elutrap apparatus (Schleicher & Schuell). The SAY plasmid fragment was labeled with ^{32}P-dATP as described above.

The recombinant plasmid, pMBS11B, containing a 4.4-kb fragment of MBS extrachromosomal DNA (Davis *et al.* 1988), was digested with *Eco*RI and *Hin*dIII and the six fragments comprising the cloned MBS plasmid DNA were gel-purified and labeled with ^{32}P-dATP as described above.

DNA hybridizations. Southern blots of DNA from MLO-infected and healthy plants and leafhoppers were prehybridized and hybridized at 42° C in solutions containing 50% formamide as previously described (Kirkpatrick *et al.* 1987). Blots were probed with either total native SAY plasmid DNA, the gel-purified 4.9-kb SAY plasmid fragment, or the gel-purified MBS extrachromosmal DNA fragments. Posthybridization washes were: two washes in 2× standard saline citrate (SSC; 1×SSC = 0.15 M NaCl, 0.15 M sodium citrate, pH 7.0), 0.1% SDS at 37° C; one wash in 0.2× SSC, 0.1% SDS at 55° C; and the final wash in 0.2× SSC, 0.1% SDS at 37° C. X-ray film (Kodak XAR) was exposed to the blots at −80° C using an intensifying screen (Lightning Plus, DuPont). In addition to the above hybridizations, Southern blots of DNA from western AY strains were probed with MBS extrachromosmal DNA fragments and washed at a higher stringency by raising the temperature of the third and fourth washes to 65° C.

RESULTS

Cloning SAY plasmid DNA. *Eco*RI digestion of the four SAY native plasmids (Kuske and Kirkpatrick 1990) produced seven linear DNA fragments. Recombinant plasmids contained five different sizes of cloned SAY-MLO plasmid DNA. Southern blot hybridization analyses of *Eco*RI-digested recombinants and native SAY plasmids produced the following results: 1) Recombinant plasmid pPSA45 contained a 4.9-kb insert corresponding in size to the native SAY plasmid pSAY2. 2) Recombinant plasmid pPSA2 contained a 3.4-kb insert that corresponded in size to SAY-MLO plasmid pSAY3. 3) Recombinant plasmids pPSA80, pPSA59, and pPSA12 contained 3.1-, 1.9-, and 1.8-kb inserts, respectively, that are fragments of either SAY plasmids pSAY1 or pSAY3. Despite several attempts, we were unable to clone the smallest SAY plasmid, pSAY4. The 4.9-kb SAY plasmid DNA fragment from pPSA45 hybridized strongly with all of the native plasmids present in SAY, DAY, and TLAY strains of western AY (Kuske 1989), and it therefore was chosen as a hybridization probe for these experiments.

Presence of extrachromosomal DNA in MLOs. MLOs were grouped into two general categories based on plant symptoms: those that cause virescence and phyllody (the virescence MLOs) and those that do not (the decline MLOs) (Table 1). ^{32}P-labeled, native SAY plasmids or the 4.9-kb

SAY plasmid fragment detected extrachromosomal DNA in many, but not all of the virescence MLOs (Table 1, Figs. 1A, 2A). Identical hybridization patterns were obtained using ^{32}P-labeled native SAY plasmids or the cloned 4.9-kb SAY plasmid fragment. Numbers and sizes of plasmids in the different MLOs varied considerably. Only the DAY and TLAY strains possessed identical plasmid profiles. Neither of the two SAY plasmid probes hybridized with DNA from any of the decline agents that were originally isolated from woody hosts, or with DNA from the two plant pathogenic spiroplasmas.

The MBS extrachromosomal DNA probe hybridized with all of the plasmids present in SAY, DAY, TLAY, and three field isolates of western AY under high stringency conditions (Figs. 1B, 2B, data for two of the field isolates is not shown), indicating that the cloned MBS extrachromosomal DNA fragment contains sequences that are present in all of the western AY plasmids. The MBS probe also hybridized with extrachromosomal DNA in some, but not all, of the virescence MLOs that hybridized with the SAY plasmid probes (Fig. 1B). In contrast to the SAY plasmid probes, the MBS probe did not hybridize with extrachromosomal DNAs present in OAY, EAY, or RV MLOs using moderately stringent conditions (Fig. 1B). No

Table 1. Presence of extrachromosomal DNAs in pathologically and geographically diverse mycoplasmalike organisms (MLOs)[a]

Code	MLO	Source	Hybridization probes: SAY	Hybridization probes: MBS
Virescence MLOs				
SAY	Severe western AY	California	+	+
DAY	Dwarf western AY	California	+	+
TLAY	Tulelake western AY	California	+[b]	+
AY-HR	Hanford AY	California	+[b]	+
AY-SC	Santa Cruz AY	California	+	+
AY-DAV	Davis AY	California	+[b]	+
DIV	*Diplotaxis* virescence	Spain	+	+
WAY	Eastern AY	Wisconsin	+[c]	+[c]
EAY	Eastern AY	New Jersey	+	−
OAY	*Oenothera* virescence	Michigan	+	−
RV	Rape virescence	France	+	−
CAY	Canadian AY	Alberta	−	−
AV	European AY	Germany	−[c]	NT[d]
CV	*Catharanthus* virescence	Peru	−	−
BLTVA	Beet leafhopper virescence	California	−[b]	−
ST	Stolbur	Yugoslavia	−[c]	NT
SP	Safflower phyllody	Israel	−[c]	NT
SESP	Sesame phyllody	Thailand	−[c]	NT
CP	Clover proliferation	Alberta	−	−
PWB	Potato witches'-broom	Alberta	−	−
Decline and other MLOs				
X	X-disease	California	−[c]	NT
PD(X)	Pear decline	California	−[c]	NT
EPN	Elm yellows	New York	−[c]	NT
LY	Coconut lethal yellows	Florida	−[c]	NT
AP	Apple proliferation	Germany	−[c]	NT
WL	Sugarcane white leaf	Thailand	−[c]	NT
BG	Bermudagrass white leaf	Thailand	−[c]	NT
MBS	Maize bushy stunt	Florida	+	+
Spiroplasmas				
	S. citri	Morocco	−[c]	NT
	S. kunkelii	California	−[c]	NT

[a] Southern blots of undigested MLO DNA from infected plants were probed with ^{32}P-labeled, native SAY plasmids or a 4.9-kb SAY plasmid fragment (SAY probe), or 4.4 kb of MBS extrachromosomal DNA (MBS probe). Autoradiographs of some of the Southern blots are illustrated in Figures 1 and 2.
[b] See also Kuske and Kirkpatrick 1990.
[c] Not illustrated in a Southern blot.
[d] NT = Not tested.

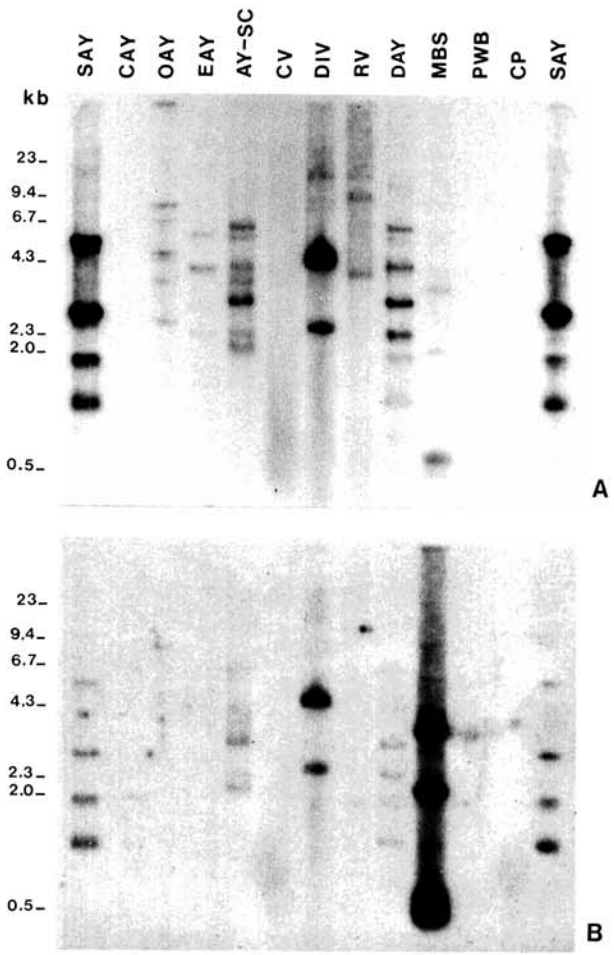

Fig. 1. Southern blot hybridization of undigested MLO DNA from infected plants probed with **A,** a ^{32}P-labeled, 4.9-kb fragment of a plasmid from the severe strain of western aster yellows (SAY) or, **B,** a ^{32}P-labeled probe derived from a cloned fragment (4.4 kb) of extrachromosomal DNA from maize bushy stunt. Blots were washed at moderate stringency as described in the text. See Table 1 for an explanation of MLO isolate codes. The six strong hybridization bands in the SAY lanes are the supercoiled and open circle forms of three of the four native SAY plasmids. The fourth plasmid is in very low titer and is only faintly visible in this exposure. Refer to Kuske and Kirkpatrick (1990) for the location of the fourth plasmid bands.

hybridization was detected between any of the three MLO DNA probes and DNA extracted from healthy celery, periwinkle, lettuce, sesame, or *Veitchia* palm. Extrachromosomal DNA was not clearly detected in DNA from SP or ST; however, background hybridization with these two preparations made definitive interpretation difficult.

DISCUSSION

Because MLOs have not yet been cultured *in vitro*, physiologic or genetic characterization of these plant pathogens has been difficult. Reliance on pathological characteristics, such as plant disease symptoms and host range, and on relationships between the MLO and its insect vector(s) have produced confusion in identifying and differentiating some MLOs. Indeed, the plant host ranges and vector relationships of most MLOs remain uncharacterized. The identification of extrachromosomal DNA in some MLOs may provide one set of genetic markers for differentiating groups of MLOs. For example, extrachromosomal DNA homologous to the SAY plasmid probe was not detected in any of the decline-inducing MLOs that were examined (Table 1). Furthermore, no extrachromasomal DNA was detected in EtBr-stained agarose gels following electrophoresis of tested DNA extracted from plants infected with the decline agents. Unlike AY strains, the decline MLOs cause disease in woody, perennial plants, and when transmitted to herbaceous plants they typically do not produce symptoms of virescence and phyllody. In addition, they are not considered to be very closely related to AY strains by serological (Sinha and Chiykowski 1984; Lin and Chen 1985; Kirkpatrick and Garrott 1986; Jiang *et al.* 1989) or DNA hybridization analyses with cloned fragments of the MLO chromosome used as probes (Kirkpatrick *et al.* 1987; Lee and Davis 1988; Kuske 1989; Sears *et al.* 1989; Lee *et al.* 1990; Kirkpatrick *et al.* 1990; Kuske *et al.*, in press).

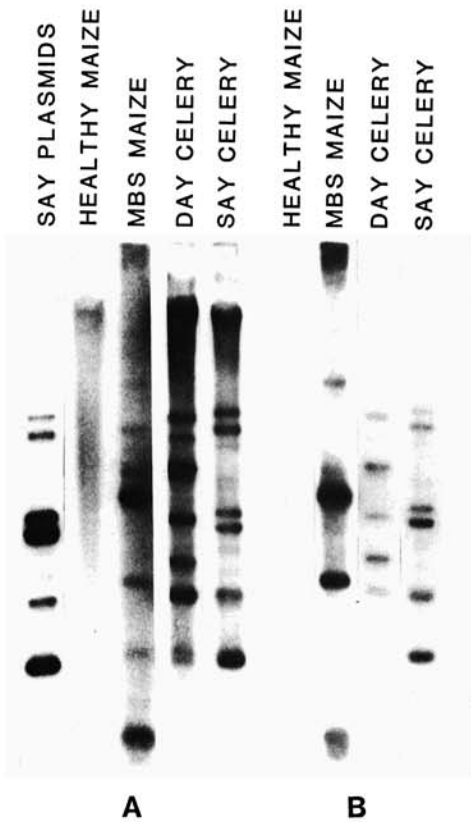

Fig. 2. Southern blot hybridization of undigested DNA extracted from healthy maize, maize infected with maize bushy stunt MLO (MBS), and celery infected with the severe (SAY) or dwarf (DAY) strains of western aster yellows MLO probed with **A,** ^{32}P-labeled, native SAY plasmids or, **B,** a ^{32}P-labeled cloned fragment (4.4 kb) of extrachromosomal DNA from MBS. The minor hybridization that occurred with chromosomal DNA of healthy celery (not shown) and maize when probed with (A) is due to a small amount of contaminating celery DNA present in the native SAY plasmids used as probe. Blots were washed at high stringency as described in the text. An explanation of plasmid forms visible in the SAY plasmids lane is presented in the Figure 1 legend.

Similarities in plant symptoms produced by genetically and serologically different virescence MLOs can make pathogen identification based solely on plant symptoms difficult or impossible. For example, the BLTVA and western AY strains, which are transmitted by different leafhopper vectors (Freitag 1964; Tsai 1979; Golino *et al.* 1987) and are serologically (Golino *et al.* 1989) and genetically (Lee and Davis 1988) distinct cause symptoms that are indistinguishable in some herbaceous hosts. Because DNA from BLTVA-infected hosts does not hybridize with western AY plasmids, the cloned SAY plasmid sequences can be used as diagnostic probes to readily distinguish between these two MLOs. Extrachromosomal DNA has been found in some, but not all MBS strains, and cloned MBS plasmid sequences have been used to differentiate among some strains of this MLO as well (Davis *et al.* 1988). Cloned MLO plasmid probes may also provide better pathogen detection than MLO chromosomal probes because there are multiple copies of some MLO plasmids (Davis *et al.* 1988).

The virescence MLOs possessing extrachromosomal DNA that hybridized with the SAY plasmids represent a geographically diverse collection of MLOs that were isolated from different plant hosts. Despite this diversity, these MLOs appear to form a cluster of genetically related organisms. Other evidence from DNA hybridization analyses using AY chromosomal DNA probes (Lee and Davis 1988; Kuske 1989; Kuske *et al.*, in press) as well as chromosomal and extrachromosomal probes derived from other MLOs (Kirkpatrick *et al.* 1987; Sears *et al.* 1989; Bertaccini *et al.* 1990; Lee *et al.* 1990) generally agree with the genetic relationships suggested by the extrachromosomal DNA homologies presented here. Comparisons of the vector specificities and plant host ranges of these diverse MLO strains may reveal additional biological similarities between the members of this group.

Many culturable bacteria possess plasmids that can be readily transferred and sometimes lost within bacterial populations. For these reasons plasmid-encoded traits are not often used for differentiating bacteria. Further characterization of MLO plasmid stability and their ability to be transferred between MLOs will be necessary to determine whether plasmid presence or composition can be used as a reliable criterion for establishing distinct groups of virescence MLOs.

Western AY strains and MBS possess extrachromosomal

DNAs that cross hybridize. Both AY and MBS cause chlorosis, stunting, and shoot proliferation in their respective plant hosts, but these MLOs differ greatly in their plant host range, vector relationships, and geographic distribution. Maize (*Zea mays* L.) and teosinte (*Zea mays* L. subsp. *mexicana* Schrader Iltis) are the only known plant hosts of MBS (Nault and Bradfute 1979). AY strains infect a large variety of plants, including some monocots (Chiykowski 1963; Banttari 1966; Westdal and Richardson 1969); however, we were not able to transmit SAY to maize (Kuske and Purcell, unpublished data). Although AY has been reported in all temperate regions worldwide (McCoy *et al.* 1989), MBS is restricted to the southern United States, Mexico, and Central America (Nault and Bradfute 1979). MBS is transmitted by *Graminella nigrifrons* and several *Dalbulus* and *Baldulus* species (Nault 1980; Madden and Nault 1983); however, these leafhoppers have not been reported as vectors of AY. Similarly, *Macrosteles fascifrons* Stål, a major vector of AY, does not transmit MBS (Nault 1980). Because of their mutually exclusive plant hosts and insect vectors, AY and MBS have been considered to be unrelated MLOs. However, the appearance of homologous sequences in the extrachromosomal DNAs of these ecologically distinct MLOs suggests they are genetically related, a conclusion that is supported by other hybridization studies using MLO chromosomal DNA probes (Lee *et al.* 1990). The observation that western AY and MBS possess extrachromosomal DNAs with similar sequences raises questions about how these MLOs acquired related plasmids. Because it is unlikely that plasmids were recently exchanged in dually infected plants or insects, these molecules may have been acquired from some ancestral MLO from which the AY and MBS MLOs evolved.

The MBS probe did not hybridize with all of the MLOs (i.e., OAY, EAY, and RIV) that hybridize with the SAY plasmid probes. Although it is possible that there was a smaller proportion of plasmid DNA in the EAY and RIV preparations, there was sufficient plasmid DNA to hybridize with the SAY probes. Furthermore, plasmid bands were readily observed in EtBr-stained gels of the OAY DNA (data not shown) and *Oenothera* tissue consistently contains high MLO titers (Sears and Klomparens 1989). These results suggest that the homologous sequences present in the OAY, EAY, and RIV MLOs and the SAY plasmids were not present in the MBS extrachromosomal probe.

Plasmids present in plant pathogenic bacteria often encode traits affecting pathogenicity (Panopolous and Peet 1985; Chatterjee and Vidaver 1986). It is possible that MLO plasmids may also carry pathogenicity determinants. Our hybridization results indicate that homology exists between extrachromosomal DNAs present in many but not all virescence-inducing MLOs from North America and Europe. This result suggests gene(s) encoding the virescence phenotype are not necessarily associated with the plasmids that were isolated from the SAY MLO. The development of methods to transfer or eliminate MLO extrachromosomal DNAs will be essential for determining the function of these molecules.

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LITERATURE CITED

Banttari, E. E. 1966. Grass hosts of aster yellows virus. Plant Dis. Rep. 50:17-21.

Bertaccini, A., Davis, R. E., Lee, I.-M., Conti, M., Dally, E. L., and Douglas, S. M. 1990. Detection of chrysanthemum yellows mycoplasmalike organism by dot hybridization and Southern blot analysis. Plant Dis. 74:40-43.

Chatterjee, A. K., and Vidaver, A. D. 1986. Advances in Plant Pathology. Vol. 4. Genetics of Pathogenicity Factors: Application to Phytopathogenic Bacteria. Academic Press, New York.

Chiykowski, L. N. 1963. *Endria inimica*, a new leafhopper vector of a celery-infecting strain of aster yellows virus in wheat and barley. Can. J. Bot. 41:669-672.

Davis, M. J., Tsai, J. H., Cox, R. L., McDaniel, L. L., and Harrison, N. A. 1988. Cloning of chromosomal and extrachromosomal DNA of the mycoplasmalike organism that causes maize bushy stunt disease. Mol. Plant-Microbe Interact. 1:295-302.

Freitag, J. H. 1964. Interaction and mutual suppression among three strains of aster yellows virus. Virology 24:401-413.

Golino, D. A., Oldfield, G. N., and Gumpf D. J. 1987. Transmission characteristics of the beet leafhopper transmitted virescence agent. Phytopathology 77:954-957.

Golino, D. A., Kirkpatrick, B. C., and Fisher, G. A. 1989. Production of a polyclonal antisera to the beet leafhopper transmitted virescence agent. Phytopathology (Abstr) 79:1138.

Jiang, Y. P., Chen, T. A., Chiykowski, L. N., and Sinha, R. C. 1989. Production of monoclonal antibodies to peach eastern X-disease agent and their use in disease detection. Can. J. Plant Pathol. 11:325-331.

Kirkpatrick, B. C. Mycoplasma-like organisms. Plant and invertebrate pathogens. In: The Prokaryotes, Vol. 2. A. Balows, G. H. Truper, M. Dworkin, W. Harder, and K. H. Schliefer, eds. Springer-Verlag, New York. In press.

Kirkpatrick, B. C., and Garrot, D. C. 1986. Detection of X-disease in plant hosts by enzyme-linked immunosorbent assay. (Abstr.) Phytopathology 74:825.

Kirkpatrick, B. C., Stenger, D. C., Morris, T. J., and Purcell, A. H. 1987. Cloning and detection of DNA from a nonculturable plant pathogenic mycoplasma-like organism. Science 238:197-200.

Kirkpatrick, B. C., Fisher, G. A., Fraser, J. D., and Purcell, A. H. 1990. Epidemiological and phylogenetic studies on western X-disease mycoplasma-like organisms. Pages 288-297 in: Recent Advances in Mycoplasmology. G. Stanek, G. H. Cassell, J. G. Tully, and R. F. Whitcomb, eds. Int. J. Med. Microbiol. (Zentral. Bakteriol. Microbiol. Hyg., Ser. A., Supplement 20). Gustaf Fisher Verlag, Stuttgart.

Kollar, A., Seemüller, E., Bonnet, F., Saillard, C., and Bové, J. M. 1990. Isolation of the DNA of various plant pathogenic mycoplasmalike organisms from infected plants. Phytopathology 80:233-237.

Kuske, C. R. 1989. Molecular characterization of the western aster yellows mycoplasma-like organisms. Ph.D. thesis. University of California, Davis.

Kuske, C. R., and Kirkpatrick, B. C. 1990. Isolation and characterization of plasmids from western aster yellows mycoplasma-like organism. J. Bacteriol. 172:1628-1633.

Kuske, C. R., Kirkpatrick, B. C., and Seemüller, E. 1991. Differentiation of virescence MLOs using western aster yellows mycoplasma-like organism chromosomal DNA probes and restriction fragment length polymorphism analysis. J. Gen. Microbiol. 137:153-159.

Lee, I.-M., and Davis, R. E. 1988. Detection and investigation of genetic relatedness among aster yellows and other mycoplasmalike organisms by using cloned DNA and RNA probes. Mol. Plant-Microbe Interact. 1:303-310.

Lee, I.-M., Davis, R. E., and DeWitt, N. D. 1990. Nonradioactive screening method for isolation of disease-specific probes to diagnose plant diseases caused by mycoplasmalike organisms. Appl. Environ. Microbiol. 56:1471-1475.

Lin, C.-P., and Chen, T. A. 1985. Monoclonal antibodies against the aster yellows agent. Science 227:1233-1235.

Madden, L., and Nault, L. R. 1983. Differential pathogenicity of corn stunting mollicutes on survival and fecundity of *Dalbulus* leafhoppers. Ann. Appl. Biol. 108:475-485.

Maniatis, T., Fritsch, G. F., and Sambrook, J. 1984. Molecular Cloning:

A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
McCoy, R. E., Cauldwell, A., Chang, C. J., Chen, T. A., Chiykowski, L. N., Cousin, M. T., Dale, J. L., de Leeuw, G. T. N., Golino, D. A., Hackett, K. J., Kirkpatrick, B. C., Marwitz, R., Petzold, H., Sinha, R. C., Sugiura, M., Whitcomb, R. F., Yong, I. L., Zhu, B. M., and Seemüller, E. 1989. Plant diseases associated with mycoplasma-like organisms. Pages 545-640 in: The Mycoplasmas, Vol. V. R. F. Whitcomb and J. G. Tully, eds. Academic Press, New York.
Nault, L. R. 1980. Maize bushy stunt and corn stunt: A comparison of disease symptoms, pathogen host ranges, and vectors. Phytopathology 70:659-662.
Nault, L. R., and Bradfute, O. E. 1979. Corn stunt: Involvement of a complex of leafhopper-borne pathogens. Pages 561-586 in: Leafhopper Vectors and Plant Disease Agents. K. Maramorosch and K. F. Harris, eds. Academic Press, New York.
Panopolous, N., and Peet, R. C. 1985. The molecular genetics of plant pathogenic bacteria and their plasmids. Annu. Rev. Phytopathol. 23:381-419.
Raju, B. C., Nyland, G., and Purcell, A. H. 1983. Current status of the etiology of pear decline. Phytopathology 73:350-353.
Sears, B. B., and Klomparens, K. L. 1989. Leaf tip cultures of the evening primrose allow stable, aseptic culture of mycoplasma-like organisms. Can. J. Plant Pathol. 11:343-348.
Sears, B. B., Lim, P.-O., Holland, N., Kirkpatrick, B. C., and Klomparens, K. L. 1989. Isolation and characterization of DNA from a mycoplasmalike organism. Mol. Plant-Microbe Interact. 2:175-180.
Sinha, R. C., and Chiykowski, L. N. 1984. Purification and serological detection of mycoplasma-like organisms from plants affected by peach eastern-X disease. Can. J. Plant Pathol. 6:200-205.
Tsai, J. H. 1979. Vector transmission of mycoplasmal agents of plant diseases. Pages 265-307 in: The Mycoplasmas, Vol. III. R. F. Whitcomb and J. G. Tully, eds. Academic Press, New York.
Westdal, P. H., and Richardson, H. P. 1969. The susceptibility of cereals and wild oats to an isolate of the aster yellows pathogen. Can. J. Bot. 47:755-760.