Bacterial Physiology

Edited by

C. H. Werkman
Department of Bacteriology, Iowa State College, Ames, Iowa

and

P. W. Wilson
Department of Bacteriology, University of Wisconsin, Madison, Wisconsin

ACADEMIC PRESS INC. · PUBLISHERS
1951 · NEW YORK

CONTRIBUTORS

H. A. BARKER, *Division of Plant Nutrition, University of California, Berkeley, California*

E. S. G. BARRON, *Chemical Division, Department of Medicine, University of Chicago, Chicago, Illinois*

C. E. CLIFTON, *Department of Bacteriology, Stanford University, Stanford, California*

JACKSON W. FOSTER, *University of Texas, Department of Bacteriology, Austin, Texas*

ERNEST F. GALE, *Medical Research Council, University of Cambridge, Cambridge, England*

I. C. GUNSALUS, *Department of Bacteriology, University of Illinois, Urbana, Illinois*

W. Z. HASSID, *Division of Plant Nutrition, University of California, Berkeley, California*

FRANK H. JOHNSON, *Department of Biology, Princeton University, Princeton, New Jersey.*

GEORGES KNAYSI, *Laboratory of Bacteriology, State College of Agriculture, Cornell University, Ithaca, New York*

STANLEY G. KNIGHT, *Department of Bacteriology, University of Wisconsin, Madison, Wisconsin*

HENRY KOFFLER, *Division of Bacteriology, Department of Biological Sciences, Purdue University, Lafayette, Indiana*

JOSHUA LEDERBERG, *Department of Genetics, University of Wisconsin, Madison, Wisconsin*

PETER MITCHELL, *School of Biochemistry, University of Cambridge, Sir William Dunn Institute, Cambridge, England*

FRITZ SCHLENK, *Department of Bacteriology, Iowa State College, Ames, Iowa*

ESMOND E. SNELL, *Department of Chemistry, University of Texas, Austin, Texas*

W. W. UMBREIT, *Merck Institute for Therapeutic Research, Rahway, New Jersey*

C. H. WERKMAN, *Department of Bacteriology, Iowa State College, Ames, Iowa*

PERRY W. WILSON, *Department of Bacteriology, University of Wisconsin, Madison, Wisconsin*

ORVILLE WYSS, *Department of Bacteriology, University of Texas, Austin, Texas*

Preface

Bacterial Physiology has been written in response to a general request for an advanced text treating the subject matter from a thought-provoking rather than a compilatory point of view. For this reason *Bacterial Physiology* is not intended to present an exhaustive survey of the literature but rather to provide the reader with modern views, theories and discussions. No attempt has been made by the editors to influence the treatment of a subject or to avoid controversial topics by the contributors, nor has any effort been made to conciliate differences presented by the contributors. The advantages of such a policy in editing an advanced text are evident.

The chemistry and the physiology of microorganisms have so advanced during the past two decades that few workers are authorities in even one field; it is, therefore, important that a selected group, each competent in his own area, provide the leadership needed to stimulate further research and discussion in this rapidly expanding branch of bacteriology. Moreover, such procedure insures a more adequate separation of the significant from the trivial. It is for these reasons that multiple authorship was decided on although the limitations were fully appreciated. The editors believe that they have been particularly fortunate in obtaining contributions from recognized leaders in the respective fields.

The major portion of *Bacterial Physiology* is devoted to the traditional, fundamental knowledge of this science. The purpose of this section is to provide the required background for critical reading of current literature through concise, authoritative discussions of specific topics. The contributors have been encouraged to submit stimulating discussions rather than historical or bibliographic reviews.

The remainder of the book was planned to present a series of short contributions illustrating the significance of bacterial physiology in the broader fields of general biology. This section is less concerned with discussions of experimental results and is somewhat more philosophical. Its purpose is to develop an appreciation of the significant contributions that bacterial physiology has made to general physiological and biochemical knowledge—and these indeed have been great.

Such a text as has been described should be welcomed by teachers and advanced students, by post graduates wishing to bring themselves

up to date, and by research workers, teachers and students in the allied fields of biochemistry, botany, zoology and veterinary medicine who wish to become acquainted with the fundamentals of bacterial physiology.

Two explanations of specific decisions for which the editors willingly assume responsibility should be mentioned here. First, this is a book on bacterial physiology, and the authors were urged to keep their material, in so far as possible, within this frame of reference, although it is recognized that discussions of corresponding work among related organisms are always valuable and sometimes necessary. A second request was to provide selected reference citations on the ground that such a list is of far greater use to the student than is a complete bibliographic compilation of all references. Often, the student does not have the background to select the significant literature and the author can aid him greatly by critical screening.

The editors wish to express their genuine appreciation to all who have contributed to make this treatise possible, particularly the authors and the publishers.

Appreciation is especially expressed to Dr. Eric Fowler for assistance in preparing the Microorganism and Subject indexes.

C. H. WERKMAN
P. W. WILSON

September, 1951

CONTENTS

Chemical Factors Affecting Growth and Death

By Orville Wyss, *Department of Bacteriology, University of Texas, Austin, Texas*

Bacterial Nutrition—Chemical Factors

By Esmond E. Snell, *Department of Chemistry, University of Texas, Austin, Texas*

Bacterial Enzymes and the Theory of Action

By Fritz Schlenk, *Department of Bacteriology, Iowa State College, Ames, Iowa*

Anaerobic Dissimilation of Carbohydrates

By C. H. Werkman and Fritz Schlenk, *Department of Bacteriology, Iowa State College, Ames, Iowa*

Bacterial Oxidations

By E. S. G. Barron, *Chemical Division, Department of Medicine, University of Chicago, Chicago, Illinois*

The Comparative Biochemistry of Molecular Hydrogen

By Henry Koffler and P. W. Wilson, *Division of Bacteriology, Department of Biological Sciences, Purdue University, Lafayette, Indiana, and Department of Bacteriology, University of Wisconsin, Madison, Wisconsin*

Assimilation by Bacteria

By C. E. Clifton, *Department of Bacteriology, Stanford University, Stanford, California*

Degradation and Synthesis of Complex Carbohydrates

By H. A. Barker and W. Z. Hassid, *Division of Plant Nutrition, University of California, Berkeley, California*

Significance of Autotrophy for Comparative Physiology

By W. W. Umbreit, *Merck Institute for Therapeutic Research, Rahway, New Jersey*

Luminous Bacteria

By Frank H. Johnson, *Department of Biology, Princeton University, Princeton, New Jersey*

CHAPTER I

CHEMISTRY OF THE BACTERIAL CELL

BY GEORGES KNAYSI

CONTENTS

I. Introduction

Morphology and physiology are as intimately related as cause and effect. Since, in the last analysis, the primary object of study in both is the cell, alone or in association with other cells, this intimate relationship

is nowhere more obvious than in cytology. Indeed, cytology is a branch of biology, not of morphology, conceived in the hope of finding common grounds for both morphology and physiology. Its domain is extensive and includes the study of form, size, structure, composition, growth, multiplication, variation and life history. Because of the spectacular contributions of cytology to genetics, cytologists have heretofore been chiefly morphologists, but as physiologists become more generally convinced of the value of cytology, and as the tools and methods of cytological research are perfected and developed, cytology will undoubtedly fulfill the mission for which it was conceived; anyone who followed developments during the last quarter of a century should share this optimistic outlook. A day will come when it will not be sufficient to state that certain bacteria, for instance, produce a certain compound under certain conditions, but where in the cell such a compound is produced, with what cellular structure it is associated, and what intracellular conditions promote its formation. Most research in metabolism is carried out on resting cells and much valuable information about life processes has been thus obtained; but what about the metabolism of growth and reproduction? and how is it related to the profound morphological changes which take place during growth and reproduction? It is difficult to see how adequate answers could be given to these questions without the help of a highly developed cytology. Since the human mind is most fertile when challenged by practical problems, the problem of cancer could not fail to stimulate positive interest in the makeup and activities of the cell and their interrelationship. In recent years, there has been evidence of such an interest. Because of the general similarity of living cells, both in construction and in behavior, the cell of a microorganism is often found to be a more convenient material for research than that of more complex organisms.

II. Form and Size

Form and size confer on the cell certain physical and physicochemical characteristics. The ratio of surface area to volume, other factors being equal, controls the rate at which nutrients diffuse into the cell and by products are eliminated; consequently, it controls the rate of metabolic activity. Indeed, a study of size and form offers some excellent illustrations of the intimate interrelation between physiology and morphology emphasized in the preceding section.

A. Form

There are three fundamental forms which can be assumed by a normal, vegetative bacterial cell: the *coccus* form in which the cell is

ellipsoidal or spherical; the *rod-like* form in which the cell is cylindrical or resembles a long ellipsoid; and the *spiral* form in which the cell is helicoidal or wavy. The terms spherical, ellipsoidal, cylindrical, or helicoidal are descriptive of what approaches the corresponding geometrical form. Rod-like cells are often slightly curved or wavy, and when free always have rounded ends.

In a young or a mature culture growing under *uniform* conditions a given form may predominate, but almost always, deviation from that form may be observed. There may be rod-like cells in a culture of *Streptococcus*, coccus and rod-like cells in cultures of *Vibrio* or *Spirillum*. In old cultures the mixture of forms becomes more common and many cells assume irregular or *involution* forms. Certain strains, called *pleomorphic*, regularly exhibit mixtures of forms even in young cultures. Variation of form in a uniform environment may be due to irregularity of cell division as in a mixture of coccus and rod-like forms, or to local irregularities in the cell wall as in a mixture of rod-like, wavy, and helicoidal forms. On the surface of agar, the form of a cell may be altered by friction and other physical factors. Motile bacteria assume, or tend to assume, helicoidal forms during motility and sometimes permanently (Ellis, 1932; Pijper, 1946, 1947). Indeed, Pijper suggests that all rod-like bacteria are in reality helicoidal.

The form of a cell determines the relation between its surface area and volume, and a change in form usually indicates a change in the physiological state. It is, therefore, desirable to give form a mathematical expression which can be analyzed, plotted graphically, and discussed. Such formulae may be found, and their use illustrated, in Henrici (1928) and Knaysi (1941a, 1951).

B. Size

Among the microorganisms described as bacteria, the cells of some approach the limit of visibility with the light microscope and those of others approach visibility with the naked eye. In the majority of bacteria, however, cell sizes occupy a narrow range intermediate between these two extremes and close to the lower limit. Among the smallest bacteria are *Spirillum parvum* (0.1 to 0.3 μ by 1 to 3 μ), *Bacterium pneumosintes* (0.15 to 0.3 μ in length and one-half to one-third of these values in width); both pass through grade V Berkefeld filter. The largest bacteria known are among the sulfur bacteria; the cells of *Beggiatoa mirabilis* are 16 to 45 μ in width and form filaments which may be several centimeters in length (Bavendamm, 1924). The resolving power of the eye may be 0.0003 of a radian or about an arc of one minute which, at the distance of 15 cm., corresponds to 45 μ. In the great majority of common bacteria, the width of the cell falls between 0.5 and 2.0 μ.

Approximate values for the volume and surface area of a cell may be calculated when the cell approaches a known geometrical form. In the case of helicoidal or wavy cells, a cell should be rectified, *i.e.*, its true length should be found, before such calculations are carried out. A number of appropriate formulae for the calculation of approximate volumes or surface areas are given in Knaysi (1951).

III. Chemical Composition of the Cell

The ultimate aim of the chemistry of the cell is to relate definite chemical compounds to structure and function in the life processes of the cell itself as well as in relation to its environment. The importance of cellular chemistry may be illustrated by the imposing literature which accumulated ever since bacteriology became a science. During the last quarter of a century much progress has been made toward that end, but the path is long, tortuous, and full of hazards and precipices of both chemical and cytological orders. Since the cell consists chiefly of

TABLE 1.1.[a] Gross chemical composition of vegetative cells.

Organism	Water %	Dry matter contains N %	C %	Ash %	Reference
Bacillus anthracis	80.0	6.3			Dyrmont, 1886; Nicolle and Alilaire, 1909
	81.7–85.4	9.2			
Corynebacterium diphtheriae	84.5	11.2	48.9	4.57	Nicolle and Alilaire, 1909; Dzierskowski and Rekowski, 1892; Tamura, 1913
		9.75			
Escherichia coli	73.3	8.3		8.05	Nicolle and Alilaire, 1909
Mycobacterium tuberculosis	85.0			8.0	Nicolle and Alilaire, 1909
	83.1–88.8	9.09	51.62	9.5	Tamura, 1913; Hammerschlag, 1891
Proteus vulgaris	80.0	10.7			Nicolle and Alilaire, 1909
Pseudomonas aeruginosa	75.5	9.8			Nicolle and Alilaire, 1909
Salmonella typhosa	78.9	8.3			Nicolle and Alilaire, 1909
Serratia marcescens	85.5	11.4		13.5	Kappes, 1891; Nicolle and Alilaire, 1909
	75.0–90.61	10.5		9.31–13.77	
Vibrio cholerae	85.6	9.96–11.08		8.35–10.68	Cramer, 1895; 1897; Nicolle and Alilaire, 1909
	86.94	9.8			

[a] Arranged from data compiled by Buchanan and Fulmer (1928), and reproduced with the permission of the Williams and Wilkins Co.

various complex compounds further associated into more complex entities in an intricate organization, there is need for considerable development in microchemistry, in structural and surface chemistry, in the technique of isolating complex biological entities in the native state, and in the knowledge of intimate cell organization. Further complications result from the variability of cell composition with the biological state, the stage of development, and other environmental factors.

The cell of bacteria consists of water and dry matter. The dry matter is a mixture of organic and mineral substances, the latter being partly in organic combinations. The absolute and relative amounts of all components vary considerably with the cultural environment. Examples of the gross chemical composition of a few common bacteria are given in Table 1.1. Additional data and references may be found in Buchanan and Fulmer (1928).

A. Water

Water is the principal constituent of the cell; it is partly free and partly bound to other cellular constituents by adsorption and, possibly, by some other loose chemical association. Bound water does not act as a solvent and, thus, plays a different physiological role from that of free water (for further discussion see Gortner, 1932). It is totally removed only by the application of heat as when cells are dried in the oven.

B. Mineral Matter

The composition of ash from several common bacteria is given in Table 1.2. The proportions of the various elements present vary considerably with the medium. Undoubtedly, there are other elements, not usually added to the medium, which may be present in traces both in the medium and in the cells and escape detection.

It is not possible to state how much of a mineral element is in organic and how much in inorganic combinations. Phosphorus, which is usually present in the highest concentration, is a part of the molecule of nucleic acids, phospholipids, and of coenzymes like adenosine phosphate and thiamine. Magnesium is combined with ribonucleic acid in gram-positive bacteria; it is also present in bacterial chlorophyll and is known to be associated with the action of enzymes such as certain phosphatases. Iron is a part of cytochrome. Sulfur is part of the molecule of glutathione and of the amino acids cystine and methionine. Trace elements are probably all in organic combinations; carbonic anhydrase, for instance, contains 0.33% zinc. On the other hand, inorganic mineral salts of elements present in large proportions probably play an indispensable, physicochemical role in the dynamics of growth, multiplication,

and other cellular processes; for instance, by producing osmotic pressure and by their effect on colloidal systems and on membrane permeability.

TABLE 1.2.[a] Composition of the ash from vegetative cells.

Organism	P_2O_5 %	K_2O %	Na_2O %	MgO %	CaO %	SiO_2 %	SO_3 %	Cl %	FeO %	Reference
Acetobacter sp.	18.4	25.59		0.7 8.0	14.0 10.7	7.76 0.6		2.29	8.15	Romegialli, 1883; Allaire, 1906
Corynebacterium xerosis	34.45	11.1	24.0	6.0	3.0	0.5		0.6		Kappes, 1891
Mycobacterium tuberculosis	43.4 46.97	7.7 8.23	11.6 11.48	5.7 9.81	9.7 8.59		22.8 10.29	1.25	Trace	Goris, 1920; Tamura, 1913
Serratia marcescens	36.0	11.0	28.0	7.0	4.0	0.5		5.0		Kappes, 1891
Vibrio cholerae[b]	9.6– 45.4	4.32– 9.01	27.5– 33.79	0.12– 0.64	0.3– 1.29		1.02– 8.55	8.87– 43.69		Cramer, 1897

[a] Arranged from data compiled by Buchanan and Fulmer (1928), and reproduced with permission of the Williams and Wilkins Co.

[b] Extensive variations with the composition of the medium.

C. ORGANIC SUBSTANCES

The organic part of the dry matter may be separated into proteins, nucleic acids, carbohydrates, lipids, and other substances which do not fall within these groups. In the native state, molecules of the various groups may be associated into larger entities, but the methods of extraction and purification, and other chemical or physical manipulations, may separate the components of a given entity and may further cause changes in the properties of these components; this is particularly true of proteins some of which have been known to undergo denaturation during concentration on ultrafilters or when subjected to sonic vibrations with a frequency of about 10,000 cps. The association of complex molecules, in their natural environment, into larger entities is probably due to residual valences, and the integrity of an entity often depends on factors like the degree of dilution, the pH, the state of reduction in the environment, and a number of other circumstances. Intact entities have properties which may be distinct from those of their separate components in toxicity, antigenicity, certain staining properties, and physiological activity. For instance, in the typhoid-paratyphoid-dysentery group type specificity is determined by a phosphorus-containing, polysaccharide-protein entity; type specificity is determined by the polysaccharide component, but the characteristic antigenicity and toxicity

appear to be properties of the intact entity. In the pneumococcus, antigenicity of the nucleoprotein molecule is modified by association with a specific carbohydrate. (For other examples and further discussion see Seibert, 1941; Mudd, 1944; Dubos, 1945.) The characteristic, gram-positive reaction of the bacterial cell seems to be largely determined by the association of magnesium ribonucleate with protein and carbohydrate (Henry and Stacey, 1943). A metabolic activity usually depends on the association of enzyme and coenzyme.

1. The Proteins

An unusually high proportion of the dry weight of the bacterial cell consists of protein distributed in the nucleus, the cytoplasm, and other fundamental cell structures. The bulk of the bacterial protein is apparently associated with nucleic acids to form nucleoproteins, important substances which are usually distinguished by the type of nucleic acid with which they are associated. Sevag, Smolens, and Lackman (1940) estimated that nucleoproteins constitute about 80% of the dry matter of *Streptococcus pyogenes*, and Belozersky (1947) reported a series of figures indicating that 50 to 80% of the dry matter of a series of species of bacteria consists of nucleoprotein. Since nucleoproteins seem to be generally associated with the transmission of characters, and are generally considered to be, directly or indirectly, the cause of differences between organisms, it is unlikely that the complex and extremely varied role they are supposed to play in the living cell is carried on only by their nucleic acid component, and greater knowledge about the protein moiety is of the utmost importance. Unfortunately, no more is known about this protein moiety than about other proteins of the cell. In the nucleus of various animal cells it seems to be chiefly a basic protein; in certain tissues it is a histone and in others a protamine (cf. review by Mirsky, 1943); a certain amount of a tryptophan-containing protein is also present (Mirsky and Pollister, 1946; see also Stedman and Stedman, 1947). The nature of the protein in cytoplasmic nucleoprotein is not known; it is said to bind less nucleic acid than does the protein of the nucleus and, consequently, is considered to be less basic. In bacteria, Ruppel (1898) reported 24.5% of nucleoprotamine and 23% of another nucleoprotein in *Mycobacterium tuberculosis;* the presence of protamine in this organism was not confirmed by Tamura (1913). Mirsky and Pollister (1946) recently reported the isolation from pneumococcus, type III, of a "chromosin" which appears to be similar to other "chromosins" isolated from various animal and plant cells, *i.e.*, it appears to consist of nucleohistone and another nucleoprotein. However, Belozersky (1947) deter-

mined the proportion of the basic amino acids arginine, histidine, and lysine in nucleoprotein obtained from a number of bacteria and concluded that the values were sufficiently low to rule out the presence of histone or protamine. Among the viruses, the protein of the tobacco mosaic virus is globulin-like; Knight (1947) found that the protein of this virus, of the viruses of influenza, and of the Shope papilloma, was low in basic amino acids. Disagreement on such an elementary point as this merely emphasizes the primitive state of protein chemistry, particularly with respect to extraction and purification.

A number of investigations on bacterial protein were carried out with the objective of finding out whether or not proteins of different species differ in amino acid composition. Serological study shows that a given antigen may be present in widely separated groups, but differences in antigenicity are demonstrable in varieties of the same species or even between the vegetative cell and endospore of the same strain. However, it should be recalled that, although antigen usually means protein and identical antigenicity means identical composition and configuration, differences in antigenicity may be the result of combination of the same protein molecule with different radicals (Landsteiner, 1944), or its association with different molecules as was already pointed out. Investigation of the amino acid composition of proteins from different bacteria (Stokes and Gunness, 1946) revealed differences in the proportions of amino acids present, but no qualitative differences in the sense that one amino acid was present in one organism and absent in another, although qualitative differences were reported in viruses (Knight, 1947), and in algae (Mazur and Clarke, 1942). The early report of Tamura (1913) that the protein of *Mycobacterium tuberculosis* was free of sulfur-containing amino acids has not been confirmed by later investigators who demonstrated the presence of cystine in the same organism. In evaluating the results of amino acid investigation, it should be kept in mind that determinations were probably made on mixtures of protein, and that qualitative differences may have existed between specific components; furthermore, only about two-fifths of the possible amino acids was determined, and differences may have existed with respect to other amino acids. Nevertheless, the quantitative differences reported are of greater significance than was realized, since they probably correspond to differences in the morphology of the protein molecule and, hence, to differences in antigenicity and other biological properties. Certainly, the same types of stones may be used to build edifices widely different in size or architecture, but the number of stones of each type in a given edifice may be widely different from that in another.