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Molecular Diagnosis of Mycoplasma-like Organisms (MLOs) in Plants

A Review

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ABSTRACT

Worldwide, yellows diseases impact plants important in human nutrition, the natural environment, and the culture and commerce of humans. Since the presumed pathogens, mycoplasma-like organisms (MLOs), have not been isolated in pure culture in vitro, their study must proceed by other experimental approaches. In a study of disease affecting grapevines in Europe and North America, polymerase chain reactions (PCR) and restriction analyses of PCR-amplified DNA were used to detect and differentiate strains of MLOs associated with grapevine yellows. MLOs were detected both in naturally diseased grapevines and in experimentally inoculated host plants. The data indicated an unexpected genomic diversity among grapevine-infecting MLOs, and supported their classification with MLOs in the aster yellows, X-disease, and elm yellows groups. The presence of diverse MLOs in grapevines provokes consideration that these MLOs may be present in overlapping geographic ranges and that multiple MLO infections may occur in individual plants, increasing the complexity of grapevine yellows epidemiology and control and the significance of sensitive MLO detection in planting stock and phytosanitary-regulated germplasm.

Index Entries: Detection; mollicutes; grapevine yellows; flavescence dorée; mycoplasmas; epidemiology; etiology; 16S rRNA gene; RFLP analysis.

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Plant yellows diseases comprise a large group of maladies characterized by extensive abnormalities in plant growth and development, suggestive of profound disturbances in hormonal balance. The effects of these maladies can vary widely depending on susceptible plant genotype and pathogen strain, but symptoms typically include yellowing of leaves, stunting, proliferation of axillary shoots, phyllody and virescence of flowers, and sterility. The impact of these diseases is global, reaching plants important in human nutrition, the natural environment, and the culture and commerce of man.

Yellows diseases are presumed to be caused by cell wall-less prokaryotes known as mycoplasma-like organisms (MLOs). In their ultrastructure and susceptibility to tetracycline antibiotics, MLOs resemble mycoplasmas known to infect humans and animals. Unfortunately, unlike mycoplasmas, the plant-inhabiting MLOs have not yet been obtained in pure culture in vitro, and their study must proceed by other experimental approaches.

Of available methods for detection of MLOs in infected hosts, perhaps the most sensitive and broadly applicable are DNA-based technologies (reviewed in ref *1*). Use of these methods has yielded highly sensitive pathogen detection and made possible a rational system for MLO classification (*1–8*). In studies of yellows disease(s) in grapevine (*Vitis vinifera* L.), several DNA-based methods have been utilized to analyze the diversity among MLOs associated with disease in different geographic regions (*5,9–13*). The methods used in our laboratory included restriction fragment length polymorphism (RFLP) analysis of total MLO chromosomal DNA, nucleotide sequencing of randomly cloned MLO DNA fragments and of MLO 16S rRNA gene regions, design of oligoprimers for use in polymerase chain reactions (PCR), PCR using a variety of primers for general or group-specific MLO detection and identification, strain- and subgroup-specific amplification of MLO DNA, and RFLP analysis of PCR amplification products. For example, amplification of MLO-specific 16S rDNA primed by oligonucleotide pair (oligopair) r16SF2/R2, previously designed for universal MLO detection (*7*), resulted in detection of MLOs in naturally diseased grapevine in the United States and in plants experimentally infected by grapevine yellows-associated MLOs (Fig. 1). RFLP analysis of amplified 16S rDNA, and group-specific DNA amplification primed by selected oligopairs, permitted group classification of the detected MLOs. The results of these studies indicated that grapevines may be naturally infected by genetically diverse MLOs that can be classified with aster yellows, X-disease, and elm yellows MLOs (Table 1).

The breadth of genomic diversity encountered among the grapevine-infecting MLOs was unexpected. The findings provoke consideration that multiple MLO infection may exist in individual plants and that such infections may be significant both for understanding yellows disease in grapevine and for eliminating the pathogens from planting stock and

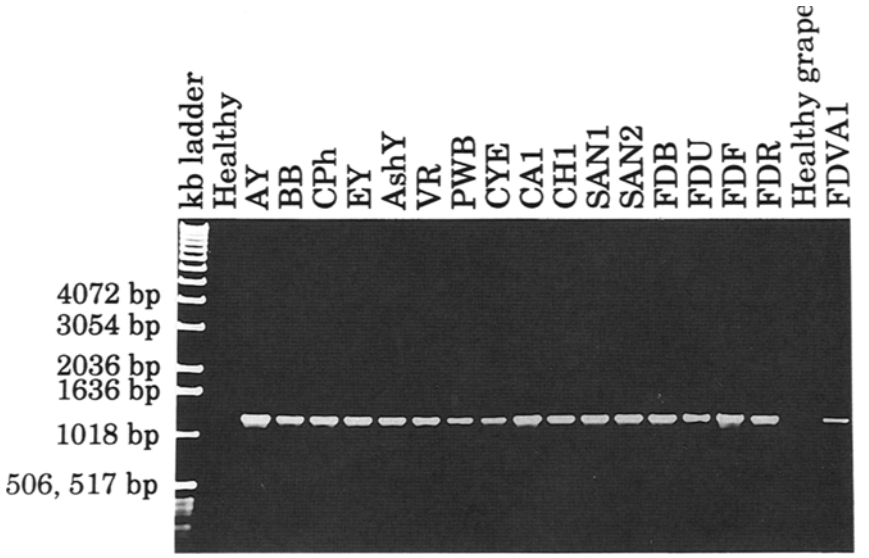

Fig. 1. Detection of mycoplasma-like organisms (MLOs) using polymerase chain reaction (PCR) amplification of a 16S ribosomal (r) RNA gene sequence primed by oligonucleotide pair r16SF2/R2. Oligonucleotides r16SF2/R2 were designed on the basis of the sequence determined for the 16S rRNA gene from an aster yellows MLO strain (7). Template DNA was extracted from healthy or diseased plants of periwinkle (*Catharanthus roseus*), broadbean (*Vicia faba*), or grapevine (*Vitis vinifera*) infected by MLOs. CA1, CH1, SAN1, SAN2, FDB, FDU, and FDR: grapevine yellows-associated MLO strains from Italy in periwinkle. FDF, grapevine yellows-associated MLO strain from France in broadbean. FDVA1, grapevine yellows-associated MLO strain in naturally infected grapevine from Virginia, USA. Healthy, healthy periwinkle. The following MLOs were in periwinkle unless otherwise indicated. AY, Maryland aster yellows. BB, tomato big bud. CPh, clover phyllody. EY, elm yellows. AshY, ash yellows. VR, beet leafhopper-transmitted virescence. PWB, potato witches' broom. CYE, clover yellow edge in clover (*Trifolium repens*). From ref. 8.

germplasms. It is abundantly evident that the availability of new biotechnologies has already opened important opportunities in fundamental and applied studies of the intriguing MLO pathogens and plant yellows diseases.

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Table 1
Group Affiliation of Grapevine Yellows-Associated MLO Strains from Europe and North America[a]

MLO strain designation	Geographic origin	Group affiliation[b]
FDB	Puglia (Southern Italy)	Aster Yellows MLO Group (I)
FDR	Lazio (Central Italy)	Aster Yellows MLO Group (I)
CA1	Emilia-Romagna (Northern Italy)	Aster Yellows MLO Group (I)
CH1	Emilia-Romagna (Northern Italy)	Aster Yellows MLO Group (I)
SAN1	Emilia-Romagna (Northern Italy)	Aster Yellows MLO Group (I)
SAN2	Emilia-Romagna (Northern Italy)	Aster Yellows MLO Group (I)
FDG	Germany	Aster Yellows MLO Group (I)
FDF	France	Elm yellows MLO Group (V)
FDU	Friuli-Venezia-Giulia (Northern Italy)	X-disease MLO Group (III)
FDVA1	Virginia, USA	X-disease MLO Group (III)

[a] Adapted from data in ref. 8.
[b] Group affiliation as delineated in ref. 7.

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