Advances in

MICROBIAL PHYSIOLOGY

Vol. 10

Advances in

MICROBIAL PHYSIOLOGY

Edited by

A. H. ROSE

School of Biological Sciences
Bath University
England

and

D. W. TEMPEST

Laboratorium voor Microbiologie,
Universiteit van Amsterdam,
Amsterdam-C
The Netherlands

VOLUME 10

1973

ACADEMIC PRESS · LONDON and NEW YORK

A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS INC. (LONDON) LTD.
24/28 Oval Road
London NW1

United States Edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue
New York, New York 10003

Library of Congress Catalog Card Number: 67-19850
ISBN: 0 12-027710-7

PRINTED IN GREAT BRITAIN BY
WILLIAM CLOWES AND SONS LIMITED
LONDON, COLCHESTER AND BECCLES

Contributors to Volume 10

E. A. Dawes, *Department of Biochemistry, University of Hull, Kingston upon Hull, England*

J. Le Gall, *University of Georgia, Athens, Georgia, U.S.A. and C.N.R.S., Marseilles, France*

J. R. Postgate, *University of Sussex, Brighton, England*

P. J. Senior, *Department of Biochemistry, University of Hull, Kingston upon Hull, England*

S. Razin, *Department of Clinical Microbiology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel*

Contents

Physiology of Mycoplasmas — SHMUEL RAZIN

Contents

Physiology of Mycoplasmas — SHMUEL RAZIN

The Physiology of Sulphate-Reducing Bacteria

JEAN LE GALL AND JOHN R. POSTGATE

The Role and Regulation of Energy Reserve Polymers in Micro-organisms — EDWIN A. DAWES AND PETER J. SENIOR

Physiology of Mycoplasmas

SHMUEL RAZIN

Department of Clinical Microbiology
The Hebrew University-Hadassah Medical School
Jerusalem, Israel

I. Introduction

The physiology of mycoplasmas, the smallest organisms capable of autonomous growth, is of special interest in view of their extremely simple structure and limited biochemical activities. In recent years they have been used quite extensively in biochemical studies, particularly those concerned with the cell membrane, and it seems worthwhile, at this juncture, to survey the new insights that have been gained since the publication of the last reviews on the subject (Razin, 1969a; Smith, 1971a).

II. Ecology

Thanks to recent improvements in cultivation and identification techniques, quite a number of further mycoplasma species could be established. By now over 40 species occurring in primates, farm and laboratory animals, and a variety of wild animals have been named (Razin, 1973; Freundt, 1973). Many more no doubt remain to be cultivated and identified as further biological materials are examined and better culture media become available.

From the highly exacting nature of the mycoplasmas, strict host specificity was initially inferred, but recent findings seem to contradict this assumption. Thus, monkeys were shown to harbour human mycoplasmas (Del Giudice *et al.*, 1969), while *Mycoplasma canis*, a dog mycoplasma, was isolated from man (Armstrong *et al.*, 1971) and *M. arginini* from a wide range of animals (Barile *et al.*, 1968). Of special interest is the isolation of *Acholeplasma laidlawii* from a variety of hosts (see Tully and Razin, 1968), casting doubt on the saprophytic nature ascribed to them because they were originally isolated from sewage and soil which they may well have reached via animal excreta. Considering also their strict nutritional requirements and osmotic sensitivity, it seems unlikely that they should be able to lead a truly saprophytic life under the variable ecological conditions of soil or sewage.

Nevertheless the recent discovery by Brock and his associates (Darland *et al.*, 1970; Belly and Brock, 1973) of mycoplasma-like organisms in self-heated coal-refuse piles seems to indicate that wall-less prokaryotes can live as true saprophytes. The thermophilic, acidophilic prokaryotes without cell walls, growing best at 55°C and pH 2·0, are somewhat larger than most animal mycoplasmas, but are like them in ultrastructure (Fig. 1), DNA base composition, and probably in mode of reproduction. Though the unusual physiological properties of the new *Thermoplasma acidophilum* suggest a rather distant relationship to the animal mycoplasmas, its inclusion in the Mollicutes class seems warranted, and its very existence considerably broadens the range of habitats in which mycoplasma-like organisms are found.

The flood of papers describing mycoplasma-like organisms in diseased plants and their insect vectors released during the past four years has been thoroughly reviewed by other authors (Maramorosch *et al.*, 1970; Davis and Whitcomb, 1971; Hull, 1971). The agents of the very large group of yellows plant diseases, long thought to be viruses, have recently been identified as mycoplasmas or, more cautiously, as mycoplasma-like organisms (MLO). Thin sections of diseased plant phloem and of tissues of infected insect vectors show numerous bodies indistinguishable from sectioned animal mycoplasmas (Fig. 1). Phase-contrast microscopy and freeze etching studies accentuate their morphological resemblance to animal mycoplasmas (Davis *et al.*, 1972), further borne out by the successful treatment of the diseased plants and vectors with tetracyclines, and the ineffectiveness of antibiotics like penicillin that specifically inhibit bacterial cell-wall synthesis (Davis and Whitcomb, 1970). Marked heat sensitivity and rapid death in buffer solutions are further points of similarity (Chen and Granados, 1970).

A major obstacle to the biochemical and serological characterization and consequent classification of the plant MLOs is the lack of adequate growth media. Sometimes viability could be sustained for a considerable period *in vitro* (Chen and Granados, 1970); in other instances the organisms isolated appeared to be laboratory contaminants (Hampton *et al.*, 1969; Lin *et al.*, 1970). Saglio *et al.* (1971) and Gianotti *et al.* (1971), however, managed to isolate MLOs from diseased plants which seem to differ from the known animal mycoplasmas, the ones cultured by Gianotti *et al.* (1971) being capable of infecting the insect vector and the plant. Several laboratories are now comparing their biochemical and serological properties with those of animal mycoplasmas to ascertain whether they really are plant mycoplasmas.

Why is it so difficult to cultivate plant MLOs? One reason may be their intracellular location in the plant and insect as distinct from animal mycoplasmas, which are rarely intracellular. This may indicate a stricter adaptation of the parasite to conditions which may be difficult to simulate in a cell-free medium.

Morphology and ultrastructural features alone may be doubtful criteria for the identification of an organism as a mycoplasma, but not for its exclusion from this group. Thus, upon careful examination of electron micrographs of thin sections, the allegedly mycoplasma-like bodies claimed to cause male sterility in *Drosophila* (Williamson *et al.*, 1971) are seen to be bounded by two rather than by one membrane (Fig. 1). Hence they seem not to be mycoplasmas, but probably belong to the rickettsia or the chlamydia group. The "Greening" agent of citrus also does not seem to qualify for membership since its membrane is about twice as thick as that of mycoplasmas and the distance between

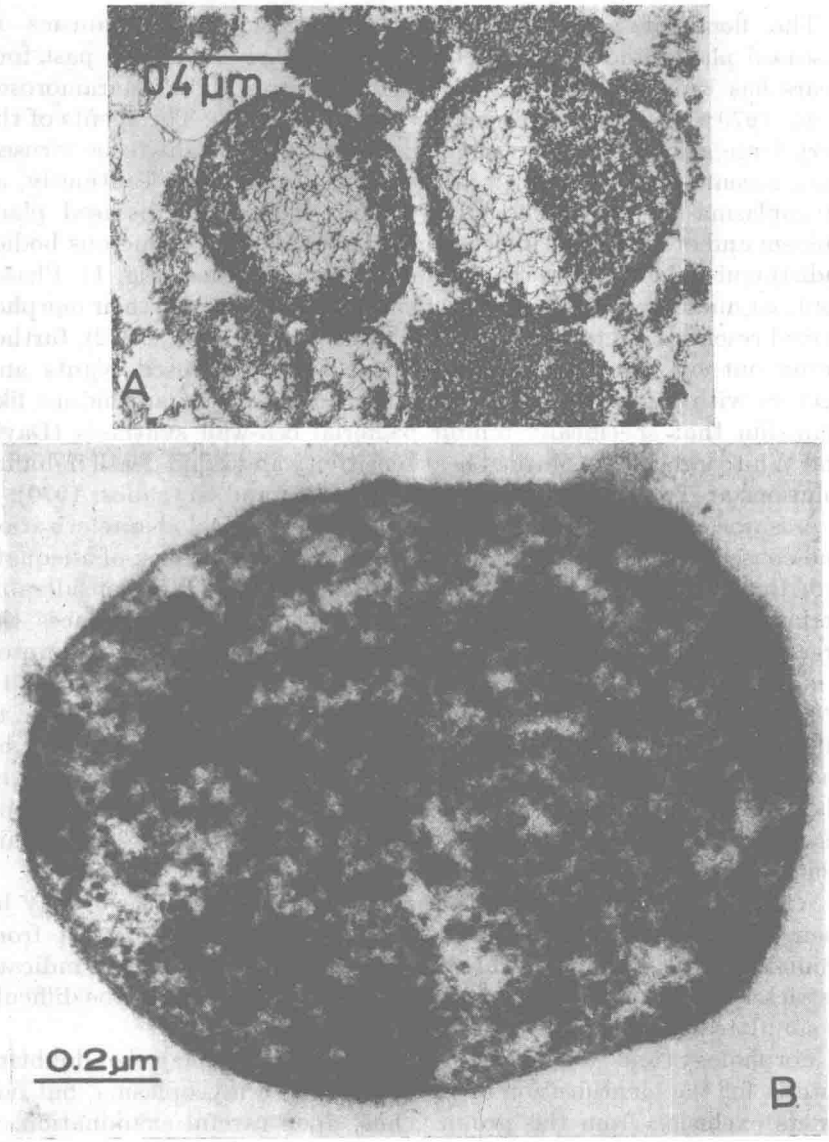
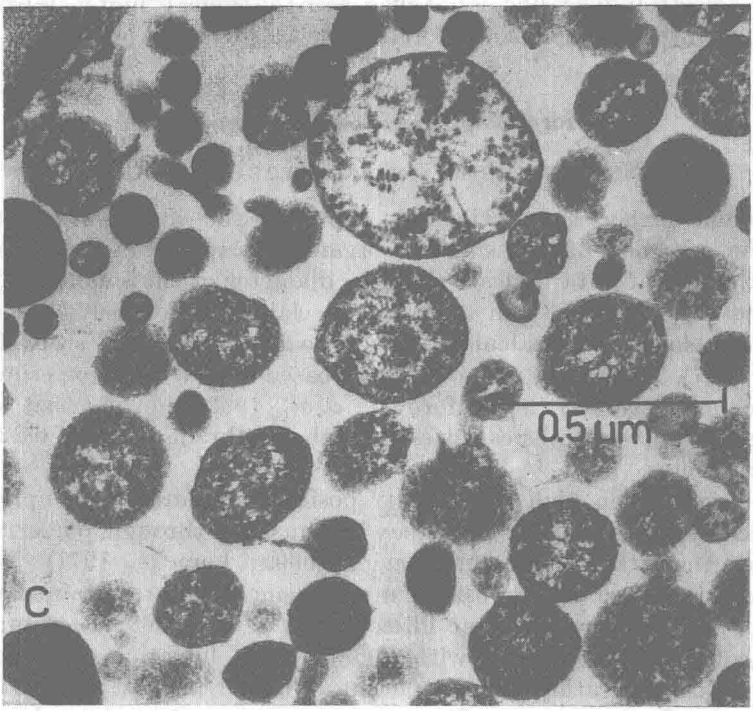
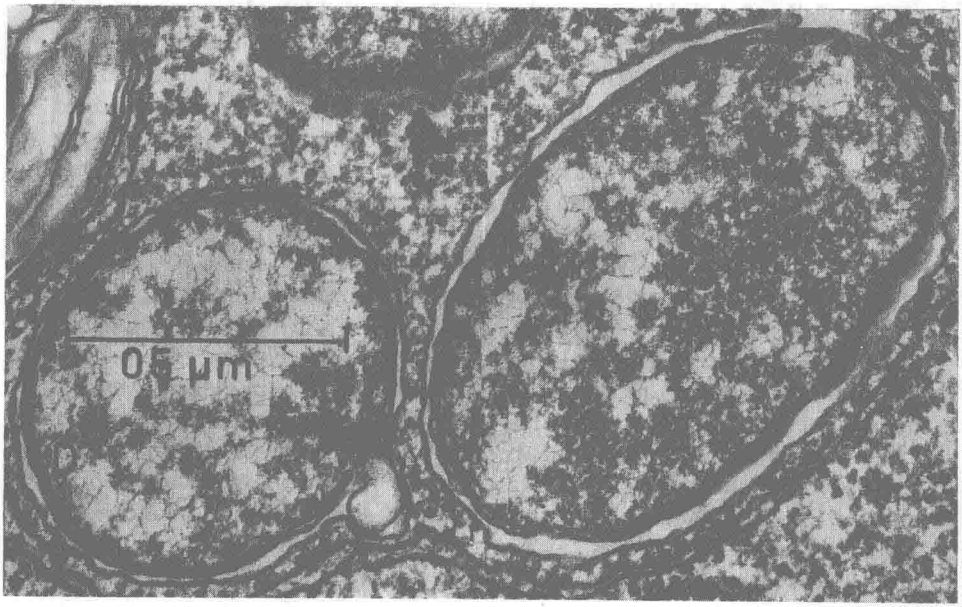


FIG. 1. Electron micrographs showing thin sections through: (A) an animal mycoplasma; taken from Anderson and Barile (1965): (B) the thermophilic *Thermoplasma acidophilum*; taken from Darland *et al.* (1970): (C) mycoplasma-like organisms in the phloem of diseased plants; an unpublished electron micrograph of Dr. H. Hirumi: (D) mycoplasma-like organisms in the testes of a sterile Drosophila male; taken from Williamson *et al.* (1971). The structural resemblance of all organisms is striking. The Drosophila micro-organism differs, however, from the others in being bounded by two unit membranes.


0.5 µm
C


0.5 µm

the two electron-dense lines is not even, as in the typical "unit membrane" of mycoplasmas (Saglio *et al.*, 1971).

III. Morphology and Mode of Reproduction

A. Size of the Minimal Reproductive Unit

Though bigger than originally thought, the mycoplasmas may still be regarded as the smallest organisms capable of autonomous growth. Recent data indicate that the diameter of the smallest mycoplasma cell is 0·2–0·3 μm and not, as claimed previously, 0·15 μm which is close to the calculated theoretical minimum cell size (Morowitz, 1967). The earlier estimates were based on filtration experiments (Klieneberger-Nobel, 1962; Morowitz *et al.*, 1963) showing that some viable mycoplasma cells can pass through membrane filters of 0·22 μm pore diameter.

However, when high negative or positive pressures are employed, the flexible mycoplasma cells may be squeezed through pores much smaller than their diameter (Razin, 1969a; Lemcke, 1971). When pressure is decreased, almost all mycoplasma cells are retained even on a 0·45 μm pore-diameter filter (Razin *et al.*, ·1968; Rottem and Razin, 1969; Cho and Morowitz, 1969) so that filtration can be used for the fast separation of mycoplasma cells in transport studies. The effect of cell plasticity on filtrability was demonstrated by Lemcke (1971). Cells of *A. laidlawii*, prefixed with glutaraldehyde, were retained on membrane filters that allowed the passage of unfixed cells, fixation presumably enhancing the rigidity of the normally soft cells. Thus filtrability through 0·45 μm pore-diameter filters, one of the standard tests recommended for the identification of an organism as a mycoplasma (Edward *et al.*, 1972), cannot be taken as an indication of size but merely of the plasticity characteristic of these organisms.

Membrane-bound bodies filled with ribosomes and having a diameter of 0·1–0·2 μm frequently observed in thin sections cannot be taken as the minimal reproductive units as they may represent a section through the tip of a spherical cell, a thin filament, or a thin thread connecting two adjacent cells (Razin, 1969a; Boatman and Kenny, 1970). The small round 0·1–0·2 μm diameter particles observed in electron micrographs of shadowed or negatively-stained mycoplasma preparations (Anderson *et al.*, 1965) seem incapable of reproduction, and may well be fragments of the fragile mycoplasma cells. To be viable, they should contain the entire cell genome, but their sections seldom show the characteristic DNA fibrils. Moreover, the minimum diameter of the sphere required to accommodate the smallest known mycoplasma

genome together with a single ribosome has been calculated at 0·13 μm (Morowitz *et al.*, 1967) so that the diameter of the minimal reproductive unit cannot be much less than 0·2 μm.

B. Morphology

In the absence of a cell wall, the mycoplasmas are so fragile and pliable that their morphology was long disputed. In preparing them for microscopy, it is obviously difficult to prevent the formation of artifacts, especially when unfixed cells are negatively stained with phosphotungstic acid. The thin filament-like protrusions often formed under these conditions as a result of osmotic damage or dessication are easily distinguishable from true filaments by their smaller calibre—less than 0·1 μm as compared with 0·3–0·4 μm (Razin *et al.*, 1967; Bredt, 1970). Structures resembling the distorted mycoplasma cells may also be obtained by the negative staining of extracts of plant or animal cells not infected with mycoplasmas, so that this is hardly the method of choice for detecting mycoplasmas in tissues (Wolansky and Maramorosch, 1970).

The tonicity of the medium also has a marked influence on mycoplasma morphology. Thin, filament-like protrusions were observed in cells of *M. gallisepticum* exposed to hypotonic conditions (Bernstein-Ziv, 1971); and fixation under grossly hypertonic conditions caused cells of *A. laidlawii* to become vacuolated or invaginated (Lemcke, 1972). To minimize artifact formation, attention should therefore be paid to the tonicity of the medium before, during and after fixation. The mechanical stress of centrifugation alone may be enough to affect morphology and cause unfixed *M. gallisepticum*, for instance, to assume a spherical, swollen shape in the electron microscope unlike their tear-shaped form—presumably their true morphology (Maniloff and Morowitz, 1967)—when prefixed with glutaraldehyde in the growth medium.

As is to be expected from plastic organisms, the coccus is the basic, though by no means the only, form in all mycoplasma cultures. In most, and under certain conditions, perhaps in all, mycoplasma cultures elongated or filamentous forms may also be discovered. The controversy about their being artifacts or not has been finally resolved upon the production of convincing evidence for filamentous growth in most species. Such evidence has been furnished by phase-contrast microscopy (Razin *et al.*, 1967; Bredt, 1970; Hubbard and Kite, 1971), negative staining (Brunner *et al.*, 1971; Rottem and Razin, 1972a), thin sectioning of mycoplasmas grown in broth (Maniloff, 1970; Metz and Bredt, 1971) or in agar (Knudson and MacLeod, 1970), scanning-beam electron

microscopy (Biberfeld and Biberfeld, 1970; Kammer *et al.*, 1970; Boatman and Kenny, 1971), and freeze etching (Davis *et al.*, 1972). While there is no longer any doubt that, given the appropriate conditions (Razin *et al.*, 1967), mycoplasma can grow in filaments, the different strains vary in their ability to do so. This may be due to differences in their ability to synthesize certain membrane components when the supply of precursors in the growth medium is limited.

C. Ultrastructural Features

The extremely simple ultrastructure found in numerous electron-microscope studies of thin mycoplasma sections supports the view that they are the simplest and most primitive organisms extant. Essentially the mycoplasma cell is built of only three organelles: the cell membrane, the ribosomes, and the characteristic prokaryotic chromosome. In several species, specialized organelles or structures have, however, recently been observed (see Section III, D, p. 9). In section, the cell membrane shows the characteristic trilaminar "unit membrane" structure, about 8·0–11·0 nm thick (Domermuth *et al.*, 1964a; Carstensen *et al.*, 1971). The frequently observed fuzziness of its outer surface (Morowitz and Terry, 1969; Maniloff, 1970; Bernstein-Ziv, 1971) disappears once the membrane is isolated and washed, and therefore probably represents material adsorbed from the growth medium, or highly polymerized material excreted from the cells, like the galactan of *M. mycoides* (Gourlay and Thrower, 1968).

There is no evidence of any intracellular membranous structures. That the membrane-bound vacuoles observed in some sectioned cells represent a section passing through a deep cup-like invagination of the cell membrane (Hirth *et al.*, 1970) has been conclusively proved by means of serial cell sections (Boatman and Kenny, 1970). Serial sections have, moreover, shown that some cells may have a hole in their centre, so that it is doubtful whether any real vacuoles are present.

In old mycoplasma cultures or in cultures growing in nutritionally inadequate media, large bodies are often seen, sometimes containing granules (Anderson and Barile, 1965) which at one time were commonly regarded as minimal reproductive units liberated into the medium after lysis of the large bodies to start a new life cycle (Klieneberger-Nobel, 1962). Very few workers, if any, still support this notion. The large bodies apparently represent damaged swollen cells of low viability, and the intracellular granules cytoplasmic degeneration products, though the possibility of some of them being viruses should also be considered (Horne, 1972).

D. Special Organelles and Structures

1. *The bleb of* Mycoplasma gallisepticum

The bleb at the tip of the worm-like cells of *M. gallisepticum* has attracted much attention since it was first discovered in the middle sixties (Maniloff *et al.*, 1965; Domermuth *et al.*, 1964b). It is not an artifact since it is observable throughout in organisms that have been negatively stained (Maniloff *et al.*, 1965; Bernstein-Ziv, 1969), sectioned (Domermuth *et al.*, 1964b; Maniloff *et al.*, 1965, Allen *et al.*, 1970; Bernstein-Ziv, 1969) or freeze-etched (Bernstein-Ziv, 1969; Maniloff, 1972). More or less like an oblate ellipsoid in shape, measuring about 80 by 130 nm without the bounding membrane which appears to be part of the plasma membrane (Fig. 2), it probably consists of protein and lipid. Nucleic acids have not been detected histochemically (Maniloff *et al.*, 1965) but, as long as this organelle has not been isolated and purified, its chemical composition remains uncertain. The function of the bleb is still unknown. One suggestion is that it is somehow associated with the organism's reproduction (Maniloff and Morowitz, 1967), but a more convincing hypothesis is that it plays a role in the adsorption of *M. gallisepticum* to cell surfaces. The excellent electron micrographs of Zucker-Franklin *et al.* (1966) show cells of *M. gallisepticum* clustered around leukocytes like iron filings around a magnetic pole or flukes attached to their host, the bleb constituting the site of contact more frequently than would be accounted for by chance alone.

2. *Terminal structure of* Mycoplasma pneumoniae

A special structure at the tip of filaments of *M. pneumoniae* was first reported by Biberfeld and Biberfeld (1970) and later confirmed by Collier and Clyde (1971). It consists of a dense central rod-like core surrounded by a lucent space enveloped by the cell membrane (Fig. 2), and measures 80 to 100 nm by 250 to 300 nm (Collier and Clyde, 1971). The electron density, fibrillar nature and affinity of the rod-like structure for uranyl and lead stains suggest that it may contain nucleic acid. Coupled with morphological evidence of filaments with two parallel terminal structures or of partly split terminal structures, this was taken to indicate participation in the initiation of reproduction by binary fission (Collier, 1972; Biberfeld, 1972). If so, however, one would expect the filament to split longitudinally along its axis, whereas all the available data point to transversal division to bead-like structures (Razin and Cosenza, 1966). It seems more probable that, like the bleb of *M. gallisepticum*, the terminal structure is instrumental in the