Essays in Microbiology

Edited by

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Preface

Microbiology shares with Geology and one or two other pure science courses taught in Universities and similar places the difficulty of being a subject not widely taught to children. Although it is certainly true that many young microbiologists begin their University Microbiology Courses with a sound enough knowledge of Chemistry and Biology, all too often there is only the haziest idea as to what they are actually about to undertake; this collection of sixteen Essays is therefore designed to throw some light on the problem, and to provide a solid basis on which to build an advanced knowledge of the subject from more specialized books and journals. Each author has been encouraged to take a simple introductory approach to his particular topic and the primary readership is defined as the early stage undergraduate student with some topics of direct relevance to the senior school pupil specializing in Biology. In addition, each author has been requested to try to place the particular aspect that concerns him in its context in the subject as a whole. In this way we hope that these Essays will also be of interest and value to those further on in their Microbiology Courses, and even to those specializing in other aspects of Biology – particularly the study of plants and animals, and their interactions.

The biological revolution that has been caused by the rise of Molecular Biology – an event in which the study of Microbiology has certainly played a central and crucial role – has left the teacher of Microbiology with a difficult problem. Should he tackle the subject in descriptive terms and try to teach by analysing the complex activities of microbial populations in Nature? Or should he begin with the structure of the component macromolecules that go to make up microbes and then attempt a synthesis that comes somewhere near to what we see in practice? Of course neither, if pursued without a leavening of the other, gives anything like a balanced picture; and indeed there is enormous advantage in leading students to look at the subject from these two alternative viewpoints in parallel. If this approach is well done, microbiologists are led to think about the subject in novel ways: two views of the same topic cross-illuminate one another, and from the paradoxes generated in this way the development of the subject by research is initiated.

There has been no attempt in these sixteen Essays to cover all aspects of Microbiology: clearly that would be foolhardy; and certainly no University attempts such a catholic approach. Rather we have chosen topics for the Essays to try to indicate the broad sweep of the subject. About half are broadly 'descriptive' while the others are 'molecular'. All, we hope, nevertheless help to throw more light on the question posed, and indeed partly answered, by Dr Stanier in his first Chapter: 'What is Microbiology?'

Textbooks are expensive and Microbiology is a vast subject taught in many different courses. Most teachers have had the experience of recommending students to study selected chapters from various books as being relevant to their particular courses. We therefore conceived the idea of publishing the Essays both as a complete book and as separate units available at the lowest possible price, so enabling the student economically to study those elements specifically required by his own programme of work.

In closing this brief Preface, we would like to thank a number of people: the patient contributors for writing and amending their scripts to fit the straightjacket imposed by the 32 pages allowed by our format; our secretaries, for all their help in getting the material ready for publication; and Janet Jones and John Wiley for agreeing to take part in an experiment in the form of textbook publication.

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What is Microbiology?

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I. Introduction

Unlike the physical sciences, biology can be subdivided in two planes: one organismal, the other functional. Organismal subdivision produces disciplines that deal with specific evolutionary branches of the biological world; for example, ornithology, entomology, mycology, bacteriology. Functional subdivision produces disciplines that cross organismal boundaries, such as cytology, genetics, and biochemistry.

Microbiology is seemingly an organismal discipline, since it is

concerned with the properties of small forms of life, or microorganisms. However, the term 'microorganism' does not define a well-circumscribed evolutionary group. According to *Webster's New International Dictionary*, a microorganism is 'any organism of microscopic (also in a broad sense, ultramicroscopic) size; applied especially to bacteria and protozoa'. Let us follow this lexigraphic lead, and see where it takes us.

The human eye cannot resolve an object less than 1 mm in diameter, and can perceive very little structural detail in objects an order of magnitude larger. Microscopic examination is therefore essential either for the very perception, or for the determination of gross structure, of any organism that is 1 mm or less in its largest dimension. Such an organism can be reasonably described as a 'microorganism'.

With very rare exceptions, cells do not have diameters in excess of 1 mm. Consequently, unicellular organisms fall into the microbial category. These include nearly all bacteria and protozoa, as noted by *Webster*. Another class of organisms which are unquestionably microorganisms are the viruses. The only stage of viral development that can be structurally defined is the infectious particle or virion, and the size range of virions extends from the lower limits for cells (0.2–0.3 μm in diameter) to objects two orders of magnitude smaller. The resolution of most virions is thus possible only by electron microscopy, which accounts for the parenthetic proviso ('ultramicroscopic') in Webster's definition.

Some members of the fungi (e.g. yeasts) and of the algae (e.g. diatoms, photosynthetic flagellates) are unicellular and can therefore be construed without ambiguity as microorganisms. Difficulties arise, however, with other members of these two taxonomic groups. Many fungi are coenocytic mycelial organisms, and there is no fixed limit to the size which can be attained during vegetative growth of a single individual. Indeed, some basidiomycetes may produce a mycelium as much as 50 metres in diameter, from which there develop, under conditions favourable for fructification, dozens of fruiting bodies, each of macroscopic dimensions. A similar problem confronts us in the algae. Some algae are either coenocytic or multicellular, and mature individuals may attain a size considerably larger than that of many flowering plants. Strict adherence to the dictionary definition would force us to conclude that some algae and fungi fall in the domain of microbiology, whereas others do not. On the other hand, certain metazoan animals (rotifers and some nematodes) may never exceed 1 mm in their largest dimension, even though they are not commonly considered as organisms that form part of the domain of microbiology.

As this analysis shows, the concept of a 'microorganism' is highly artificial. It is at the same time remarkably broad, in the sense of covering the protozoa, the bacteria, and the viruses, three groups which

differ profoundly in their biological properties; and unduly restrictive, in the sense of cutting through reasonably well-defined natural groups, such as fungi and algae. Microbiology is accordingly not comparable to most disciplines of organismal biology. Nevertheless it is without question a branch of biology that possesses both unity and coherence. These are derived from the fact that it possesses some of the attributes of a functional discipline: the diverse taxonomic groups that fall into the microbiological domain are all susceptible to analysis by a special methodology. It is the methods of microbiology that determine, in the last analysis, the kinds of organisms that constitute its biological subject matter: viruses, bacteria, protozoa, fungi, and algae.

II. The Methods of Microbiology

Microorganisms are ubiquitous in the biosphere; every natural habitat contains an extremely diverse microbial population. On very rare occasions, a population that consists predominantly of one type of microorganism may develop, but such microbial 'blooms' are typically localized in both space and time. Although the relative abundances of different microorganisms fluctuate continuously, the heterogeneity of natural populations is normally so great that any given kind of microorganism represents, at most, a very small fraction of the total. This makes it virtually impossible to study the properties of a specific microorganism in its natural habitat. The problem can be solved only by isolating it from the natural habitat, freeing it from all accompanying organisms, and propagating it as a pure strain in a suitable artificial medium which has been sterilized prior to inoculation, and protected from subsequent contamination by the other microorganisms that are omnipresent in the environment.

A. Sterilization

Fundamental to pure culture technique is the preliminary sterilization (and protection from subsequent contamination) of the media, culture vessels, and implements employed in isolating and manipulating pure cultures. Sterilization can be achieved by exposure to lethal agents, either physical or chemical; or, in the special case of solutions, by filtration. Filtration, the only means of sterilizing solutions of highly labile compounds, has an intrinsic limitation. Although filters that will retain all cellular organisms are readily available, filters sufficiently fine to retain even the smallest viruses are not, since some virions have the dimensions of large protein molecules. Filtration can be used to make a solution cell-free, without necessarily making it virus-free.

The most convenient and widely used agent of sterilization is a physical one: heat. The principles of heat sterilization are not always

clearly understood and merit brief discussion. When a pure culture of a microorganism is exposed to heat (or to any other lethal agent) the kinetics of death are typically exponential: a plot of the logarithm of the number of survivors as a function of time gives a straight line, the negative slope of which expresses the rate of killing. These kinetics reflect the fact that, in a homogeneous population, probability alone determines the time of death of any given individual. The death rate defines what *fraction* of the initial population will survive any given period of exposure to the lethal agent; it does not, of itself, provide information as to the *number* of survivors at this time. This is determined by another parameter, the initial population size. The larger the initial population, the larger the number of survivors after any given period of exposure to the lethal agent. Thus, a much longer period of heating is necessary to sterilize 10 litres of a culture medium than to sterilize 10 millilitres, assuming that they both contain microbial populations of the same initial density. Long experience has shown that the endospores produced by certain bacteria are the most highly resistant of all microbial cells; bacterial spore suspensions are therefore frequently used to calibrate heat sterilization procedures.

In the light of the foregoing discussion, the goal of sterilization can be reformulated in a somewhat more sophisticated way: *the probability that the object subjected to treatment contains even one viable surviving cell should be infinitesimally small.* The sterilization procedures employed by microbiologists are designed to meet this goal, and provide a wide margin of safety.

B. Microbial Nutrition and the Design of Culture Media

In order to grow a cellular microorganism, a culture medium must be prepared which contains an adequate supply of nutrients, i.e. the various chemical substances required for the synthesis of cell materials and for the generation of ATP. The culture of viruses is a different problem which will not be further considered here; their growth is dependent on the provision of suitable conditions for the development of the cellular host.

Cellular microorganisms display an extraordinary nutritional diversity, which reflects their extreme diversity in physiological and biochemical respects. Consequently, it is impossible to prepare a universal culture medium, suitable for the growth of all kinds of microorganisms. The design of a suitable culture medium must be worked out for each particular microbial group, taking into account its special physiological and biochemical properties. For bacteria alone, literally thousands of different culture media have been proposed. Nevertheless, certain general principles govern the design of them all.

In the first place, a culture medium should contain a *balanced*

Table 1. Approximate elementary composition of the microbial cell[a]

Element	Dry weight (%)
Carbon	50
Oxygen	20
Nitrogen	14
Hydrogen	8
Phosphorus	3
Sulfur	1
Potassium	1
Sodium	1
Calcium	0.5
Magnesium	0.5
Chlorine	0.5
Iron	0.2
All others	~0.3

[a]Data for a bacterium, *Escherichia coli*, assembled by S. E. Luria, in *The Bacteria* (I. C. Gunsalus and R. Y. Stanier, eds.), Vol. I, Chap. 1 (New York: Academic Press, 1960).

mixture of the different nutrients, each being furnished in a relative amount roughly proportional to biosynthetic requirements; some nutrients are required only in traces, others in much larger amounts. This principle is of critical importance, since depletion of any one nutrient, whatever its nature, will arrest growth, and arrest is sometimes preceded by a short period of unbalanced growth which makes the population physiologically abnormal.

The chemical composition of cells, which varies little, provides a useful insight into general nutritional requirements (Table 1). Water is always the principal molecular component (80–90 per cent by weight) of the living cell, and therefore a major essential nutrient. In addition to hydrogen and oxygen (derivable metabolically from water), the dry matter of cells contains four principal non-metallic elements: carbon, nitrogen, phosphorus, and sulphur. It also contains a variety of metals, of which potassium, sodium, calcium, magnesium, and iron are quantitatively the most important. However, several additional metals, present only in traces in cells, play indispensable roles in cellular metabolism, and are therefore essential nutrients. They include manganese, cobalt, copper, molybdenum, and zinc. Although both sodium and chlorine are normally present at fairly high levels in the dry matter of cells, neither of these elements can be demonstrated to be essential for the growth of most microorganisms, with the exception of those which inhabit marine or hypersaline environments. Indigenous marine

microorganisms have readily demonstrable Na^+ and Cl^- requirements, as well as quantitative requirements for Ca^{2+} and Mg^{2+} considerably higher than those of terrestrial and freshwater forms. With the partial exception of Na^+ and Cl^-, all the above mentioned elements are essential nutrients, and must be provided in any culture medium in a suitable chemical form.

All metals, together with phosphorus (as phosphate) can be provided as nutrients in the form of inorganic salts. The nutritional diversity of microorganisms largely reflects the different molecular forms in which four elements – carbon, nitrogen, sulphur, and oxygen – must be provided.

Most photosynthetic microorganisms (exception: some photosynthetic bacteria) can use the most highly oxidized form of carbon, CO_2, as a carbon source. In these groups, ATP is derived from a physical source, by the conversion of light energy into chemical bond energy. Carbon dioxide can also be used as a carbon source by certain groups of non-photosynthetic bacteria (chemoautotrophs), which can couple the oxidation of reduced inorganic compounds (e.g. NH_3, H_2, H_2S) with ATP synthesis.

All other microorganisms belong to the nutritional category of chemoheterotrophs, which require at least one organic compound as a major nutrient, from which they derive cell carbon. This substance also has a second metabolic role, as a source of ATP; it is in part decomposed by a respiratory or fermentative pathway, the operation of which is coupled with ATP synthesis.

Many chemoheterotrophic bacteria and fungi, as well as a few protozoa, can derive both carbon and energy from the metabolism of a single organic compound. Microbial diversity with respect to the organic substances utilizable for this purpose is extreme. *Every naturally occurring organic compound can be used as a carbon and energy source by at least one type of microorganism.* Consequently the number of different organic compounds utilizable by the totality of microorganisms runs into tens of thousands. Not surprisingly the nutritional spectrum of any given microorganism is narrow, relative to the immense total range. Nevertheless, some bacteria possess remarkably wide nutritional spectra; for example, representatives of the genus *Pseudomonas* can use as single carbon and energy sources at least 100 different organic compounds, including sugars, fatty acids, dicarboxylic acids, hydroxyacids, aminoacids, amines, benzenoid compounds, and sterols. Other bacteria have extremely limited and specialized nutritional spectra; for example, one physiological group, the obligate methylotrophs, can use only two carbon and energy sources: methane and methanol. Methane cannot be utilized by any other microorganisms, and methanol is very rarely utilized by members of other microbial groups.

In the cell, nitrogen and sulphur occur principally in a reduced organic state, as amino ($R-NH_2$) and sulphhydryl ($R-SH$) compounds, respectively. Many microorganisms can use as sources of these elements the anions NO_3^- and SO_4^{2-}; their incorporation into organic form within the cell is preceded by a reduction to ammonia and sulphide, respectively. Microorganisms unable to perform one of these reductions must be furnished with either ammonia (or sulphide) as a nitrogen (or sulphur) source. Inability to reduce nitrate is relatively common; inability to reduce sulphate is much rarer. Many bacteria (but not other microorganisms) can use N_2 as an inorganic nitrogen source.

Simple nutrient requirements necessarily reflect a high degree of biosynthetic ability. A microorganism able to grow at the expense of a single carbon compound, nitrate, and sulphate, must be able to synthesize from these three nutrients a wide diversity of metabolic intermediates, all essential for cellular function. They include the coenzymes, as well as the monomers required for the synthesis of proteins, nucleic acids, lipids, and polysaccharides. If an organism does not possess the enzymic machinery necessary for the synthesis of any one of these coenzymes or monomers, the compound in question (or its immediate metabolic precursor) becomes an essential nutrient, and must be furnished in the growth medium. Such nutrients, individually required in quantities that are small relative to the principal carbon source, are termed *growth factors.* Growth factors can be divided by virtue of their chemical structures and biological functions into three categories: amino acids, the building blocks of proteins; purines and pyrimidines, the building blocks of nucleic acids; and vitamins, a chemically diverse array of compounds, each of which is a metabolic precursor of a particular type of coenzyme.

In microorganisms that require growth factors, the number and nature of the requirements vary widely. For many microorganisms, the requirement can be met by the provision of a single growth factor, for example, a specific amino acid or vitamin. Other groups of microorganisms have extensive growth factor requirements; this is an evolutionary expression of multiple losses of biosynthetic capacity, resulting from existence in ecological niches where these biosynthetic intermediates are readily available in the external milieu. Among bacteria, the most extreme example of growth factor dependence occur among the lactic acid bacteria. Some species of this group have absolute requirements for as many as 16 of the 20 amino acids that enter into the composition of proteins; four purines and pyrimidines, and numerous vitamins. This nutritional complexity reflects the nutrient-rich natural habitats of lactic acid bacteria, which develop in decaying plant materials, in milk, and in the body cavities of animals. Many protozoa have growth factor requirements of equivalent complexity. This often reflects their predacious mode of life (phagotrophy);

such protozoa normally use as food sources smaller microorganisms, which they ingest by phagocytosis, and digest within intracellular vacuoles.

Even when the precise growth factor requirements of such microorganisms have been determined, the preparation of a chemically defined medium for their cultivation is rarely attempted. There is a much simpler and more expeditious solution: the preparation of a *complex* medium, which contains (in addition to the necessary minerals and a suitable organic carbon and energy source) a product of natural origin rich in growth factors, but of undefined chemical composition. Yeast extract and extracts of plant and of animal tissues, are often used for this purpose. Phagotrophic protozoa can be conveniently grown either as two-membered cultures with an appropriate microbial prey, or as pure cultures, furnished with a heat-killed suspension of the prey. This is often necessary, because many phagotrophic protozoa grow poorly or not at all at the expense of dissolved nutrients, and appear to require food materials in particulate form.

The role of oxygen in microbial nutrition requires special discussion. Although oxygen can be derived metabolically from water, all organisms that obtain energy from oxygen-linked respiration must also be furnished with another molecular form of this element, O_2, essential as a terminal electron acceptor. Organisms of this physiological type (*strict aerobes*) are widespread among bacteria, fungi, and protozoa. However, some fungi (e.g. many yeasts) and bacteria (e.g. members of the enteric group) are facultative aerobes, since they can obtain energy from either the fermentative or the respiratory dissimilation of organic compounds, and thus can grow in the absence of oxygen, provided that they are furnished with a *fermentable* organic substrate. The proviso is important: a facultative anaerobe such as the bacterium *Escherichia coli* behaves as a strict aerobe if provided with a substrate (e.g. acetate or lactate) which can be metabolized only through the respiratory pathway; on the other hand, it behaves as a facultative anaerobe if the organic substrate is a fermentable sugar.

Molecular oxygen is a very reactive compound, and all organisms that live in contact with air possess enzymic devices to prevent the accumulation in the cell of the highly toxic derivatives formed from it. The most damaging derivative is the superoxide free radical, O_2^-. Organisms that can tolerate exposure to molecular oxygen contain an enzyme, superoxide dismutase, which eliminates the free radical by the reaction:

$$2O_2^- + 2H^+ \longrightarrow O_2 + H_2O_2$$

Most organisms also contain catalase, which decomposes the much less toxic product, hydrogen peroxide, to oxygen and water:

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$