

MYCOPLASMAS : THE NEW CHAPTER IN PLANT PATHOLOGY

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MYCOPLASMAS ("Fungus-forms"), the free-living or parasitic organisms, are not new to the microbiologists and veterinary pathologists. *Mycoplasma mycoides* was cultured *in vitro* some seventy-five years ago in Pasteur's laboratory (Nocard et al., 1898), from cattle suffering from bovine pleuropneumonia. Similar type of organisms were later on isolated from a number of other animals and birds, and all these organisms were grouped together and called pleuro-

and Freundt proposed to retain only the valid genus *Mycoplasma* on the ground of priority. Now it is a universally established and accepted genus of the order Mycoplasmatales under the class Schizomycetes. Though they resemble bacteria to a greater extent, they cannot be classified with the so-called "true bacteria" (Table I). Edward and Freundt³⁴ and Edward et al.³⁵ proposed a new class Mollicutales (*mollis*: soft and pliable and *cutis*: skin), parallel to, but distinct from Schizomycetes.

TABLE I
Differentiation between Mycoplasmas, L-forms of bacteria, True bacteria and viruses

Mycoplasma	L-Form bacteria	True bacteria	Viruses
Occur in nature	Usually artificially created in laboratory	Naturally occurring	Naturally occurring
Do not have any rigid cell-wall or cell mucopeptide, generally pleomorphic in nature and cells are delimited by a unit lipoprotein membrane	Do not have rigid cell-wall but are surrounded by cell membrane only	Presence of rigid mucopeptide cell-wall and multi-layered membrane	No cell-wall or membrane, only naked nucleoproteins
Filterable through bacterial filters	Filterable	Non-filterable	Filterable
Mostly free living	Free living	Free living	Obligate parasites
Except <i>M. laidlawii</i> and <i>M. granulorum</i> most of them are sterol requiring	No absolute requirement for sterol	No absolute requirement	..
Don't revert to the bacterial forms if grown in antibiotic free media	Unstable L-forms revert to the parent bacteria in antibiotics free media
Limited metabolic activities	Metabolic activities similar to their parent bacteria	Large number of enzyme activities shown	No enzyme of their own grow on the energy of host cells
Response to Dines' stain developed by Hayflick (1967)
Both the nucleic acids (RNA & DNA) are present in the cells	Both RNA & DNA present in the cells	Same	Two types are known, i.e., with RNA or with DNA

pneumonia-like organisms (PPLOs). Turner¹¹⁷ and Kleiberger-Nobel⁶⁵ have given a detailed account of these organisms.

The first binominal given to these organisms was *Asterococcus mycoides* by Borrel et al.⁵, later on they were put under the genus *Mycoplasma* (86) and *Borrelomyces* (116). The Editorial Board of the International Bulletin of Bacterial Nomenclature and Taxonomy (29), discarded the genus *Asterococcus* on the basis that it is an algal genus, and decided to use either of the other two genera. It was only in 1956 that Edward

CHARACTERISTICS

The organisms can be characterized as follows :

1. They are known both as parasites as well as saprophytes.
2. Cells are non-motile and usually form typical "poached-egg"-shaped minute colonies with central nipple, on agar media.
3. They are highly pleomorphic and their forms vary with the cultural conditions.
4. Cells are delimited by a unit lipo protein membrane and lack rigid mucopeptide

cell-wall, which is responsible for the plastic nature of the cell.

5. Due to their plastic nature they can easily be sucked out through bacterial filters (cf. viruses).
6. Cells are usually resistant to the antibiotics which act on cell-wall, e.g., penicillin, cephaloridine, vancomycin, etc. However, there are certain mycoplasmas, which are inhibited even with a very low dose of penicillin and cephaloridine, due to some unknown reasons (59, 114).
7. Antibiotics, which act on various metabolic path, inhibit a wide range of mycoplasmas, e.g., tetracyclines.
8. Usually they do not grow in abundance in artificial media and most of them require very complex media for growth.
9. Growth of mycoplasmas, either in broth or on solid media, can be inhibited by specific antisera.

LIFE-CYCLE

The life-cycle of mycoplasmas has been determined by correlating morphological changes at different growth phases. Ultrastructures, morphology and reproduction of mycoplasmas have recently been reviewed by Freundt³⁹ and Anderson¹. Morowitz and Maniloff⁸², Maniloff and Morowitz⁷⁴ and Maniloff⁷⁵ could analyse the life-cycle of *M. gallisepticum* and *M. laidlawii* respectively, using phasecontrast and electron microscopy (thin sectioning and negative staining). Morowitz and Tourtellotte⁸⁰ reported four types of cells from *M. laidlawii* culture using density gradient centrifugation, as follows:

1. Smallest cells called elementary bodies, spherical, 0.1–0.2 μ in diameter.
2. Second types were somewhat larger than this, called intermediate cells.
3. Third ones still larger, upto 1.0 μ in diameter.
4. Cells similar to large cells but containing a lot of inclusions.

They hypothesised that elementary bodies (1) transform to intermediate cells (2) and then to large cells (3 or 4) which again give the elementary bodies (1). The large cells either develop inclusions and get released in the medium or undergo binary-fission, develop inclusions and then get released. Razin *et al.*⁹⁴ reported that elementary bodies enlarge to form filamentous forms which may further give rise to elementary bodies (33). Morowitz and

Maniloff⁸¹ and Furness *et al.*⁴⁰ demonstrated binary-fission of the large cells.

METABOLISM OF CELLS

The cell membrane is composed of protein-lipid and lipid-protein layers, and about 12% of total weight of the cell is nucleic acids (RNA & DNA), which are distributed both in ribosomes as well as in other soluble particles (80 & 81).

More than forty different enzymatic reactions in mycoplasmal cells, including the enzymes of glycolysis, have been demonstrated. Kandler *et al.*⁶² and Zehender¹²⁶ found that these organisms are unable to break organic acids and that the cells are inhibited with moniodoacetic acid and fluoride but are completely resistant to potassium cyanide, 2, 4-dinitrophenol, azide, arsenite and arsenate. Sensitivity of organisms towards moniodoacetic acid and fluoride indicated an operation of glycolysis (upto the formation of pyruvic acid), while insensitivity towards KCN, 2, 4-DNP, $-N_3$, arsenite and arsenate showed complete absence of TCA-cycle and cytochrome systems. Demark²⁶ reviewed and gave an account of the metabolic pathways in mycoplasmas, while Razin⁹³ discussed the cell membrane in details.

ISOLATION, CULTURING AND MEDIA FOR GROWTH

The subject has been well reviewed (36, 65). Most of the mycoplasmas can be isolated under aerobic conditions but sometimes incubation under 5–10% carbon dioxide or 95% nitrogen and 5% CO_2 is preferable. Sometimes semi-solid biphasic media are suitable for the primary isolation of certain mycoplasmas.

From the very beginning these organisms were known to be extremely fastidious, requiring very complex media with serum, yeast extract, etc., for saprophytic growth *in vitro*. One of the most general type of media was that formulated by Hayflick⁵².

Except *M. laidlawii* and *M. granularum* (115) most of the mycoplasmas are cholesterol requiring (8, 30, 96). Cholesterol is required for the synthesis of cell membranes, and due to its presence in the membrane, *M. laidlawii* is inhibited by polyene antibiotics, filipin (119) and amphotericin B (37). Smith¹⁰⁸ suggested that carotenoids may serve the same function as sterols, in non-sterol requiring strains.

In media generally 50 units/ml of penicillin and 0.25 mg/L of thallium acetate are added to

keep off Grampositive and Gramnegative bacteria, respectively. Sometimes media are supplemented with 20 µg/ml of sodium salt of deoxyribonucleic acid, which is essential for primary isolation of several pathogenic mycoplasmas (31). There is an antagonistic relationship between the two nucleic acids, and DNA in these media is necessary to overcome the excess of RNA (18, 91).

Several workers have tried to define media for mycoplasmas by analysing serum and other complex materials in it. Thus Lund and Shorb⁷² and Kurzepa *et al.*⁶⁶ could replace serum with the addition of yeast extract, cholesterol, lecithine and cardiolipin while Smith and Boughton¹⁰⁷ and Rodwell and Abbot⁹⁷ discussed the role of protein, phospholipids, glycolipids, glycerol, cholesterol, long chain fatty acids in the nutrition of PPLOs. Tauraso¹¹³ reported that diethylaminoethyl dextran (DEAE), 0.1 mg/ml of media, enhanced the growth of some mycoplasma strains. Razin and Knight⁹¹ suggested a partially defined media.

SPECIES IDENTIFICATION

Mycoplasmas can be broadly identified on the basis of their metabolic activities, but they have mostly been classified by their serological reactions (13, 27, 69). Certain serological tests are so sensitive that they not only classify mycoplasmas into species but also sometimes differentiate between the strains. Recently Stewart¹¹¹ used fluorescent-antibody technique while Razin⁹⁵ and Armstrong and Yu³ used gel electrophoretic techniques for specific identifications of different mycoplasmas.

MAINTENANCE OF CULTURES

Mycoplasma spp. in broth or on agar media can be kept at -60° to -75° C for over twelve months even sometimes for twelve years (64, 99). Kelton⁶⁴, lyophilized 18 hr old cultures in some special media (63), mixed with equal parts of sterile skim milk. Lyophilized cultures stored at -26° to -65° C remained viable for 3-4 years.

MYCOPLASMAS AND PLANT DISEASES

Two recent reports from Prof. Asuyama's laboratory (28 and 60), suspecting the association of Mycoplasma-like or PLT-like (psittacosis-lymphogranuloma-trachoma-like) organisms with mulberry dwarf disease, have turned up enthusiasm among the plant pathologists in this direction. Their evidences are based on (1) presence of mycoplasma-like bodies (under

electron microscope) only in the phloem cells of infected plants and (2) disappearance of these bodies from the cells when treated with tetracycline antibiotics.

Maramorosch *et al.*⁷⁶ claimed that these pleomorphic bodies (Mycoplasma-like) were observed previously in ultrathin sections of yellows infected phloem cells by a number of workers but their significance in yellows disease etiology was not recognised till Asuyama's report. Since this report, most, if not all, of yellows type, and some of the other controversial plant diseases which were considered to be due to viruses, are now suspected to be due to mycoplasmas (78). Most of these etiological evidences are indirect, based on electron microscopic and tetracycline therapeutic studies. The organisms have also been cultivated in cell-free media, in few cases only.

(i) *Electron microscopic studies.*—Electron microscopic observation revealed the presence of pleomorphic bodies both in plants (6, 11, 15, 16, 19, 28, 46, 47, 50, 51, 55, 56, 58, 59a, 70, 76, 77, 83, 87, 88, 100-104, 112, 118, 124, 125) and in some of their insect vectors (7, 42, 43, 48, 57, 76, 77, 83, 89, 101, 105). Pleomorphic organisms in plants apparently restricted to the phloem cells, but they have also been reported from intracellular spaces, in phloem parenchyma (28), in companion cells (70) and also in ground tissue (125). In plants these bodies have been suspected to pass from cell to cell through pores in sieve elements (56, 101). In infected insects they have been found from salivary glands (46, 47, 57, 88, 104), intestine (7, 42, 45, 48, 76, 104), fat bodies (101), nervous system (48, 76) and brain cells (83).

Hirumi⁵⁸ reviewed the ultra-thin structure of mycoplasma-like bodies associated with some plant diseases and their insect vectors and found that these bodies are fundamentally similar to animal mycoplasmas, i.e., the bodies are highly pleomorphic, bounded by a unit membrane, varied from 0.5-1.0 µ in diameter with ribosomes and nuclear material (but no nucleoli). These bodies he failed to classify morphologically, but concluded confidently that they belong to the order Mycoplasmatales.

(ii) *Chemotherapeutic studies.*—Some of these diseases could be checked or delayed by the application of antibiotics to the host plants or to their insect vectors (2, 20-23, 38, 49, 60, 61, 90, 102, 103, 109, 122, 123). Most of the effective antibiotics belong to tetracycline group,

which have its multiple sites of action in the cells, other than the cell-wall (67). This has been taken to be one of the strongest supports for mycoplasma etiology of these diseases, as these antibiotics are also effective against animal mycoplasmas (53, 84). Both pre-inoculation as well as post-inoculation treatments are effective. In plants they are administered as foliar spray or through roots while in insects they are either fed through membrane or are directly injected. Our findings on the tetracycline therapy of citrus greening and Sandal spike are remarkable (83 a and 90 a).

Two-year old sweet orange plants (*Citrus sinensis*) infected by grafting, when sprayed with 500 p.p.m. of demethylchlortetracycline (ledermycin) and tetracycline hydrochloride (achromycin), showed recovery from greening symptoms. The antibiotics were sprayed at weekly intervals for ten weeks. The spike disease of Sandal could be suppressed by applying ledermycin (approx. 1 gm/tree) paste by girdling method. The recovered trees of Sandal in the forest also flowered. Transmission of Western-X (WX) disease of peach and Corn stunt (CS) disease are also inhibited by tetracycline antibiotics as shown by Jensen and Nasu⁶¹ and Granados⁴⁹ respectively. Penicillin, Kannamycine, Cycloserine, Spectinomycine, Vancomycine, and Streptomycine are found to be ineffective against either plants (23) or insects (123). Gold sodium thiomalate which inhibited *M. pneumoniae* (79) was found to be ineffective against aster yellows (AY) agents (23).

(iii) *Extraction and purification of plant mycoplasma.*—Giannotti et al.⁴⁸ reported the isolation of mycoplasma-like bodies from "Flavescence dorée" disease of grapes by differential centrifugation. Streere¹¹⁰ could purify AY agents by gel filtration after extracting it, in 0.3 M glycine-0.3 M MgCl₂ buffer, at pH 8.0 and then passing it through 7% agar-gel. Whitcomb and Davis¹²¹ showed infectivity of this gel eluent. Cohen et al.¹⁴ showed that AY agents from plant, passed through 300 m μ pore size myllipore filters, could retain infectivity. Infectivity of WX was sedimented after 10 min at 25,000 g, and in rate zonal density gradient centrifugation infectivity was found throughout the gradient column, after 25 min at 25,000 rpm, with most infectivity in the bottom one-third of the column. WX infectivity was best recovered from gel filtration columns when a buffer containing glycine and Mg was

used. Whitcomb et al.¹²⁰ found that WX-agents retain infectivity even after freezing.

(iv) *Cultivation of plant mycoplasmas in cell-free media.*—To confirm the mycoplasmal etiology of the above-mentioned plant diseases attempts have been made to bring these organisms in pure culture. Hampton et al.⁵⁰ were the first to report on the isolation and cultivation of a mechanically transmissible *Mycoplasma*, associated with naturally infected pea plants, in Hayflick's media (52). They claimed that their *Mycoplasma* was antigenically related to human (*M. salvarium*) and avian (*M. gallisepticum* and *M. meleagridis*) mycoplasmas. Chen and Granados¹⁰ cultivated corn stunt (CS). *Mycoplasma* in some very complex media and could reproduce the disease through vectors (*Dalbulus elimatus*) by injecting them with pure culture.

Lin et al.⁷¹ reported the cultivation of *Mycoplasma* from "White leaf" disease of sugarcane in Morton's PPLO media (Difco Manual, 1965). They could reproduce the disease mechanically by inoculating the plants with 24 hr old culture. Recently Saglio et al.⁹⁸ and Calavan et al.⁹ reported the cultivation of *Mycoplasma* associated with 'Citrus stubborn' diseases, at the 2nd International Symposium on Plant Pathology, India.

Preliminary attempts have also been made in our laboratory to cultivate these organisms from Citrus greening and Little leaf of Brinjal and *Mycoplasma*-like colonies have been obtained from Citrus greening (41).

(v) *Viability test.*—Davis et al.²⁴ studied the viability of AY agents *in vitro*. In 0.3 M glycine-0.03 M MgCl₂ buffer at pH 4.8, organisms lost their viability within 3 hrs at 22° C. In a basal media containing amino-acids, vitamins, inorganic salts, sucrose, cholesterol and 5% horse serum, AY agents could survive upto 48 hrs but not upto 72 hrs. Survival could be improved by incubating them under N₂ or CO₂-N₂ atmosphere. AY agents were viable for two days when stored in liquid nitrogen in Grace's medium supplemented with sucrose and horse serum.

(vi) *Concluding remarks.*—Uptill now nearly fifty plant diseases, graft or insect transmissible, with characteristic symptoms like abnormalities in floral and vegetative parts (virescence and phyllody), yellowing, proliferation of axillary buds, reduction of leaf lamina, general stunting, etc., have been reported to be due to *Mycoplasma*-like bodies from various parts of

TABLE II

Suspected *Mycoplasma* diseases of plants

Alfalfa witches' broom	Papaya bunchy top
Apple proliferation	Para stolbur
Asle witches' broom	Paulownia witches' broom
Aster yellows	Pea and green pea yellow dwarf
Blueberry stunt	Peach yellows
Brinjal little leaf	Peanut witches' broom
Cassava witches' broom	Pear decline
<i>Cheiranthus allioni</i> virescence	Phloem necrosis of elm
Cherry buckskin	Phormium yellow leaf
Citrus greening and stubborn	Potato purple top
Clover dwarf	Potato stolbur
Clover phyllody	Potato witches' broom
Clover stolbur	Rice stripe
Clover virescence	Rice yellow dwarf
Corn stunt	Rubus stunt
Cotton virescence	Safflower phyllody
Cranberry false blossom	Sandal spike
Crimean yellows	Sesame phyllody
<i>Cryptotaenia</i> witches' broom	Stolbur
"Flavescence dorée (Dwarf)"	Strawberry green petal
Giallume yellows of rice	Sugarcane white leaf
Grassy stunt of rice	Sweetpotato little leaf
Legume little leaf	Sweetpotato witches' broom
Legume witches' broom	Tobacco yellow dwarf
Little cherry	Tomato big bud
Little peach	<i>Vicia faba</i> virescence
Lucerne witches' broom	<i>Vinca rosea</i> yellows
<i>Mal Azul</i> (blue) diseases of tomato	Western X-disease of peaches and cherries
Mulberry dwarf	Yellow wilt of sugarbeet
<i>Opuntia tuna</i> witches' broom	

the world (Table II). Mycoplasmal bodies have been found in the most common diseases in India like brinjal little leaf and Sandal spike which are of considerable economic importance, and they have been shown to be due to these organisms. The main criterion for 'Mycoplasmas' as etiologic agent for these diseases is the association of Mycoplasma-like bodies (under electron microscope) with the diseased tissue only. Emphasis should be given more towards fundamental researches in the cultivation of these organisms, pathogenicity tests and mode of transmission in field, than to merely increasing the list.

Regarding control of mycoplasmal diseases of plants, antibiotic therapy has been successfully used only in laboratory experiments. These compounds as well as some other ones like inhibitors, promoters, etc., are yet to be tested in field trials. Since most of these diseases are spread by their insect vectors, there are possibilities to check these diseases by controlling its vectors. Henne⁵⁴ actually successfully checked the incidence of AY disease in carrot, using some insecticides against their leaf hopper vectors. The third possibility of

control is by the killing of weeds, which may serve as alternate hosts.

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1. Anderson, D. R., "Ultrastructural studies of mycoplasmas and the L-phase of bacteria," In L. Hayflic's (ed.), *The Mycoplasmatales and the L-phase of Bacteria*. Appleton-Century-Crofts, N.Y., 1969, p. 365.
2. Anjaneyulu, A. and Ramkrishnan, K., *Curr. Sci.*, 1969, 38, 271.
3. Armstrong, D. and Yu, B., *J. Bact.*, 1970, 104, 295.
4. Borges, M. de. L. V. and David-Ferreira, J. F., *Biol. Soc. Brotheriana* (Ser. 2), 1968, 42, 321.
5. Borrel, A., Dujardin-Beaumetz, E., Jeantel and Jouan, *Ann. Inst. Pasteur*, 1910, 24, 168.
6. Bowyer, J. W., Antherton, J. G., Teakle, D. S. and Ahern, G. A., *Aust. J. Biol. Sci.*, 1969, 22, 271.
7. Brack, J. and Kralik, O., *Biol. Plantum*, 1969, 11, 95.
8. Butler, M. and Knight, B. C. J. C., *J. gen. Microbiol.*, 1960, 22, 483.
9. Calavan, E. C., Igwegbe, E. C. K. and Fudl-Allah, A. E., "Recent developments in the etiology and control of stubborn disease of citrus," *2nd International Symposium in Plant Pathology*, I.A.R.I., India (Abstr.), 1971.
10. Chen, T. A. and Granados, R. R., *Science*, 1970, 167, 1633.
11. —, *Phytopathol.*, 1971, 61, 233.
12. Clark, H. W., *J. Bact.*, 1965, 90, 1373.
13. Clyde, W. A. (Jr.), *J. Immun.*, 1964, 92, 958.
14. Cohen, R., Purcell, R. and Steere, R. L., *Phytopathol.* (Abstr.), 1969, 59, 1555.
15. Cousin, M. T., Schweisguth, B., Faivre-Amiot, A., Kartha, K. K., Staron, T. and Moreau, J., *C.R. Acad. Sci., Paris*, 1971, 272, 830.
16. —, Kartha, K. K. and Delattre, R., *Coton Fibres Tropicales*, 1970, 25, 525.
17. Craford, Y. E. and Krabill, W. H., *Ann. N.Y. Acad. Sci.*, 1967, 143, (Art 1), 411.
18. Crowthers, S. and Knight, B. C. J. C., *J. gen. Microbiol.*, 1956, 14, Proc. VII.
19. Dale, J. L. and Kim, K. S., *Phytopathol.*, 1969, 59, 1765.
20. Davis, R. E., Whitcomb, R. F. and Steere, R. L., *Ibid.* (Abstr.), 1968 a, 58, 884.
21. —, — and —, *Science*, 1968 b, 161, 793.
22. — and —, *Phytopathol.* (Abstr.), 1969, 59, 1556.

23. Davis, R. E. and Whitecomb, R. F., *Infect. Immun.*, 1970, 2, 201.
24. —, — and Purcell, R., *Phytopathol.* (Abstr.), 1970, 60, 573.
25. Del Giudice, R. A., Robillard, N. F. and Craski, T. R., *J. Bact.*, 1967, 93, 1205.
26. Demark Van, P. J., *Ann. N.Y. Acad. Sci.*, 1967, 143, 77.
27. Dinter, Z., Danielsson, D. and Bakos, K., *J. gen. Microbiol.*, 1965, 41, 77.
28. Doi, Y., Teranaka, M., Yora, K. and Asuyama, H., *Anna. Phytopath. Soc., Japan*, 1967, 33, 259.
29. Editorial Board, *Inst. Bull. Bact. Nomen. Taxon.*, 1955, 5, 13.
30. Edward, D. G. ff. and Fitzgerald, W. A., *J. gen. Microbiol.*, 1951, 5, 576.
31. —, and —, *Vet. Rec.*, 1952, 64, 395.
32. —, and Freundt, E. A., *J. gen. Microbiol.*, 1956, 14, 197.
33. —, *Ann. N.Y. Acad. Sci.*, 1967, 143 (Art. 1), 7.
34. —, and Freundt, E. A., *Inst. J. syst. Bact.*, 1967, 17, 267.
35. —, —, Chanock, R. M., Fabricant, J., Hayflick, L., Lemcke, R. M., Razin, S., Somerson, N. L. and Wittler, R. G., *Science*, 1967, 155, 1694.
36. Fallon, R. J., and Whittlestone, P., "Isolation, cultivation and maintenance of *Mycoplasma*," In J. R. Norris and D. W. Ribbons, (ed.), *Methods in Microbiology*, Academic Press, N.Y., 1969, 3 B, 211.
37. Feingold, D. S., *Biochem. Biophys. Res. Comm.*, 1965, 19, 261.
38. Freitag, H. G. and Smith, S. H., *Phytopathol.*, 1969, 59, 1820.
39. Freundt, E. A., "Cellular morphology and mode of reproduction of mycoplasmas," In L. Hayflick (ed.), *The Mycoplasmatales and the L-phase of Bacteria*. Appleton-Century-Crofts, N.Y., 1969, p. 281.
40. Furness, G., Pipes, F. J. and Mutrey, M. J. Mc., *J. infect. Dis.*, 1968, 118, 1.
41. Ghosh, S. K., Raychaudhuri, S. P., Anupam Varma, and Nariani, T. K., *Curr. Sci.*, 1971, 40, 299.
42. Giannotti, J., Devauchella, G. and Vago, C., *Compt. Rend. (Ser. D)*, 1968 a, 266, 2168.
48. Granados, R. R., Maramorosch, K., and Shikata, *Ibid.*, 1968 b, 267, 454.
44. —, Moruan, G. and Vago, C., *Ibid.*, 1968 c, 267, 76.
45. —, Vago, C., Devauchelle, G. and Marchoux, G., *Entomol. Exptl. Appl.*, 1968 d, 11, 470.
46. —, Caudwell, A., Vago, C. and Duthoit, I., *Compt. Rend. (Ser. D)*, 1969 a, 268, 845.
47. —, Devauchelle, D., Marchoux, G. and Vago, C., *Ibid.*, 1969 b, 268, 1354.
48. Granados, R. R., Maramorosch, K., and Shikata, E., *Proc. Nat. Acad. Sci.*, 1968, 60, 841.
49. —, *Phytopathol.* (Abstr.), 1969, 59, 1556.
50. Hampton, R. G., Stevens, I. O. and Allen, T. C., *Pl. Dis. Repr.*, 1969, 53, 499.
51. Harrison, B. D. and Roberts, I. M., *Ann. Appl. Biol.*, 1969, 63, 347.
52. Hayflick, L., *Texas Rept. Biol. Med.* (Suppl. 1), 1965, 23, 285.
53. —, and Chanoch, R. M., *Bacteriol. Rev.*, 1965, 29, 186.
54. Henne, R. C., *Can. J. Plant Sci.*, 1970, 50, 169.
55. Hibben, C. R. and Wolanski, B., *Phytopathol.*, 1970, 61, 151.
56. Hibino, H. and Schneider, H., *Ibid.*, 1970, 60, 499.
57. Hirumi, H. and Maramorosch, K., *J. Virology*, 1969, 3, 82.
58. —, *X Int. Cong. Biol.*, (Abstr.), 1970, p. 233.
59. Hottle, G. A. and Wright, D. N., *J. Bact.*, 1966, 91, 1834.
- 59 a. Hull, R., Horne, R. W. and Nayar, R. M., *Nature*, 1969, 224, 1121.
60. Ishie, T., Doi, Y., Yora, K. and Asuyama, H., *Ann. Phytopath. Soc., Japan*, 1967, 33, 267.
61. Jenson, D. D. and Nasu, S., *X Int. Cong. Microbiol.* (Abstr.), 1970, p. 223.
62. Kandler, O., Zehender, C. and Miller, J., *Arch. Microbiol.*, 1956, 24, 219.
63. Kelron, W. H., *Ann. N.Y. Acad. Sci.* (Art. 10), 1960, 79, 422.
64. —, *J. Bact.*, 1964, 87, 588.
65. Klienberger-Nobel, K., *Pleuropneumonia-like Organisms (PPLO)*, *Mycoplasmataceae*, Academic Press, London and N.Y., 1962, p. 157.
66. Kurzepa, H., Flinton, L. and Demark, P. J., *J. Bact.*, 1969, 99, 908.
67. Laskin, A. I., "Tetracyclines." In D. Gottlieb and P. D. Shaw (ed.), *Antibiotics: Vol. 1: Mechanism of Action*. Springer Verlag, N.Y., Inc., 1967, p. 331.
68. Lawson, R. H., Khan, R. P., Heron, S. and Smith, F. F., *Phytopathol.* (Abstr.), 1970, 60, 1016.
69. Lemcke, R. M., *J. Hyg.*, 1964, 62, 199.
70. Lin, S. and Lee, C., *Ann. Rept. Taiwan Sugar Exptl. Sta.*, 1967-68, p. 17.
71. Lin, Shu-Chen, Lee, Ching-Shiou and Chiu, Ren-Jong, *Phytopathol.*, 1970, 60, 795.
72. Lund, P. G. and Shorb, M. S., *Soc. Exptl. Biol. Med.*, 1966, 121, 1070.
73. Maillet, P. L., Gourret, J. and Hamon, C., *Compt. Rend. (Ser. D)*, 1968, 266, 2309.
74. Maniloff, J. and Morowitz, H., *Ann. N.Y. Sci.*, 1967, 143, 59.
75. —, *J. Bacteriol.*, 1970, 102, 561.
76. Maramorosch, K., Shikata, E. and Granados, R., *Trans. N.Y. Acad. Sci.*, 1968 a, 30, 841.
77. —, — and —, *Phytopathol.* (Abstr.), 1968 b, 58, 886.
78. —, Granados, R. R. and Hirumi, H., *Adv. Virus Res.*, 1970, 16, 135.
79. Marimion, B. P. and Goodburn, G. M., *Nature*, 1961, 189, 247.
80. Morowitz, H. J. and Tourtellorree, M. E., *Sci. Amer.*, 1962, 206, 117.
81. —, —, Guild, W. R., Castro, E., Woese, C. and Cleverdon, R. C., *J. Mol. Biol.*, 1962, 4, 93.
82. —, and Maniloff, J., *J. Bact.*, 1966, 91, 1638.
83. Nasur, S., Jensen, D. D. and Richardson, J., *Virology*, 1970, 41, 583.
- 83 a. Nariani, T. K., Raychaudhuri, S. P. and Viswanath, S. M., *Curr. Sci.*, 1971, 40, 552.
84. Newham, A. G. and Chu, H. P., *J. Hyg.*, 1965, 63, 1.
85. Nocard, E., Roux, E. R., Borrel, Mn., Salimbeni and Dujardin Beasumet, *Ann. Inst. Pasteur.*, 1898, 12, 240.
86. Nowak, J., *Annls. Inst. Pasteur*, 1929, 43, 1330.

87. Ploaie, R., Granados, R. R. and Maramorosch, K., *Phytopathol.* (Abstr.), 1968, 58, 1063.
88. Ploaie, P. and Maramorosch, K., *Ibid.*, 1969, 59, 536.
89. Raine, J. and Forbes, A. R., *Can J. Microbiol.*, 1969, 15, 1105.
90. Raychaudhuri, S. P., Varma, A., Chenulu, V. V., Prakash, N. and Singh, S., *X Int. Cong. Microbiol.* (Abstr.), 1970, p. 222.
- 90 a. —, Chenulu, V. V., Ghosh, S. K., Varma, A., Rao, P. S., Srimathi, R. A. and Nag, K. C., *Curr. Sci.*, 1972, 41, 72.
91. Razin, S. and Knight, B. C. J. C., *J. gen. Microbiol.*, 22, 504.
92. — and —, *Ibid.*, 1960 a, 22, 1942.
93. —, *Ann. N.Y. Acad. Sci.*, 1967, 143, 115.
94. —, Consenza, B. J. and Tourtellotte, M. E., *Ibid.*, 1967, 143, 66.
95. —, *J. Bact.*, 1968, 96, 687.
96. — and Tully, J. G., *Ibid.*, 1970, 102, 306.
97. Rodwell, A. W. and Abbot, A., *J. gen. Microbiol.*, 1961, 25, 201.
98. Saglio, P., Lafleche, D., Bonissol, C. and Bove, J. M., "Isolation and culture of Mycoplasma-like organism associated with strubborn disease of Citrus," *2nd International Symposium on Plant Pathology*, I.A.R.I., India (Abstr.), 1971.
99. Shepard, M. C., *Ann. N.Y. Acad. Sci.*, 1967, 143, 505.
100. Shikata, E., Marmorosch, K., Ling, K. C. and Matsumoto, T., *Ann. Phytopath. Soc. Japan*, 1968, 34, 208.
101. — and —, *Phytopathol.* (Abstr.), 1969, 59, 1559.
102. —, Teng, W. S. and Matsumoto, T., *J. Fac. Agr. Hokkaido Univ.*, 1969 a, 56, 79.
103. —, Maramorosch, K. and Ling, K. C., *Plant Prot. Bull.*, FAO, 1969 b, 17, 121.
104. Sinha, R. C. and Paliwal, Y. C., *Virology*, 1969, 39, 759.
105. — and —, *Ibid.*, 1970, 40, 665.
106. Smith, P. F., *Appl. Microbiol.*, 1956, 4, 254.
107. — and Boughton, J. E., *J. Bacteriol.*, 1960, 80, 851.
108. —, *J. ben. Microbiol.*, 1963, 32, 307.
109. Staron, T., Cousin, M. T. and Crison, G., *C.R. Acad. Sci.*, (Ser. D), 1969, 267, 2328.
110. Steers, R. L., *Phytopathol.*, 1967, 57, 832.
111. Stewart, S. M., *Immunology*, 1967, 13, 513.
112. Story, G. E. and Halliwell, R. S., *Phytopathol.* (Abstr.), 1969, 59, 118.
113. Taruaso, N. M., *J. Bacteriol.*, 1967, 93, 1559.
114. Taylor Robinson, G., *Post grad. med. J.* (Suppl.), 1967, 43, 100.
115. Tully, J. G. and Razin, S., *J. Bact.*, 1968, 95, 1504.
116. Turner, A. W., *J. Path. Bact.*, 1935, 41, 1.
117. —, In *Infectious Diseases of Animals, Diseases due to Bacteria* (A. W. Stableforth and I. A. Galloway, ed.), Butterworth, London, 1959, 2, 437.
118. Varma, A., Chenulu, V. V., Raychaudhuri, S. P., Prakash, N. and Rao, P. S., *Indian Phytopathol.*, 1969, 22, 289.
119. Weber, M. N. and Kinsky, S. C., *J. Bacteriol.*, 1956, 89, 306.
120. Whitcomb, R. F., Jenson, D. D. and Richardson, J., *J. Invertebr. Pathol.*, 1968, 12, 192.
121. — and Davis, R. E., *Phytopathol.* (Abstr.), 1969, 59, 1561.
122. —, and —, *Ann. Rev. Entomol.*, 1970 a, 15, 405.
123. — and —, *Infec. Immun.*, 1970 b, 2, 209.
124. Worley, J. F., *Phytopathol.* (Abstr.), 1969, 59, 1561.
125. —, *Ibid.*, 1970, 60, 284.
126. Zehender, C., *Zbl. Bakt. Abt. II.* 1956, 109, 337.

STUDIES ON MINERAL CONSTITUENTS OF SOME SPECIES OF CORALS

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INTRODUCTION

CALCAREOUS rocks formed by various corals have occupied the attention of mineralogists along different lines of research¹. Calcium carbonate was known earlier but its presence in the skeleton of corals was confirmed at the end of the 18th century. Qualitative analyses of corals were done by Morozzo², Fourcroy and Vauquelin³, John⁴ and others. Silliman⁵ first studied the chemical composition of corals. Bowen and Sutton⁶ examined the mineral constituents of marine sponges, while Turekian and Armstrong⁷ have analysed about 100 molluscan shells for the contents of magnesium, strontium and barium. Rao et al.⁸ analysed

molluscan shells from Indian coastal water for strontium, radium and calcium contents.

No detailed study of the major, minor and trace elements in corals nor any attempt to correlate the trace elements in sea-water with those in corals appears to have been carried out. A preliminary investigation was undertaken to study the distribution of the elements in the skeletal material of the coral samples collected off Mandapam (Lat. 9° 15' N, Long. 79° 08' E). The following is a brief resume of the work that has been done in this field.

COLLECTION AND PROCESSING OF SAMPLES

The samples were collected in October 1968 off Mandapam. Corals were washed with distilled