ENCYCLOPEDIA OF MICROBIOLOGY

VOLUME 1 A-C

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ENCYCLOPEDIA OF Microbiology

Volume 1 A–C

ACADEMIC PRESS, INC.
A Division of Harcourt Brace & Company
San Diego New York Boston London Sydney Tokyo Toronto

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ACADEMIC PRESS, INC.
525 B Street, Suite 1900
San Diego, California 92101-4495

United Kingdom Edition published by
Academic Press Limited
24–28 Oval Road, London NW1 7DX

Library of Congress Cataloging-in-Publication Data

Encyclopedia of microbiology / edited by Joshua Lederberg
p. cm.
Includes bibliographical references and indexes.
ISBN 0-12-226891-1 (v. 1). -- ISBN 0-12-226892-X (v. 2). -- ISBN 0-12-226893-8 (v. 3). -- ISBN 0-12-226894-6 (v. 4)
1. Microbiology--Encyclopedias. I. Lederberg, Joshua.
QR9E53 1992
576'.03--dc20 92-4429
CIP

PRINTED IN THE UNITED STATES OF AMERICA
94 95 96 97 EB 9 8 7 6 5 4 3

ENCYCLOPEDIA OF
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Preface

For the purposes of this encyclopedia, microbiology has been understood to embrace the study of "microorganisms," including the basic science and the roles of these organisms in practical arts (agriculture and technology) and in disease (public health and medicine). Microorganisms do not constitute a well-defined taxonomic group; they include the two kingdoms of Archaebacteria and Eubacteria, as well as protozoa and those fungi and algae that are predominantly unicellular in their habit. Viruses are also an important constituent, albeit they are not quite "organisms." Whether to include the mitochondria and chloroplasts of higher eukaryotes is a matter of choice, since these organelles are believed to be descended from free-living bacteria. Cell biology is practiced extensively with tissue cells in culture, where the cells are manipulated very much as though they were autonomous microbes; however, we shall exclude this branch of research. Microbiology also is enmeshed thoroughly with biotechnology, biochemistry, and genetics, since microbes are the canonical substrates for many investigations of genes, enzymes, and metabolic pathways, as well as the technical vehicles for discovery and manufacture of new biological products, for example, recombinant human insulin.

Within these arbitrarily designated limits, let us consider the overall volume of published literature in microbiology, where to find its core, and strategies for searching for current information on particular topics. Most of the data for this preface are derived from the 1988 Journal Citation Reports Current Contents (T) of the Institute for Scientific Information (ISI). Table I lists the 53 most consequential journals in microbiology, assessed by citation impact factor, the average number of literature citations per article published in a given journal. Table II presents that list sorted by the total number of articles printed in each journal in 1988. Table III shows the distribution of journals citing the *Journal of Bacteriology* and the distribution of journals cited in it.

Obviously, the publications of the American Society for Microbiology (indicated by AMS in the tables) play a commanding role. The society is now making its journals available in electronically searchable form (on optical disks), which will greatly facilitate locating and retrieving the most up-to-date information on any given subject. In addition, interdisciplinary journals such as *Nature (London), Science*, and the *Proceedings of the National Academy of Sciences, U.S.A.* are important sources of prompt news of scientific developments in microbiology. It is difficult to assess how much of their total publication addresses microbiology. As seen in Table III, the bibliographies in the *Journal of Bacteriology* cite half as many articles from the *Proceedings* (2348) as from the *Journal of Bacteriology* itself (5708). The 7038 articles indicated in Table II probably reach some 10,000 per year when these interdisciplinary and other dispersed sources are taken into account. An equal number might be added from overlapping aspects of molecular biology and genetics. To find and read all these titles would tax any scholar, although it could be done as a near full-time occupation with the help of the weekly Current Contents (T) of the ISI. To start afresh, with perhaps a decade's accumulation of timely background, would be beyond reasonable human competence. No one person would intelligently peruse more than a small fraction of the total texts.

The "Encyclopedia of Microbiology" is intended to survey the entire field coherently, complementing material that would be included in an advanced undergraduate and graduate major course of university study. Particular topics should be accessible to talented high school and college students, as well as graduates involved in teaching, research, and technical practice of microbiology.

Even these hefty volumes cannot embrace all current knowledge in the field. Each article does provide key references to the literature available at the time of writing. Acquisition of more detailed and up-to-date knowledge depends on (1) exploiting the review and monographic literature and (2) bibliographic retrieval of the preceding and current research literature. To make greatest use of review literature and monographs, the journals listed in Table II are invaluable. Titles such as *Annual Reviews* should not be misunderstood: these journals appear at annual intervals, but 5 or 10 years of accumulated research is necessary for the inclusion of a focused treatment of a given subject.

To access bibliographic materials in microbiol-

ogy, the main retrieval resources are Medline, sponsored by the U.S. National Library of Medicine, and the Science Citation Index of the ISI. With governmental subsidy, Medline is widely available at modest cost: terminals are available at every medical school and at many other academic centers. Medline provides searches of the recent literature by author, title, and key word, and offers on-line displays of the relevant bibliographies and abstracts. Medical aspects of microbiology are covered exhaustively; general microbiology is covered in reasonable depth. The Science Citation Index must recover its costs from user fees, but is widely available at major research centers. It offers additional search capabilities, especially by citation linkage. Therefore, starting with the bibliography of a given encyclopedia article, one can quickly find (1) all articles more recently published that have cited those bibliographic reference starting points and (2) all other recent articles that share bibliographic information with the others. With luck, one of these articles may be identified as another comprehensive review that has digested more recent or broader primary material.

On a weekly basis, services such as Current Contents on Diskette (ISI) and Reference Update offer still more timely access to current literature as well as abstracts with a variety of useful features. Under the impetus of intense competition, these services are evolving rapidly, to the great benefit of a user community desperate for electronic assistance in coping with the rapidly growing and intertwined networks of discovery. The bibliographic services of Chemical Abstracts and Biological Abstracts would also be potentially invaluable; however, their coverage of microbiology is rather limited.

In addition, major monographs have appeared from time to time—"The Bacteria," "The Prokaryotes," and many others. Your local reference library should be consulted for these volumes.

Valuable collections of reviews also include *Critical Reviews for Microbiology*, *Symposia of the Society for General Microbiology*, *Monographs of the ASM*, and *Proceedings of the International Congresses of Microbiology*.

The articles in this encyclopedia are intended to be accessible to a broader audience, not to take the place of review articles with comprehensive bibliographies. Citations should be sufficient to give the reader access to the latter, as may be required. We do apologize to many individuals whose contributions to the growth of microbiology could not be adequately embraced by the secondary bibliographies included here.

The organization of encyclopedic knowledge is a daunting task in any discipline; it is all the more complex in such a diversified and rapidly moving domain as microbiology. The best way to anticipate the rapid further growth that we can expect in the near future is unclear. Perhaps more specialized series in subfields of microbiology would be more appropriate. The publishers and editors would welcome readers' comments on these points, as well as on any deficiencies that may be perceived in the current effort.

My personal thanks are extended to Kathryn Linenger at Academic Press for her diligent, patient, and professional work in overseeing this series; to my coeditors, Martin Alexander, David A. Hopwood, Barbara H. Iglewski, and Allen I. Laskin; and above all, to the many very busy scientists who took time to draft and review each of these articles.

Joshua Lederberg

Table I The Top Journals in Microbiology Listed by Impact Factor

Citation impact rank	Journal title	Number of articles published in 1988	Citation impact rank	Journal title	Number of articles published in 1988
1	*Microbiol. Rev.*	28	28	*FEMS Microbiol. Lett.*	365
2	*Adv. Microb. Ecol.*	10	29	*Am. J. Reprod. Immunol.*	50
3	*Annu. Rev. Microbiol.*	29	30	*Infection*	103
4	*FEMS Microbiol. Rev.*	13	31	*Can. J. Microbiol.*	236
5	*Yeast*	NA	32	*Curr. Microbiol.*	87
6	*J. Bacteriol.*	915	33	*J. Appl. Bacteriol.*	125
7	*Mol. Microbiol.*	94	34	*J. Microbiol. Meth.*	34
8	*Antimicrob. Agents Ch.*	408	35	*B. I. Pasteur*	20
9	*Rev. Infect. Dis.*	213	36	*ZBL Bakt. Mikr. Hyg. A*	164
10	*CRC Crit. Rev. Microbiol.*	12	37	*Ann. Inst. Pasteur Mic.*	58
11	*Syst. Appl. Microbiol.*	52	38	*Vet. Microbiol.*	104
12	*Int. J. Syst. Bacteriol.*	83	39	*Acta Path. Micro. Im. B*	NA
13	*J. Antimicrob. Chemoth.*	352	40	*Protistologica*	NA
14	*Appl. Environ. Microb.*	588	41	*Med. Microbiol. Immun.*	37
15	*J. Clin. Microbiol.*	619	42	*Diagn. Micr. Infec. Dis.*	60
16	*Adv. Appl. Microbiol.*	8	43	*Int. J. Food Microbiol.*	66
17	*Curr. Top. Microbiol.*	53	44	*J. Gen. Appl. Microbiol.*	27
18	*Arch. Microbiol.*	173	45	*Microbiol. Immunol.*	122
19	*J. Gen. Microbiol.*	367	46	*Lett. Appl. Microbiol.*	81
20	*Enzyme Microb. Tech.*	108	47	*Gen. Physiol. Biophys.*	57
21	*Eur. J. Clin. Microbiol.*	161	48	*A. Van Leeuw. J. Microb.*	51
22	*FEMS Microbiol. Ecol.*	42	49	*Symbiosis*	14
23	*J. Med. Microbiol.*	124	50	*Comp. Immunol. Microb.*	27
24	*J. Infection*	68	51	*Microbios.*	61
25	*Eur. J. Protistol.*	37	52	*ZBL Bakt. Mikr. Hyg. B*	76
26	*Microbiol. Sci.*	70	53	*J. Basic Microb.*	69
27	*Appl. Microbiol. Biot.*	270			

NA, Not available.

Table II Microbiology Journals Listed by Total Number of Articles Published per Year (1988)

Journal title	Number of articles published in 1988	Journal title	Number of articles published in 1988
J. Bacteriol.	915	*Int. J. Food Microbiol.*	66
J. Clin. Microbiol.	619	*Microbios.*	61
Appl. Environ. Microb.	588	*Diagn. Micr. Infec. Dis.*	60
Antimicrob. Agents Ch.	408	*Ann. Inst. Pasteur Mic.*	58
J. Gen. Microbiol.	367	*Gen. Physiol. Biophys.*	57
FEMS Microbiol. Lett.	365	*Curr. Top. Microbiol.*	53
J. Antimicrob. Chemoth.	352	*Syst. Appl. Microbiol.*	52
Appl. Microbiol. Biot.	270	*A. Van Leeuw. J. Microb.*	51
ZBL Bakt. Mikr. Hyg. A	240	*Am. J. Reprod. Immunol.*	50
Can. J. Microbiol.	236	*FEMS Microbiol. Ecol.*	42
Rev. Infect. Dis.	213	*Med. Microbiol. Immun.*	37
Arch. Microbiol.	173	*Eur. J. Protistol.*	37
Eur. J. Clin. Microbiol.	161	*J. Microbiol. Meth.*	34
J. Appl. Bacteriol.	125	*Eur. J. Protistology*	29
J. Med. Microbiol.	124	*Annu. Rev. Microbiol.*	29
Microbiol Immunol.	122	*Microbiol. Rev.*	28
Enzyme Microb. Tech.	108	*J. Gen. Appl. Microbiol.*	27
Vet. Microbiol.	104	*Comp. Immunol. Microb.*	27
Infection	103	*B. I. Pasteur*	20
Mol. Microbiol.	94	*Acta Path. Micro. Im.*	18
Curr. Microbiol.	87	*Symbiosis*	14
Int. J. Syst. Bacteriol.	83	*FEMS Microbiol. Rev.*	13
Lett. Appl. Microbiol.	81	*CRC Crit. R. Microbiol.*	12
Microbiol. Sci.	70	*Adv. Microb. Ecol.*	10
J. Basic Microb.	69	*Adv. Appl. Microbiol.*	8
		Total	7038

Table III.A Distribution of Journals Cited in *Journal of Bacteriology*, 1979–1988

Journal cited	Number of citations	Journal cited	Number of citations
J. Bacteriol.	5708	*Genetics*	183
P. Natl. Acad. Sci. U.S.A.	2348	*Can. J. Microbiol.*	139
J. Biol. Chem.	1698	*Arch. Biochem. Biophys.*	127
Mol. Gen. Genet.	1157	*Virology*	123
J. Mol. Biol.	1148	*Bacteriol. Rev.*	118
Gene	902	*Cold Spring Harb. Sym.*	110
Nature (London)	820	*Antimicrob. Agents Ch.*	109
Nucleic Acids Res.	804	*Escherichia Coli Sal.*	95
Cell	802	*Plant Physiol.*	80
J. Gen. Microbiol.	701	*J. Biochem.-Tokyo*	78
Infect. Immun.	478	*J. Virol.*	78
Methods Enzymol.	434	*Mol. Cell. Biol.*	68
Anal. Biochem.	411	*J. Infect. Dis.*	67
Biochim. Biophys. Acta	401	*Bio-Technol.*	61
Eur. J. Biochem.	376	*Exp. Gene Fusions*	60
Mol. Cloning Laboratory	363	*Trends Biochem. Sci.*	60
Microbiol. Rev.	361	*Mutat. Res.*	59
Arch. Microbiol.	347	*Syst. Appl. Microbiol.*	55
Embo J.	327	*Phytopathology*	51
Biochemistry-U.S.	310	*Adv. Bacterial Genet.*	50
Science	301	*Photochem. Photobiol.*	50
Appl. Environ. Microb.	294	*Biochimie*	49
FEMS Microbiol. Lett.	257	*J. Exp. Med.*	48
Exp. Mol. Genetics	234	*Agr. Biol. Chem. Tokyo*	47
Plasmid	234	*Int. J. Syst. Bacteriol.*	44
Biochem. Bioph. Res. Commun.	224	*FEMS Microbiol. Rev.*	43
FEBS Lett.	213	*J. Clin. Microbiol.*	42
Biochem. J.	207	*Curr. Microbiol.*	41
Annu. Rev. Microbiol.	194	*J. Cell Biol.*	41
Annu. Rev. Biochem.	188		
Annu. Rev. Genet.	187	All other (1301)	4311

(continues)

Table III.B (*continued*) Distribution of Journals Citing *Journal of Bacteriology*, 1979–1988

Journal citing	Number of citations	Journal citing	Number of citations
J. Bacteriol.	5708	*Curr. Genet.*	117
J. Biol. Chem.	1119	*FEMS Microbiol. Rev.*	115
J. Gen. Microbiol.	963	*J. Basic Microb.*	115
Mol. Gen. Genet.	896	*J. Antimicrob. Chemoth.*	112
Appl. Environ. Microb.	890	*Microb. Pathogenesis*	110
Microbiol. Rev.	759	*Science*	104
Infect. Immun.	663	*Ann. Inst. Pasteur Mic.*	101
FEMS Microbiol. Lett.	648	*Methods Enzymol.*	99
Gene	599	*ZBl. Bakt. Mikr. Hyg. A*	98
P. Natl. Acad. Sci. U.S.A.	588	*A. Van Leeuw. J. Microb.*	95
Can. J. Microbiol.	579	*Annu. Rev. Biochem.*	94
Arch. Microbiol.	484	*Plant Physiol.*	88
Mol. Microbiol.	452	*J. Infect. Dis.*	86
J. Mol. Biol.	434	*J. Med. Microbiol.*	85
Nucleic Acids Res.	431	*Folia Microbiol.*	79
Biochim. Biophys. Acta	378	*Genetika*	79
Eur. J. Biochem.	350	*Gene Dev.*	78
Antimicrob. Agents Ch.	340	*Microbios.*	77
Annu. Rev. Microbiol.	316	*Arch. Biochem. Biophys.*	75
Cell	246	*Biotechnol. Bioeng.*	73
Biochimie	238	*Nature (London)*	69
Biochemistry-U.S.	236	*Syst. Appl. Microbiol.*	69
Plasmid	236	*Zh. Mikrob. Epid. Immun.*	67
Embo J.	234	*J. Antibiot.*	66
J. Clin. Microbiol.	214	*Annu. Rev. Genet.*	65
Genetics	201	*Microbiol. Immunol.*	65
Adv. Microb. Physiol.	199	*J. Biochem.-Tokyo*	64
Agr. Biol. Chem. Tokyo	198	*Microbial Ecol.*	60
Mol. Cell. Biol.	197	*Plant Soil*	58
CRC Crit. R. Microbiol.	194	*Anal. Biochem*	56
Curr. Microbiol	193	*Annu. Rev. Cell Biol.*	55
Appl. Microbiol. Biot.	183	*Biotechnol. Lett.*	54
J. Appl. Bacteriol.	169	*Adv. Microb. Ecol.*	53
Mutat. Res.	160	*Enzyme Microb. Tech.*	53
Biochem. Bioph. Res. Commun.	152	*Curr. Sci. India*	52
Rev. Infect. Dis.	141	*Eur. J. Clin. Microbiol.*	51
Biochem. J.	137	*J. Theor. Biol.*	51
Microbiol. Sci.	135	*Bot. Acta*	50
Int. J. Syst. Bacteriol.	128	*Photochem. Photobiol.*	50
FEBS Lett	125		

How to Use the Encyclopedia

This encyclopedia is organized in a manner that we believe will be the most useful to you, and we would like to acquaint you with some of its features.

The volumes are organized alphabetically as you would expect to find them in, for example, magazine articles. Thus, "Foodborne Illness" is listed as such and would not be found under "Illness, Foodborne." If the first words in a title are not the primary subject matter contained in an article, the main subject of the title is listed first (e.g., "Heavy Metals, Bacterial Resistances," "Marine Habitats, Bacteria," "Method, Philosophy," "Transcription, Viral"). This is also true if the primary word of a title is too general (e.g.,"Bacteriocins, Molecular Biology"). Here, the word "bacteriocins" is listed first because "molecular biology" is a very broad topic. Titles are alphabetized letter-by-letter so that "Cell Membrane: Structure and Function" is followed by "Cellulases" and then by "Cell Walls of Bacteria."

Each article contains a brief introductory Glossary wherein terms that may be unfamiliar to you are defined *in the context of their use in the article*. Thus, a term may appear in another article defined in a slightly different manner or with a subtle pedagogic nuance that is specific to that particular article. For clarity, we have allowed these differences in definition to remain so that the terms are defined relative to the context of each article.

Articles about closely related subjects are identified in the Index of Related Titles at the end of the last volume (Volume 4). The article titles that are cross-referenced within each article may be found in this index, along with other articles on related topics.

The Subject Index contains specific, detailed information about any subject discussed in the *Encyclopedia*. Entries appear with the source volume number in boldface followed by a colon and the page number in that volume where the information occurs (e.g., "DNA repair by bacterial cells, **2**:9"). Each article is also indexed by its title (or a shortened version thereof), and the page ranges of the article appear in boldface (e.g. "Lyme disease, **2:639–646**" means that the primary coverage of the topic of Lyme disease occurs on pages 639–646 of Volume 2).

If a topic is covered primarily under one heading but additional related information may be found elsewhere, a cross-reference is given to the related material. For example, "Biodegradation" would contain all the page numbers where relevant information occurs, followed by "*See also* Bioremediation; Pesticide biodegradation" for different but related information. Similarly, a "*See*" reference refers the reader from a less-used synonym (or acronym) to a more specific or descriptive subject heading. For example, "Immunogens, synthetic. *See* Vaccines, synthetic." A *See under* cross-reference guides the reader to a specific subheading under a term. For example, "Mixis. *See under* Genome rearrangement."

An additional feature of the Subject Index is the identification of Glossary terms. These appear in the index where the word "defined" (or the words "definition of") follows an entry. As we noted earlier, there may be more than one definition for a particular term, and as when using a dictionary, you will be able to choose among several different usages to find the particular meaning that is specifically of interest to you.

Contents

C

A

Acetogenesis and Acetogenic Bacteria

Harold L. Drake
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Glossary

Autotroph Organism that uses CO_2 as its sole source of carbon
Chemolithoautotroph Organism that uses CO_2 as its sole source of carbon and an inorganic substrate for energy
CO_2 fixation Process in which CO_2 is covalently bonded to an organic compound
Heterotroph Organism that uses organic forms of carbon
Methanogens Obligate anaerobes that form methane as their end product
Obligate anaerobe Organism that cannot grow in the presence of oxygen
Sulfur-reducing bacteria Obligate anaerobes that use inorganic forms of sulfur as terminal electron acceptors
Syntrophy Relationship between two organisms in which each syntrophic partner is nutritionally dependent on the other

ACETOGENESIS is a term used to describe the metabolic processes by which certain bacteria form acetate from CO_2. There are three known processes by which acetogenesis occurs: the acetyl-CoA pathway, the glycine synthase–dependent pathway, and the reductive citric acid cycle. This article focuses on the acetyl-CoA pathway and those bacteria that are dependent on this pathway for the conservation of energy and the synthesis of biomass. These bacteria are defined in the present statement as acetogenic bacteria, or acetogens for short. Acetogens are obligate anaerobes and usually form acetate as their main end product. Many are chemolithoautotrophs. Ecologically, they play important roles in the turnover of carbon and energy in anaerobic habitats that range from soils and sediments to the gastrointestinal tract of many animals, including termites and humans. Three identifying features of acetogenic bacteria are (1) the use of chemolithoautotrophic substrates [carbon dioxide (CO_2) plus molecular hydrogen (H_2), or carbon monoxide (CO) alone] as sole sources of carbon and energy under strictly anaerobic conditions; (2) the capacity to convert certain sugars (e.g., glucose) stoichiometrically to acetate during heterotrophic growth; and (3) the use of aromatic compounds under acetogenic conditions. However, any single acetogen may not display all three of these metabolic features. Although other bacterial groups (e.g., methanogens and sulfur-reducing bacteria) also make use of the acetyl-CoA pathway for the synthesis of biomass or the oxidation of carbon, these bacterial groups use pathways that are slightly different from the acetyl-CoA pathway of acetogens, are dependent on other energy-yielding processes, and are not acetogenic.

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It is nonetheless important to understand that the acetyl-CoA pathway is not restricted to a single bacterial group and is widespread in nature.

I. Acetogenic Bacteria

A. Definition of the Term "Acetogenic Bacterium"

When should the term "acetogenic bacterium" be used to describe an organism? There are many acetate-forming processes used by bacteria, and any bacterium that forms acetate could be termed an acetogen or an acetogenic organism. However, such broad usage of the term "acetogen" does not adequately distinquish between the different processes by which acetate is formed. For the purpose of this article, the following definition has been applied:

> An acetogenic bacterium is an obligately anaerobic bacterium that uses the acetyl-CoA pathway for (i) the synthesis of acetyl-CoA, (ii) the conservation of energy, and (iii) growth.

The following organisms are excluded by this definition: (i) purinolytic clostridia (e.g., *Clostridium acidiurici*) and other organisms that use the glycine synthase pathway for the formation of acetate; (ii) bacteria that employ the reductive citric acid cycle; and (iii) all other acetate-forming bacteria.

Nonetheless, this definition may not be as straightforward as it appears. For example, even though the capacity to form acetate as the sole or major product is viewed as synonymous with acetogenesis, the formation of acetate *per se* is not even required by the above definition. In other words, the fate of acetyl-CoA is less important than how it is formed. Even though the production of acetate by a true acetogen is an important observation, it is not the primary reason why these organisms should be defined as acetogens: the production of acetate is a manifestation of the reason, not the reason itself. To understand this point more clearly, several points can be examined:

1. Per the above definition, it follows that whether an acetogenic bacterium is chemolithoautotrophic (capable of growth at the expense of H_2/CO_2 or CO) or heterotrophic (dependent on organic carbon) is not a critical point of classification. Even true acetogens may not be capable of chemolithoautotrophic growth because they lack hydrogenase or autotrophic anabolic processes (e.g., *Clostridium magnum*). It should also be noted that many actogens isolated as strict heterotrophs have been later shown to be capable of chemolithoautotrophic growth. A classic example of this is *Clostridium thermoaceticum*. [*See* HETEROTROPHIC MICROORGANISMS.]

2. Some acetogens that are only shown to form acetate as a reduced end product during initial characterizations and thus termed "homoacetogens" may be later found to form alternative end products. An excellent example of this is *Acetobacterium woodii*. Originally shown to only form acetate, this acetogen has recently been found to reduce acetyl-CoA to ethanol under conditions of phosphate limitation. Another similar example is the capacity of *C. thermoaceticum* to form H_2 during CO-coupled growth. Furthermore, certain acetogens may never form acetate as their sole fermentation product, yet they are obligate anaerobes and use the acetyl-CoA pathway as defined above. For example, butyrate is formed by condensation of 2 acetyl-CoA's by the acetogens *Eubacterium limosum* and *Butyribacterium methylotrophicum* and under certain conditions can be the predominant end product. Can one safely apply the term "homoacetogen"?

3. Many anaerobic organisms use the acetyl-CoA pathway for purposes that are dissimilar to that of acetogenic bacteria; that is, the pathway is not used in the direction of acetyl-CoA synthesis or it is used primarily in an assimilatory fashion for biosynthesis [e.g., autotrophic methanogens and sulfur-reducing bacteria that use the pathway for assimilation of cell carbon, or organisms that may oxidize organic carbon via a reverse-type acetyl-CoA pathway (e.g., species of *Desulfobacterium* and *Methanosarcina*)]. Furthermore, portions of the acetyl-CoA pathway may (i) exist in organisms in a cryptic state and have no (known) function in the cell or (ii) perform non-acetogenic functions. Such organisms need not be considered acetogens (see Section II.E).

4. The above definition does not exclude the possibility that acetogens can also use other forms of energy conservation. Thus, although CO_2 is considered the primary terminal electron acceptor of acetogens, recent studies have shown that alternative acceptors might also be used in concert with CO_2 and give rise to other end products (e.g., the reduction of aromatic acrylate groups and fumarate appear to be coupled to the conservation of energy by *A. woodii* and *Clostridium formicoaceticum*, respectively, and yield products other than acetate).

To summarize these points, what is most critical in determining if an anaerobic bacterium is an acetogen per the above definition is demonstrating that it uses the acetyl-CoA pathway in the direction of acetyl-CoA synthesis and that this function is coupled to both the conservation of energy and growth. That an anaerobe grows chemolithoautotrophically and forms acetate as its sole product is extremely good evidence that the organism is indeed an acetogen. Equally compelling is the observation that acetate is the sole product obtained from the fermentation of certain carbohydrates. But, in general, and especially in cases where such results are not obtained, these lines of evidence should ideally be supplimented with additional tests to verify that the acetyl-CoA pathyway is used per the above definition. Perhaps the most conclusive evidence of this type would be demonstrating that acetyl-CoA synthase (often referred to as carbon monoxide dehydrogenase, as discussed in section II.B) is functional in the direction of acetyl-CoA synthesis.

Nonetheless, as many new and unusual organisms are isolated, one must also leave room for the possibility that the above definition is not absolutely appropriate for all acetogenic isolates. For example, a recent isolate appears to have the capacity to use a reversible acetyl-CoA pathway, depending on the conditions of growth. In such cases, the organism is only acetogenic when the pathway is used in the direction of acetate synthesis.

B. Origins of Acetogenic Bacteria

Approximately 40 different species have been isolated from extremely diverse anaerobic habitats (Table I). Given the diverse origins of isolates to date, it seems likely that acetogens occur in most anaerobic habitats. Although most isolates to date are mesophilic, psychrophilic and thermophilic species have also been isolated.

Most isolates are rod shaped, but coccus forms have also been studied. Staining properties vary, and both gram-positive and gram-negative species have been reported. Some acetogens have flagella and are therefore motile. Some form spores that remain viable for long periods; the thermophilic spore-forming species are fairly resistant to high temperatures. Thus, the ultrastructural features of acetogens are highly variable. The guanine-plus-cytosine (G+C) content of the genome of acetogens is also highly variable between species.

C. Isolation and Growth of Acetogenic Bacteria

Acetogens are strict anaerobes, and attempts to isolate or cultivate these organisms must take this fact into account. The basic Hungate technique is thus widely used for the cultivation of acetogenic bacteria. The Hungate technique is also used to cultivate other obligately anaerobic bacteria. Many versions of the basic technique are in use, but all have the same basic protocol: preparation of sterile anaerobic media and transfer of cultures under strictly anaerobic conditions. The basic approach involves the use of oxygen-free gases and air-tight culture vessels. Alternatively, anaerobic cultivation can also be achieved in anaerobic glove boxes, chambers that provide oxygen-free environments for the study and growth of obligate anaerobes.

Although all acetogens use the acetyl-CoA pathway, they vary in their capacity to use different substrates. In general, various carbohydrates (e.g., glucose, xylose, and cellobiose), alcohols (e.g., methanol and ethanol), methoxylated aromatic compounds (e.g., vanillate and trimethoxybenzene), and H_2/CO_2 or CO are growth-supportive substrates for acetogens and can be used to enrich for acetogens. Recent studies indicate that certain halogenated compounds can also be used as substrates for enrichment.

Not many acetogens have been studied relative to nitrogen metabolism, but *C. formicoaceticum* has been shown to catalyze the fixation of nitrogen (N_2). It is thus likely that other acetogens also have this capacity and are therefore able to assimilate N_2 during growth under certain conditions.

In most laboratory studies, growth of acetogens is under undefined conditions (i.e., in complex, undefined growth medium), and supplemental CO_2 is often supplied in the gas phase or in the form of dissolved carbonates (e.g., bicarbonate). Although many metabolic processes, including acetogenesis under certain conditions, give rise to CO_2, studies under defined conditions have shown that some acetogens need supplemental levels of CO_2 for growth. The reason for this requirement may be very complex, but it is likely due to the fact that CO_2 is the normal terminal electron acceptor for acetogenic bacteria and that under laboratory conditions the cell is unable to generate CO_2 in adequate amounts or rates. This fact must be taken into account when designing a growth medium for acetogens.