Letter to the Editor
The Enigma of Mycoplasma in Plants and Insects

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Considerable confusion exists regarding the causal relationships of various mycoplasmalike organisms (MLO) observed in plants and insect vectors, and the diseases with which they are associated. The discovery of MLO in phloem elements of yellows-diseased plants, their disappearance after treatment with certain tetracycline antibiotics, and the concomitant remission of disease symptoms, announced in three papers in Japan in 1967, have revolutionized work on yellows-type diseases earlier presumed to be caused by viruses. Many research groups have followed the leads provided by Doi et al. (5), Ishiie et al. (14), and Nasu et al. (19). In order to avoid some of the mistakes made with virus diseases on the inferential relationships, it is desirable to culture the MLO apart from their hosts and carry out inoculation tests with the axenic cultures. Furthermore, such cultures are essential for identification of the different species and races, and for establishing a logical system of classification based upon morphology, chemistry, physiology, and serology.

Several reports have been made on the cultivation of MLO from Taiwan (16, 17), Canada (3), United States (2, 4, 7, 13), France (6, 9, 10, 11, 12, 21, 22, 23), Italy (18), and India (8, 20). Some of these reports indicate maintenance of MLO in vitro, whereas others report successful subculturing and even inoculation and reisolation of comparable cultures. I have no intention of questioning the veracity of competent investigators, but at present very few cultures have been made available to animal mycoplasmatologists for comparative tests, and no cultures have been deposited at any central repository such as a type culture collection. The animal mycoplasma workers require for the description of a new mycoplasma species the deposition of the isolate in a recognized culture collection and the designation of an accession number. Only after this procedure has been completed can a new species be named.

During the past 3 years I have become aware of many pitfalls that can be encountered in attempts to isolate MLO. Some are discussed below.

1) Contamination from outside sources, including the mouth of the person who carries out the isolation, may result in *Mycoplasma hominis* or *M. orale* contamination.

2) All commercial horse sera as well as bovine sera may contain contaminations with animal mycoplasma species (1). It seems conceivable that these species would grow more actively in response to growth-promoting substances present in the sap of diseased but not of healthy plants. If so, contaminants would be demonstrable in mycoplasma media containing extracts from diseased plants, but not necessarily extracts from healthy controls.

3) Filtration of media and plant or insect extracts through 0.45 micron filters does not prevent passage of mycoplasmas: the higher the pressure, the faster mycoplasmas pass through standard bacterial filters.

4) Pseudocolonies, that is, nonliving formations of sterol salts of calcium and magnesium have been observed and described in the literature (15) for nearly 50 years, but sometimes they were mistakenly identified as mycoplasma colonies. When material from pseudocolonies is inoculated into healthy plants, chlorosis follows, but there is no vein-clearing or witches'-broom effect.

5) Pathogenicity tests in the case of yellows-type diseases usually require inoculation of vectors that, in turn, are used to inoculate plants. Let us assume that the plant pathogen has failed to grow but did retain infectivity in a serum-containing medium, whereas a contaminant such as *M. arginini* formed typical "fried-egg" colonies. The latter could be identified mistakenly as MLO, and broth containing both the MLO and the contaminant could be used for injection of vectors. The insects would be rendered infective and plants would be successfully inoculated even though MLO had failed to grow. Koch's postulates would thus be mimicked, but not proven. If proper subculturing, not mere dilution of the original inoculum, were carried out and the same tests successfully performed, the evidence would be more convincing, but the naming of the isolate would still require adherence to rules established for the taxonomy of Mycoplasmatales, including deposition in a type culture collection.

Identification of a known animal-infecting or saprophytic member of the Mycoplasmatales in plant or insect extracts does not preclude the possibility that the isolate is, indeed, plant-pathogenic. A known species of mycoplasma could perhaps infect a plant, although this would seem unlikely in view of the known specificity of the yellows-type disease agents as well as that of animal-parasitic mycoplasmas. Since antisera are available for all cultured mycoplasma species, serological tests could establish the identity of the MLO in situ in phloem sections of diseased plants. Should plant MLO cause animal diseases and plants serve as reservoirs of mycoplasmas that cause human and animal diseases, the implications would have an enormous bearing on public health, but there is little reason to suspect this at present.

This letter is not intended to detract from the valuable contributions made during the past 4 years, and it does not question the feasibility of isolation and cultivation of the MLO. It is likely that some MLO have already been cultured, but the reports have

not always proven this adequately.

Recently, the following have generously offered to examine, and compare, isolates obtained from diseased plants and/or insect vectors: E. A. Freundt, FAO/WHO Virus Reference Centre, Institute of Medical Microbiology, University of Aarhus, Aarhus, Denmark; D. Taylor-Robinson, MRC Clinical Research Centre, Northwick Park Hospital, Watford Road, Harrow, Middlesex, Great Britain; and Michael F. Barile, Mycoplasma Section, Division of Biologic Standards, NIH, Bethesda, Maryland 20014, USA.

The American Type Culture Collection has offered to receive cultures of new species, and shipments can be addressed to ATCC, Margaret C. Norman, 12301 Parklawn Drive, Rockville, Md. 20852, USA. At the ATCC there is someone on duty at all times to receive cultures.

There now exist over 45 antigenically distinct species of Mycoplasmatales. An examination by experts could easily clarify whether a culture represents one of the known species or whether it is a new, as yet undescribed, member of this group.

LITERATURE CITED

1. BARILE, M. F., & J. KERN. 1972. Isolation of Mycoplasma arginini from commercial bovine sera and its implication in contaminated cell cultures. Proc. Soc. Exp. Biol. Med. 138:432-437.
2. CALAVAN, E. C., E. C. K. IGWEGBE, & A. E. FUDL-ALLAH. 1971. Recent developments in the etiology and control of stubborn disease of citrus, p. 132-133. Proc. Second Int. Symp. Plant Pathol. New Delhi. (Abstr.).
3. CANADA DEPARTMENT OF AGRICULTURE RESEARCH STATION, VANCOUVER, BRITISH COLUMBIA. 1969. Canada Department of Agriculture, Research Branch, Research Report 1969, Ottawa, p. 351.
4. CHEN, T.-A., & R. R. GRANADOS. 1970. Plant-pathogenic mycosplasma-like organism: maintenance in vitro and transmission to Zea mays L. Science 167:1633-1636.
5. DOI, Y., M. TERENAKA, K. YORA, & H. ASUYAMA. 1967. Mycoplasma- or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or paulownia witches' broom (in Japanese, English Abstr.). Ann. Phytopathol. Soc. Japan 33:259-266.
6. FAIVRE-AMIOT, A., J.-P. MOREAU, M.-T. COUSIN, & T. STARON. 1970. Essai de mise en culture de l'agent de la Phyllodie du Trèfle. Ann. Phytopathol. 2:251-258.
7. FUDL-ALLAH, A. E., E. C. CALAVAN, & E. C. K. IGWEGBE. 1971. Culture of a mycoplasmalike organism associated with stubborn disease of citrus. Phytopathology 61:1321 (Abstr.).
8. GHOSH, S. K., S. P. RAYCHAUDHURI, A. VARMA, & T. K. NARIANI. 1971. Isolation and culture of mycoplasma associated with citrus greening disease. Curr. Sci. (India) 40: 299-300.
9. GIANNOTTI, J., C. LOUIS, & C. VAGO. 1971. Ultrastructure de mycoplasmas phytopathogènes cultivés in vitro. J. Microsc. (Paris) 11:61 (Abstr.).
10. GIANNOTTI, J., & C. VAGO. 1971. Role des mycoplasmes dans l'étiologie de la phyllodie du Trèfle: culture et transmission expérimentale de la maladie. Physiol. Veg. 9:541-553.
11. GIANNOTTI, J., C. VAGO, F. LECLANT, G. MARCHOUX, & D. CZARNECKY. 1972. Obtention de cultures de mycoplàsmes a partir de Lavendula hybrida Reverchon atteinte de "dépérissement" et de Hyalestes obsoletus Sign., un vecteur probable de la maladie. C. R. Hebd. Seances Acad. Sci. Ser. D Sci. Nat. (Paris) 274:394-397.
12. GIANNOTTI, J., C. VAGO, G. MARCHOUX, G. DEVAUCHELLE, & D. CZARNECKY. 1972. Caracterisation par la culture in vitro de souches de mycoplasmes correspondant a huit maladies differentes de plantes. C. R. Hebd. Seances Acad. Sci. Ser. D Sci. Nat. (Paris) 274:330-333.
13. HAMPTON, R. O., J. O. STEVENS, & T. C. ALLEN. 1969. Mechanically transmissible mycoplasma from naturally infected peas. Plant Dis. Reptr. 53:499-503.
14. ISHIIE, T., Y. DOI, K. YORA, & H. ASUYAMA. 1967. Suppressive effects of antibiotics of tetracycline group on symptom development of mulberry dwarf disease (in Japanese, English Abstr.). Ann. Phytopathol. Soc. Japan 33:267-275.
15. LAIDLAW, P. P. 1925. On structures which develop in certain culture media and resemble colonies of microorganisms. Brit. J. Exp. Pathol. 6:36-39.
16. LIN, S.-C., & C.-S. LEE. 1969. White leaf disease of sugarcane. Sugarcane Pathologists Newsletter 3:2-3.
17. LIN, S.-C., C.-S. LEE, & R.-J. CHIU. 1970. Isolation and cultivation of, and inoculation with, a mycoplasma causing white leaf disease of sugarcane. Phytopathology 60:795-797.
18. LOMBARDO, G., & P. PIGNATTELLI. 1970. Cultivation in a cell-free medium of a mycoplasma-like organism from Vinca rosea with phyllody symptoms of the flowers. Ann. Microbiol. 20:83-88.
19. NASU, S., M. SUGIURA, T. WAKIMOTO, & T. T. IIDA. 1967. On the etiologic agent of rice yellow dwarf disease (in Japanese). Ann. Phytopathol. Soc. Japan 33:343-344 (Abstr.).
20. NAYAR, R. M., & H. S. ANANTHAPADMANABHA. 1970. Isolation, cultivation and pathogenicity trials with mycoplasma-like bodies associated with sandal spike disease. J. Indian Acad. Wood Sci. 1:59-61.
21. SAGLIO, P., D. LAFLÈCHE, C. BONISSOL, & J. M. BOVÉ. 1971. Isolement et culture in vitro des mycoplasmes associés au "Stubborn" des agrumes et leur observation au microscope électronique. C. R. Hebd. Seances Acad. Sci. Ser. D Sci. Nat. (Paris) 272:1387-1390.
22. SAGLIO, P., D. LAFLÈCHE, C. BONISSOL, & J. M. BOVÉ. 1971. Isolement, culture et observation au microscope électronique des structures de type mycoplasme associés à la maladie du Stubborn des agrumes et leur comparaison avec les structures observées dans le cas de la maladie du Greening des agrumes. Physiol. Veg. 9:569-582.
23. VAGO, C., & J. GIANNOTTI. 1971. Évolution et état actuel des recherches sur les mycoplasmes des végétaux et des invertébrés. Bull. Soc. Sci. Vet. Med. Comp., Lyon 73:69-82.