Methods in Mycoplasmology

Volume I

MYCOPLASMA CHARACTERIZATION

Edited by

SHMUEL RAZIN

Department of Membrane and Ultrastructure Research
The Hebrew University—Hadassah Medical School
Jerusalem, Israel

JOSEPH G. TULLY

Mycoplasma Section
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Frederick Cancer Research Facility
Frederick, Maryland

With the cooperation and assistance of participants in the International Research Program on Comparative Mycoplasmology of the International Organization for Mycoplasmology

1983

ACADEMIC PRESS

A Subsidiary of Harcourt Brace Jovanovich, Publishers

New York London
Paris San Diego San Francisco São Paulo Sydney Tokyo Toronto

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data
Main entry under title:

Mycoplasma characterization.

(Methods in mycoplasmology ; v. 1)
Includes index.
1. Mycoplasmatales. I. Razin, Shmuel.
II. Tully, Joseph G. III. Series.
QR352.M47 1983 vol. 1 589.9s [589.9] 82-24384
ISBN 0-12-583801-8

PRINTED IN THE UNITED STATES OF AMERICA

83 84 85 86 9 8 7 6 5 4 3 2 1

Preface

In 1971, a small group of mycoplasmologists met in Paris under the auspices of the World Health Organization and the Food and Agriculture Organization to establish an international program on animal mycoplasma characterization. Shortly thereafter, the coordinating Board of the program organized a series of working teams on mycoplasmas of various animal hosts.* In 1978, this program, the name of which has been changed to the International Research Program on Comparative Mycoplasmology (IRPCM), became an operating component of the International Organization for Mycoplasmology (IOM). Today, the IRPCM program is composed of ten working teams and mycoplasmologists from more than 60 laboratories around the world collaborating on the development of improved techniques for the isolation and characterization of mycoplasmas from man, animals, plants, and insects.

One of the major objectives of the early IRPCM program was the preparation and distribution of standardized technical procedures used in mycoplasma characterization. As a result, five documents on various methods were published by WHO. The dissemination of these documents to mycoplasma workers around the world and their general acceptance as practical working procedures soon led the Board to formulate plans for a general methodology manual covering the very broad range of techniques currently employed in mycoplasma research and diagnosis. These plans received further stimulation in 1979 when the IOM, in conjunction with the Université de Bordeaux II and the Institut National Recherche Agronomique in France, organized a mycoplasma techniques course in Bordeaux, France. The laboratory manual prepared for this course, as a collaborative effort of more than 20 mycoplasmologists, and the experience gained through the use of this manual facilitated considerably the formulation of the outline for the "Methods in Mycoplasmology" volumes.

The two volumes of "Methods in Mycoplasmology" are the result of cumulative efforts to meet the need for standardized techniques in mycoplasmology. We have had the counsel and guidance of the IRPCM Board, and most of the authors of the individual chapters are members of the Board or one of the Working Teams in the Program. We have also had the generous assistance of other experts in the field in an endeavor to provide a comprehensive manual for laboratories working on mycoplasmas. We are well aware that techniques out-

*Members of the Board (1974). The FAO/WHO programme on comparative mycoplasmology. *Vet. Record* **95**, 457–461.

lined for various rapidly moving subdisciplines in the field, such as those described here for plant and arthropod mycoplasmas, may soon become outdated. However, it is our feeling that the great majority of the techniques detailed here form the solid basis of well-tried and standardized procedures that will continue to be useful in recovery, identification, and characterization of mycoplasmas. Finally, it seems obvious at this point in time that the field of mycoplasmology will continue to expand rapidly, covering newly recognized mycoplasma species, new hosts, and new diseases. It is hoped that the present methodology manual will fulfill an important role in these developments.

Shmuel Razin
Joseph G. Tully

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SECTION A

Introduction

A1

CHARACTERISTICS OF THE MYCOPLASMAS AS A GROUP

Shmuel Razin

The single most important characteristic that distinguishes the mycoplasmas from other prokaryotes is their total lack of a cell wall. In fact, demonstration of a single membrane having a trilaminar shape in properly fixed and sectioned cells is an essential requirement for defining a new isolate as a mycoplasma (Subcommittee, 1979). This anatomical feature is responsible for most of the peculiar properties of the mycoplasmas as a group, including their typical morphology and plasticity; their sensitivity to lysis by osmotic shock, alcohols, organic solvents, detergents, antibody and complement; their filterability through 0.45-μm pore diameter filters (Chapter C12 and D3, this volume); their fried-egg colony shape (Chapter B11, this volume); and their total resistance to penicillin and other antibacterial substances that degrade or specifically inhibit peptidoglycan synthesis (Chapter C16, this volume). Many of the aforementioned features are shared by the L-phase variants (L forms) of bacteria, in which the cell wall is either defective or is totally missing. However, the L-phase variants, which are usually laboratory artifacts, can revert to the wall-covered bacterial forms once the inducing substance (e.g., penicillin, lysozyme, antibody) is removed from the growth medium (Chapter C16, this volume). Moreover, the L-phase variants, unlike mycoplasmas, are capable of synthesizing precursors of bacterial cell wall polymers and penicillin-binding proteins (Martin *et al.*, 1980).

The lack of a cell wall constitutes the basis for the inclusion of the mycoplasmas in a separate class: Mollicutes (*mollis*, soft; *cutis*, skin). The common term *mycoplasmas* has been used rather loosely to denote any species in the class Mollicutes, whereas the terms *acholeplasmas*, *ureaplasmas*, *spiroplasmas*, and *anaeroplasmas* are used when reference is made to members of the correspond-

METHODS IN MYCOPLASMOLOGY, VOL. I

ISBN 0-12-583801-8

ing genus, rather than to a defined species within the genus. The term *mollicutes* has been proposed as a common name for any member of the class. In this case, the common name mycoplasmas will be retained for *Mycoplasma* species only. The principles of mycoplasma classification and taxonomy are discussed in Chapter A2 in this volume.

The mycoplasmas are the smallest self-replicating prokaryotes. Mycoplasma cultures contain coccoid cells that have diameters as small as 300 nm and are still capable of reproduction. Moreover, mycoplasmas usually exhibit an extremely simple ultrastructure. The mycoplasma cell is bounded by a plasma membrane, and the enclosed cytoplasm contains ribosomes and a circular double-stranded DNA molecule. A fibrillar network, probably representing a primitive "cytoskeleton" and participating in contractile processes and shape formation, has been demonstrated in a few mollicutes but may be a common feature of these plastic microorganisms. In some mycoplasmas unique polar organelles shaped as tapered tips or blebs built around a central striated rod have been observed. These tips appear to play a role in the attachment of mycoplasmas to host cells and to inert surfaces (see Chapters B12 and E11 in this volume and Chapter E10 in Vol. II).

The mycoplasma genome is typically prokaryotic. However, the size of the *Mycoplasma* and *Ureaplasma* genome (approximately 5×10^8 daltons) is the smallest recorded for any self-replicating prokaryote, being approximately one-sixth the size of the *Escherichia coli* genome and approximately one-half the size of the genome of *Rickettsia* (Myers *et al.*, 1980). The extremely low guanine + cytosine (G + C) content of mycoplasmal DNA (23 to 30 moles % G + C in most species) imposes further restrictions on the amount of genetic information available to mycoplasma cells, in line with their complex nutritional requirements and their parasitic or saprophytic mode of life.

The replication of the mycoplasma genome resembles that of other prokaryotes in being semiconservative. However, cytoplasmic division in mycoplasmas frequently lags behind genome replication, resulting in the formation of multinuclear filaments (Chapter B1, this volume). The protein-synthesizing machinery in mycoplasmas is also prokaryotic in nature so that antibiotics such as chloramphenicol and the tetracyclines inhibit protein synthesis and growth of mycoplasmas. Mycoplasma ribosomes resemble those of other prokaryotes in size and RNA species. Ribosomal RNA synthesis in *M. capricolum* is subject to the same stringent control mechanism found in *E. coli* (Glaser *et al.*, 1981), but the number of cistrons involved in ribosomal RNA synthesis in mycoplasmas is only 1 or 2, compared to at least 7 in *E. coli* and 10 in *Bacillus subtilis* (Sawada *et al.*, 1981; Amikam *et al.*, 1982). Hence, it appears that the minute mycoplasma cells contain only the minimum sets of organelles and metabolic pathways essential for cellular growth and replication. This extreme simplicity has