Applied Mycology and Bacteriology

L. D. GALLOWAY

R. BURGESS

APPLIED MYCOLOGY AND BACTERIOLOGY

ORIGINALLY COMPILED BY

L. D. GALLOWAY, M.A.(CANTAB.)

Imperial Mycologist, New Delhi, 1934-1937

Mycologist to British Cotton Industry Research Association, 1927-1934

AND

R. BURGESS, M.SC., PH.D.(LOND.)

Microbiologist, Geigy Co. Ltd.

Dairy Bacteriologist to Lancs. County Council, 1935-45

Microbiologist to British Woollen Industry Research Association, 1927-1935

REVISED BY

L. D. GALLOWAY

LONDON

LEONARD HILL [BOOKS] LIMITED

9 EDEN STREET, N.W.1

1957

Applied Mycology and Bacteriology

FOREWORD TO THE FIRST EDITION

IT is obviously not possible to compress into one small volume a really adequate account of fungi and bacteria, together with a description of their applications to human affairs, a guide to laboratory technique, and a bibliography. Nevertheless some such attempt seems desirable. It is hoped that the following brief survey of the field of mycology and bacteriology will be of some service to biologists and chemists in co-ordinating their studies with those of other workers, and in indicating the scope and methods of economic microbiology.

The industrial chemist, in particular, is expected to cope with any problem, from plumbing to entomology. He would often like to examine in more detail some micro-organism that is affecting his work, but is at a loss how to begin; and it is a regrettable fact that the early training of a chemist seldom includes even the most elementary biology, although the converse is not the case. If he attempts to extract the details he requires from textbooks chosen at random among the extensive literature available, he is likely to be appalled at the enormous variety of organisms, the multiplicity of staining methods, and the technical jargon of books intended for those with a biological training. An even worse danger is that he should be over-confident, and lend the support of his learning to statements unacceptable to the expert.

This volume is intended only as an elementary review of essentials, and as an introduction and supplement to the fuller literature quoted. Medical microbiology has been barely touched on, and admittedly deserves a larger space than it receives here. The authors have endeavoured not to waste space in repeating material that may be found in any of the standard textbooks; readers wishing to see illustrations of incubators and other laboratory equipment are referred to the makers' catalogues. The bibliography has been kept small, and is limited for the most part to literature that is readily available and in which further references will be found. As regards names of bacteria, Bergey's system has been followed except that common species like *Escherichia* (*Bacterium*) *coli* are sometimes referred to by the older and more familiar names.

The problem has been not what to put in, but what to leave out, and every reader will find some omissions that he will consider inexcusable. Errors may have been included, and undoubtedly many important references have been missed. The authors would be grateful for details of such errors and omissions.

The authors are deeply indebted to the following friends for advice in connection with this book:

Professor F. T. Brooks and Dr. R. St. John-Brooks (general criticism); Dr. L. R. Bishop (Chapter X.); Dr. W. C. V. Brothwood (Chapter XII.); Mr. W. C. Moore (Chapter XIII.); Dr. B. B. Mundkur (Chapters II. and XIII.); Dr. R. G. Tomkins (Chapter IX.); Dr. T. K. Walker (Chapters VII. and X.).

L. D. G.
1937. R. B.

FOREWORD TO THE THIRD EDITION

THE last ten years have seen notable advances in Microbiology, and an awakening of public interest in the subject. There has been a corresponding increase in the number of laboratory workers employed on specialised branches of microbiology. Some years' experience in consulting and lecturing work has confirmed the reviser in his belief that most junior workers in this field have an insufficiently wide general knowledge of the activities and potentialities of micro-organisms. It was to provide such a background that this book was written, and a new edition seems justified.

The present edition has been very considerably re-written. Chapter bibliographies have been revised, but reduced in bulk by the omission of references to original papers—often ephemeral in value and difficult of selection. Authors' names have been deleted from the text in order to make easier reading.

I am indebted to Mr. A. W. Rule for the microphotographs reproduced in Fig. 3(*e*) and Fig. 9; and to Mr. T. McLachlan for permission to publish those in Fig. 5(*b*) and (*c*).

Changes in the book are intended for the better, but for all errors of judgment or fact the reviser alone is responsible.

Welbeck Way, W. 1. L. D. G.
1950.

CONTENTS

LIST OF ILLUSTRATIONS

PART ONE

CHAPTER I

INTRODUCTORY

THE organisms included under the names of fungi and bacteria affect our lives very closely. The normal decomposition of organic matter is largely brought about by them, and without their aid the world would be strewn to an embarrassing extent with unrotted vegetation and undecayed animal corpses. Micro-organisms play an essential part in plant nutrition, whilst individual types are utilised by us in the preparation of many food products and for an increasing number of industrial processes.

On the other hand, we suffer considerably if the activities of micro-organisms are uncontrolled. "Perishable" substances become damaged or completely ruined, whilst our crops and even our bodies and those of our domestic animals are subject to diseases caused by them.

It is therefore of importance not only to the scientific worker, but also to the agriculturist, the industrialist, the housewife, and the man in the street, to know something more of micro-organisms than is at present included in a general education.

Leeuwenhoek, the seventeenth century Dutchman whose hobby was the construction of simple microscopes, was probably the first man to see bacteria, although his highest magnifications were of the order of $\times 200$. About 1683 he submitted to the Royal Society drawings of rod-like organisms, found in deposit on teeth, that were probably large bacteria. He also figured yeasts and protozoa seen in various infusions and fermenting liquids.

Later, doubts began to be expressed as to the "spontaneous generation" of micro-organisms from non-living matter. The experiments of the eighteenth century priests Needham and Spallanzani on the sterilising effect of heat were not directed to practical ends; they did, however, pave the way for Pasteur's researches, and incidentally for the modern canning industry.

The best way to acquire an enthusiasm for bacteriology is to read one of the many excellent biographies of the famous Frenchman, Louis Pasteur. Few men can claim to have stimulated the advance of applied science in so many directions as did Pasteur (1822-1895). Originally trained as a chemist, he carried out a well-known piece of research on the stereo-isomerism of racemic acid, during which he observed that certain green moulds (*Penicillium* spp.) utilised *d*-tartaric acid in preference to *l*-tartaric. His interest thus aroused by the chemical activities of micro-organisms, he turned to the study of alcoholic, lactic, and butyric fermentations, which for the first time were shown to be due to living organisms. He then laid the bogey of "spontaneous generation," and carried out pioneer work on "diseases" of vinegar, wines and beers, and a protozoal disease of silkworms. Lister's antiseptic system that revolutionised surgery arose directly from this earlier work of Pasteur, and Pasteur's own later work laid the foundations of the study and treatment of infectious diseases. Following up the principle of Jenner's vaccine treatment, he devised methods of inoculation against anthrax and rabies.

It is perhaps not surprising that microbiological studies concerned with the alleviation of human suffering took precedence, and that the development of bacteriology since 1870 has been largely confined to the study of pathogenic organisms. In fact, the word Bacteriology is still associated in the public mind with hospitals and disease "germs." Nevertheless, the pathogenic bacteria represent a small minority, and the study of other types, with a view to their utilisation or suppression, offers a vast field for research. It is probable that even now the good effects of micro-organisms far outweigh the harm they do, and as our knowledge extends it will be found increasingly possible to make use of the harmless types and to keep the harmful types under strict control. The recent development of penicillin and other antibiotics is a step in this direction.

The *Fungi*—with the exception of the yeasts, which have much in common with the bacteria—were a subject of study long before the bacteria. By reason of their larger size, they did not have to wait on the progress of microscopy. But it was not until about 1845 that certain of the more minute fungi were realised to be the cause of severe crop diseases. About the same time, favus and certain other human "ringworm" diseases were shown to be due to fungi. Fungi still remain to some extent the prerogative of the botanist, and just as bacteriology is often taken to mean medical bacteriology, so mycology (*i.e.*, the study of fungi) often implies plant pathology. Yet later studies have shown that the activities of fungi and bacteria are closely interwoven; both are equally active in producing soil fertility, fermentation, and disease in plants and animals.

As a convenient term to cover the study of bacteria and of the more microscopic fungi (which include yeasts) the word Microbiology has of late years come into use; this in its industrial aspects covers the same ground as the terms Technical Mycology and Industrial Bacteriology. The term Microbiology is often used in a somewhat wider sense to include viruses, protozoa, and the more minute forms of algae.

It will be seen that the chemist, the botanist, and the pathologist have all played a part in developing our knowledge of micro-organisms, and a sympathetic co-operation by these three classes offers the best hope of further advances.

It is very desirable that the worker in applied microbiology should possess some knowledge of forms of life other than bacteria and fungi. He should be able to recognise the presence of algae or protozoa in liquids he is examining for bacteria, and to detect mites if they occur as an infection of his fungal cultures and food products. A knowledge of plant and animal structures is often of considerable assistance in examining material under the microscope. Such a warning against too narrow specialisation is probably unnecessary, but it is given here, since the limits of the present volume preclude anything beyond a brief sketch of the fungi and bacteria.

Classification.

The primary object of classification is to secure an orderly arrangement of an otherwise unwieldy mass of material. The terms used should be in some universal language—since science is international—and the classification should as far as possible indicate the relationships considered to exist among the various forms—*i.e.*, it should be a " natural " classification.

What constitutes a basic unit of classification, or *species*, is very difficult to define, especially in the case of organisms which have a simple life history. In fact, it is so difficult to formulate a satisfactory definition that the following has been put forward: " A species is that which has been accepted as a species by a competent systematist "! In practice the limit of the species is fairly well appreciated, though some workers tend to segregate into species forms that other workers regard as merely varieties of one species.

Each organism is given a scientific name, usually more or less based on Latin or Greek roots, according to the binomial system introduced by Linnaeus. Thus a certain common mould fungus is called *Cladosporium herbarum*; here *Cladosporium* is the *genus*, which is subdivided into a number of *species*, of which *C. herbarum* is one. It is incorrect to use generic names in the plural—*e.g.*, *Aspergilli* should read *Aspergillus* spp.

The grouping of genera into *families*, and families into *orders*, presents further doubts and difficulties, and no system of classification should be regarded as anything but a provisional one, subject to alteration as our knowledge increases.

Practical Importance of Identification.

In considering the steps necessary to check some destructive manifestation of micro-organisms, the practical man is apt to say: " Why on earth waste time in identifying the organism ? All I want to do is to suppress it." But the time spent in identification is usually—though not always—well spent. Even an approximate identification at once places the worker in touch with all the important information in the literature concerning this organism, or (showing the advantage of a " natural " classification) related organisms of similar habits. Such information is frequently of value in suggesting a method of control. Conversely, research done on any particular organism is practically valueless to later workers unless the organism is accurately identified. As an example of this, one may quote the vast amount of chemical work that has been done on " *Penicillium glaucum*," a term that has been used in the past to mean practically any species of green *Penicillium*. Incidentally, such work is of little value unless precautions are taken, *and recorded*, to ensure that the work was started and completed under pure culture conditions—*i.e.*, in the absence of other organisms.

The best means of specifying the organism used in any published work is to obtain it from, or deposit it with, one of the standard collections which are maintained. In England the following sources are available:

National Collection of Type Cultures, London, N.W.9 (bacteria pathogenic to man and animals).

D.S.I.R. Chemical Research Laboratory, Teddington (bacteria of industrial importance).

Commonwealth Mycological Institute, Kew (fungi parasitic on plants; industrial mould fungi, etc.).

London School of Hygiene and Tropical Medicine, W.C.1 (fungi pathogenic to man and animals).

Institute of Brewing (non-pathogenic yeasts).

Forest Products Research Laboratory, Princes Risborough (wood-rotting fungi).

Botany School, Cambridge (bacteria pathogenic to plants).

A very large collection of yeasts and other fungi is maintained at the Centraalbureau voor Schimmelcultures, Baarn, Holland. Workers in U.S.A. are catered for by the American Type Culture Collection at Washington, and by the collection of the North Regional Research Laboratory at Peoria.

Systematic Position of Fungi and Bacteria.

The old distinction between plants and animals is firmly rooted in our minds, but in reality no sharp dividing line exists. In general, of course, animals move and plants are stationary; animals require complex food, whilst green plants utilise the sun's energy to build up proteins, carbohydrates, and fats from inorganic sources; best distinction of all, animals ingest solid food by means of a gullet, whereas plants must absorb their food in solution through a cell wall.

Among the simpler organisms, however, exceptions can be found to all these criteria. Many forms obviously related to green plants have lost their chlorophyll, and in consequence require organic food material; certain Myxomycetes (which are classed with plants) differ very little from the simpler Protozoa (which are classed with animals); whilst the bacteria show so little structural detail that it is difficult to say what their systematic position should be.

With these reservations, the following scheme may be given as a rough guide to the position of bacteria and fungi in the scheme of living organisms:

ANIMALS	Single celled ..	*Protozoa.*	
	Many celled ..	*Metazoa* (including Mammals, Insects, Fishes, etc.).	
PLANTS	Seed plants ..	*Phanerogams* (including Flowering Plants, Conifers, etc.).	
	Seedless plants .. *Cryptogams*	Pteridophytes (Ferns, etc.).	
		Bryophytes (Mosses and Liverworts).	
		Thallophytes	**Algae** } Combined as Lichens.
			Fungi }
			Bacteria.
			? Viruses.

BIBLIOGRAPHY

1. Bulloch, W. (1938): History of Bacteriology. London.
2. Dobell, C. (1932): Leeuwenhoek and His Little Animals. London.
3. Henrici, A. T. (1948): The Biology of Bacteria. New York.
4. Vallery-Radot, R. (1919): Life of Pasteur. London.

CHAPTER II

THE FUNGI

THE foundations of Mycology, or the study of fungi, were well laid before the study of bacteria was begun. On the other hand, the technique used in dealing with the micro-fungi that concern the industrial or agricultural microbiologist has largely been borrowed—often without sufficient discrimination—from the bacteriologist.

To give an indication of the economic importance of the fungi, one may list the following activities of this group:

(i.) As cause of disease in animals or plants, and particularly crop plants.

(ii.) As soil organisms bringing about the necessary decomposition of organic matter.

(iii.) As spoilage organisms affecting foodstuffs, timber, textiles, etc.

(iv.) As fermentation organisms—*e.g.*, for the production of citric acid and penicillin.

(v.) As food—from "mushrooms" to food yeast and animal fodder.

The fungi form too vast a group to be described in a few pages, but a rough background is desirable even for those whose interests are concerned with one particular sub-group.

Structure of Fungi.

Some fungi—*e.g.*, the yeasts—are unicellular, but most are multicellular organisms. The active part of a fungus usually consists of a system of filaments of microscopic thickness —the *hyphae*—known collectively as the *mycelium*. This may form a compact mass of definite form, as in the familiar "mushrooms," or it may be simply a loose cottony mass ramifying in or on the substratum. In certain instances the hyphae may be aggregated loosely into rope-like strands (*rhizomorphs*), or they may unite to form hard compact masses (*sclerotia*) capable of acting as a resting stage.

A fungus possesses no chlorophyll, and to secure growth it must absorb suitable organic nutrient material in soluble form. Within the cell wall are contents similar to those found in cells of higher plants—protoplasm, nuclei, and reserve food substances. In most groups of fungi the hyphae are divided at intervals by cross-walls, whilst in other groups these occur but rarely. The cross-walls usually possess a central pore, through which there is a constant slow movement of protoplasm from the older to the younger parts of the fungus. Fresh mycelium arises by the growth of existing mycelium, or by the germination of a structure known as a *spore* which is usually capable of undergoing a resting stage; reproduction and dissemination are ensured by the production of a fresh supply of spores later in the life history of the organism.

Spores may be of two types, sexual—preceded by a nuclear fusion—and asexual. The processes leading to sexual reproduction in fungi are still imperfectly understood; but the different types of sexual spore serve as a basis for the classification of the fungi into three main groups, with a fourth group in which sexual spores are unknown. In the first of the main groups outlined on p. 9, the Phycomycetes, the process is fairly clear; in the Ascomycetes it is less clear; in the Basidiomycetes nuclear fusions occur which may represent the beginnings of sexual fusion. The resulting spore form is known variously as *oospore*, *zygospore*, *ascospore* or *basidiospore*, according to its methods of formation. In the Fungi Imperfecti (by definition) sexual reproduction is unknown.

The **asexual spores** are usually produced simply by a budding-off process. When such budding takes place from a modified external hyphal branch, the spores are known more correctly as *conidia*, and the branch as a *conidiophore*. Conidiophores may be formed in clusters known as *coremia*. Other types of spore exist. *Chlamydospores* are formed by rounding off and thickening of portions of the vegetative hyphae; *zoospores* are motile asexual spores liberated from an enclosing *sporangium*; and non-motile spores may be produced in a closed *sporangium*, in a flask-shaped *pycnidium*, or on a cushion-like *sporodochium*. Several types of spore frequently occur in one and the same fungus.

The variety of shape in spores and spore-bearing structures is limitless. Spores may be single-celled or multicellular, colourless or coloured. The common feature is that the spores are microscopically small, light structures which are readily detached when ripe, and are thus suitable for dissemination by wind, water or animal agency. In size fungal spores vary from spheres 2 μ in diameter to structures 100 μ or more in length (1 μ or micron being one-thousandth of a millimetre) It is quite incorrect to regard spores as the seeds of fungi. They are much less complex structures than seeds, though analogous to the seeds of higher plants in that they can undergo a resting stage and withstand desiccation, factors that enable the fungus to survive exposure to unfavourable conditions.

The majority of fungi are *saprophytes*—that is to say, they are capable of existing on dead organic matter. Many, however, are *parasites* on animals or plants—more especially on plants—and of these parasites some are quite incapable of existing saprophytically. The fungi causing *Rusts* of wheat, for example, have never been cultivated on laboratory culture media. Parasitism by a species of fungus may be possible on a range of host organisms, or may be confined to a single host species; the parasite may pass the whole of its life cycle on one host, or two distinct parts of its life cycle on two different species of host.

The Main Groups of Fungi.

All classification systems are largely arbitrary, and differences of opinion arise as to the best orderly arrangement of the many thousands of fungal species. The scheme outlined below gives the generally accepted basis for the separation of the larger groups, excluding the Myxomycetes, whose affinity with the fungi is doubtful.

Classification of Fungi.

Vegetative mycelium mostly without cross-walls; sexual spore an oospore or zygospore; asexual spores usually in sporangia	.. .. ..	**Phycomycetes.**
Vegetative mycelium divided by cross-walls	Sexual spores in asci	**Ascomycetes.**
	Sexual spores on basidia ..	**Basidiomycetes.**
	Sexual spores lacking ..	**Fungi Imperfecti.**

The **Myxomycetes,** which are not included in the above scheme, are organisms whose vegetative form, known in the aggregate as a *plasmodium*, is simply a mass of protoplasm with no surrounding cell wall. Later in the life history spores are produced. Many myxomycetes occur on old logs, where they become noticeable as slimy masses after wet weather. In general, the group has little economic significance except for *Plasmodiophora*, which is the cause of "Club-root" or "Finger and Toe" in cabbage and turnip; and even this is now considered by many systematists to belong to the lower Phycomycetes.

The **Phycomycetes** may be divided into the *Archimycetes*, in which the mycelium is rudimentary or absent; the *Oomycetes*, in which the mycelium is well developed, motile accessory spores may be found, and sexual reproduction is by means of differentiated organs that give rise to an *oospore*; and the *Zygomycetes*, in which the mycelium is also well developed, but sexual reproduction is brought about by the union of organs which are not differentiated.

The general name of the group, *Phycomycetes*, is given because the group comprises forms which resemble the green algae in general structure, although possessing no chlorophyll. The *Archimycetes* include a large number of species parasitic on plants and aquatic animals, but are for the most part of no great importance to man. The chief exception is *Synchytrium*, a species of which causes the dreaded "Wart Disease" of potatoes.

Among the *Oomycetes* are a number of aquatic groups saprophytic or parasitic on plants or aquatic animals; a few species of *Achlya* and *Saprolegnia* are destructive to young fish. The *Peronosporales* are mostly parasites of flowering plants, linked up with aquatic types by the genus *Pythium*, the cause of various "damping off" diseases of seedlings. *Pythium* merges into the genus *Phytophthora*, which is still more terrestrial in habit. Of the many species of this genus causing plant diseases, one of the most harmful is *Phytophthora infestans*, causing "Late Blight" of potato. A closely related type (*Plasmopara viticola*) causes a serious disease of grape vine. These diseases are so widespread that the spraying of potatoes and grape vine with fungicides has become a routine preventive measure. Abundant conidia are produced by such types.

The remaining group, the *Zygomycetes*, includes, in addition to a group of insect parasites, the widely distributed *Mucorales*, practically all of which are saprophytic moulds. *Mucor*, *Rhizopus*, and a few related genera are among the commonest types developing on damp foodstuffs, rotting fruit, or other substrata, and possess characteristic knob-like sporangia. Many possess a marked starch-decomposing ability which has led to the utilisation of such fungi in the fermentation industries. *Thamnidium* develops on meat in cold storage. The phenomenon of *heterothallism* was first observed among the Mucorales; in certain species a mycelium derived from a single spore is one of two types, termed + and − because it is only when hyphae of opposite sign approach each other that sexual fusion occurs. This is probably equivalent to sex difference, although without visible differentiation of form. In other groups, however, cases of heterothallism are known which cannot be easily explained on a sex basis, and "physiological heterothallism," affecting purely vegetative characteristics, is also known.

The **Ascomycetes** are an enormous group characterised by the possession of a special spore form contained in a specialised cell known as an *ascus*. Asci are usually club-shaped cells each containing eight ascospores. The simplest group includes the *Endomycetales*, or Yeasts. Here mycelium is vestigial or absent, the fungus existing in the form of round or oval cells, which usually multiply in a characteristic manner by budding.

With certain yeasts, under certain conditions of environment, the contents of individual cells may divide up into one to eight endospores; these represent ascospores of the yeasts, and are the ground on which this very characteristic group is placed among the Ascomycetes. Ascospores of yeasts, on germination, may either form small (haploid) yeast cells, or may fuse in pairs to give larger (diploid) cells possessing double the number of chromosomes.

All yeasts are not capable of forming spores, and strictly speaking those that do not should be classed under the Fungi Imperfecti. However, the group is sufficiently well defined, and it is convenient to consider sporing and non-sporing yeasts together. Classification is difficult. A rough division for practical purposes is that into "wild" yeasts that show a tendency to form mycelium and have little fermentative