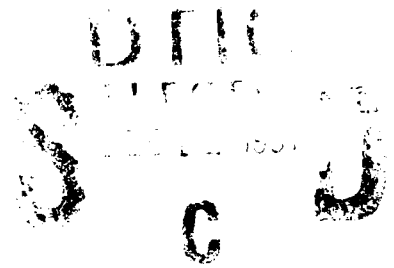


AD-A243 464



1991 COURSE REPORT
MICROBIAL DIVERSITY

Co-Directors: John A. Breznak, Michigan State University.
: Martin Dworkin, University of Minnesota



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I. Objectives of the Course

A. Philosophy

The philosophy of the course is to underscore the essence of microbiology which is diversity: diversity of morphology and cellular development, behavior, and metabolic and physiological functions. Emphasis is on microbes other than those customarily covered in conventional microbiology courses and includes: the archaebacteria, extremophiles, an array of obligate anaerobes, various phototrophs, and those microbes exhibiting complex developmental cycles. Also included are microbes carrying out a variety of transformations of organic and inorganic compounds, as well as those which normally occur in symbiotic association with other microbes or with higher forms of life.

Inasmuch as the course emphasizes nature as a tremendous reservoir of microbial diversity, a special effort is made to exploit the array of terrestrial, freshwater and marine habitats in the vicinity of Woods Hole as the source of microbes to be isolated in the laboratory portion of the course. Additional samples from unusual habitats (eg. deep sea hydrothermal vent areas; deep subsurface cores) are obtained from other investigators. Subsequent to isolation, experiments are designed to examine some of the unique biochemical and physiological properties of the organisms in pure culture and, where appropriate, when placed in defined co-culture with other microbes. To accomplish this, students are taught various techniques for cultivation of microorganisms (including the "Hungate technique" for strict anaerobes), as well as various methods for observing and studying the isolated microbes (phase contrast, fluorescence, and DIC microscopy; gas-liquid and high performance liquid chromatography; spectroscopy; PCR methodology and gel electrophoresis; etc.). Such knowledge is then integrated, during the 2nd half of the course, into the pursuit of independent research projects which are developed by the students in consultation with the instructional staff.

B. History and Tradition

The course was originally developed by Holger Jannasch and Harlyn Halvorson at Woods Hole in the early 1970's. Patterned after the famous van Niel course taught at the Hopkins Marine Station in Pacific Grove, California, the course is an intensive six-and-a-half week offering intended to emphasize and explore the tremendous metabolic, morphologic, and behavioral diversity that exists in the microbial world, but which is often overlooked or underemphasized in conventional microbiology courses. Indeed, it is intended that the concept of the incredible and almost unlimited diversity of microbes emerges as a dominant theme.

Traditionally, students in the course have come from all over the world, and have ranged from graduate students to university professors, as well as researchers in government laboratories and private industry. Undergraduates have also been admitted to the course, but they have been few in number. The students are a highly select group that includes microbiologists and individuals for whom a better understanding of microbial diversity is important, eg. marine biologists, biochemists, geneticists, molecular biologists, biotechnologists, etc. It has also been

a tradition that the position of Course Director (or Co-Director) is one that turns over approximately every 5 years. This provides for both a period of continuity and development, as well as fresh perspective and further evolution every 5 years. As a consequence of the excellent and dedicated instructional staff, as well as of the quality of the students, the course has evolved into one of the finest of its type in the world.

C. Training Goals

The training goals of the course are two-fold. The first is to teach students (via lectures, mini-symposia, etc.) about the diversity of organisms that constitute the microbial world. In so doing, it is hoped to demonstrate that the great strength of microbiology lies in the wealth of microbial types that may be exploited for basic and applied research. The second goal is to train students in the classical, as well as newly developed, strategies for enrichment, isolation, and laboratory investigation of diverse microorganisms from Nature, including strict anaerobes and other organisms for which somewhat specialized culture and/or experimental techniques are necessary (eg. obligate syntrophs, symbionts).

II. Course Organization

A. Instructional Staff

The resident instructional staff for 1991 included the course co-directors (Breznak & Dworkin); an invited faculty member (Prof. Hans G. Trüper, Universität Bonn, FRG); a course coordinator (Dr. Pamela R. Contag, Stanford Univ.); 2 postdoctoral teaching assistants (Joseph Calabrese, West Virginia Univ. & Stefan Wagener, Michigan State Univ.); a graduate teaching assistant (Christiane Dahl, Univ. Bonn); and 2 graduate TA trainees (Courtney Frasch and Dan Smith, Univ. Minnesota).

The responsibility of the course co-directors is to define the scope and format of the course, to participate in lecture and laboratory instruction, to recruit other instructional staff and invited lecturers where necessary and appropriate, and to participate in the acquisition of funding for the course. Invited faculty members participate in lecture presentation and laboratory instruction. The course coordinator helps to coordinate the day-to-day activities of the students; supervises teaching assistants and the dishwasher; solves logistical problems associated with field trips; and expedites the acquisition of equipment and supplies. Teaching assistants function primarily in laboratory instruction of students and assuring that necessary materials are prepared, gathered, and/or otherwise made ready for the laboratory portion of the course. The course coordinator and postdoctoral TA's may also present lectures upon invitation by the co-directors. Full names and addresses of the 1991 instructional staff are included in Appendix I.

The non-resident instructional staff includes those individuals who are invited to present one or more guest lectures. These include scientists affiliated with the MBL or WHOI, as well as others who may be available owing to other commitments in the vicinity of Woods Hole. A list of the non-resident instructors and their contribution to the 1991 course is included in the lecture schedule (Appendix II).

B. Instructional Format

The instructional format for the course consists of three principal components: lectures, laboratory experiments, and mini-symposia.

Lectures: 54 Lectures were presented as part of the 1991 course. A list of lecture topics and lecturers is presented in **Appendix II**.

Laboratory: Laboratory exercises are presented in two phases.

The first phase (lasting approximately 2-3 weeks) consists of **formal laboratory experiments** and group rotations to familiarize students with the fundamental techniques of enrichment, isolation and cultivation of microorganisms. After isolation of microbes in pure culture, some of the major biochemical, physiological, and morphological properties of the organisms are examined in experiments in which are also integrated instructions on the use of relevant analytical equipment, eg. gas chromatographs, video microscopes, HPLC, recording spectrophotometer, etc. A list of formal laboratory exercises presented in 1991 is given in **Appendix III**. The purpose of this phase is to provide students with the fundamental knowledge and technical skills that facilitate the execution of their independent research projects.

The second phase (continuing through the remainder of the course) consists of **student independent projects**. In this phase, the initiation, design, and execution of experiments is largely the responsibility of the students, although the instructional staff provides advice on feasibility of experimental design and discusses with the students the interpretation of experimental results. Students are encouraged to initiate two independent projects: one that is relatively easy and has a more-or-less good chance for success in the time available; and one that is more difficult and challenging with perhaps a debatable chance of success. Although some students come to the course with an independent project in mind, the instructional staff also prepares a "menu" of projects of varying degrees of difficulty from which the students may also select. All students are expected to submit a short report of their results, and these reports are bound and kept on file in the course reading room/library for future reference. A list of student independent projects in 1991 (with summaries of results) is given in **Appendix IV**.

Mini-Symposia: The purpose of the mini-symposia is two-fold. The first is to provide participants of the course with new knowledge - knowledge at the "cutting edge" of a particular research area - by experts in the field. The second purpose is to allow students to have extended, personal contact with some of the most distinguished scientists in the world. Through such contact, students acquire a sense of how research areas are developed and advanced, and how successful investigators attack a research problem.

Four mini-symposia were presented as part of the 1991 course. Themes of the mini-symposia were: Microbial Adhesion; Bioremediation; Microbial Positioning; and Biopolymer Degradation. Usually, mini-symposia are held on Saturday mornings and consist of 3 presentations by distinguished researchers in the field. After lunch, presenters are available for informal discussion with students in the laboratory. This is continued, with refreshments, after dinner in an informal atmosphere. Due to

scheduling difficulties, the latter two mini-symposia were presented in one day (am & pm of July 27). A list of mini-symposia, speakers, and lecture titles is presented in Appendix V.

C. Students (Recruitment, Applicant Review, Admission)

The course is normally limited to 20 total students (primarily, but not exclusively, graduate students, postdoctoral fellows, and independent investigators) and restricted to those who will benefit from this unique experience and who are prepared to undertake a rigorous program. The limitation to 20 students is based on past experience and is considered to be optimal for the number of instructors and facilities and supplies available. This year, however, the course also accommodated a Science Writing Fellow (Blake Edgar) for ca. 2/3 of the course, including laboratory experience. The two TA trainees (Frasch and Smith) also participated to some extent as "students", to gain experience, as well as TA's.

Applicants are reviewed by an admissions committee composed of two or more scientists, selected by the MBL, and which conduct a preliminary screening, followed by the course co-directors which make a final recommendation for admission. The composition of admissions committees must be approved by the Director of the MBL. A working member of the admissions committee must disqualify himself/herself from voting on any application submitted by a student or member of his/her own department, or of any other department of the same institution with which he/she has working relationships during the academic year. Students are admitted to the microbiology course on the basis of their academic records (eg. grade point average and GRE scores for graduate students), demonstrated research ability and originality, recommendations, and stated career goals. For some applicants (eg. college or university faculty members, or Ph.D.-level researchers in industry) a *curriculum vitae* may be submitted in lieu of academic transcripts and GRE scores. Students are advised that the required two letters of recommendation accompanying each application should not come from faculty members of the microbiology course.

The MBL has an active program for recruitment and support of underrepresented minorities in science and these applicants are identified during the application procedure. All applications and advertisements state clearly that "the MBL is an EEO Affirmative Action Institution". Moreover, one fellowship for a woman or other underrepresented minority, is made available from the outreach/education program of the NSF-Sponsored Center for Microbial Ecology at Michigan State University of, which one of the course co-directors (Breznak) is a member.

A list of students who participated in the 1991 course is presented in Appendix VI.

D. Contact Hours

A typical day consists of 2 x 1h lectures (with time for special announcements and a short break) in the morning followed by laboratory work in the afternoon and into the evening. This is modified on days when mini-symposia are held, and on Sundays when no formal lectures are given. In the second half of the course, lectures begin to taper off as more effort is

devoted to independent research projects. As all instructional staff are generally present during lectures and are available during laboratory work, the contact hours for the instructional staff amount to about 60-70 hours per week per staff member.

E. Other Attendees

Since course lecture schedules are posted in the MBL, anyone in the Woods Hole scientific community may attend the lectures. Those individuals who do attend are usually investigators or students affiliated with the MBL or WHOI, although no sign-up sheet is circulated and no record is kept. Nevertheless, casual observations and recollections suggest that perhaps 1-4 individuals not formally affiliated with the course attend lectures.

III. Description of the Course

A. Lectures: As stated above, the intended function of lectures is to educate students in microbial diversity. Accordingly, lectures seek to provide students with the most up-to-date information on the diversity of morphologic, physiologic, metabolic, behavioral and developmental properties of a wide variety of organisms representative of the microbial world. Lectures also underscore the importance of microbes in various ecological habitats and indicate why certain microbial activities (eg. photosynthesis, anaerobic fermentations, inorganic oxidations and reductions, etc.) are absolutely essential for continued life in our biosphere. Notwithstanding our current knowledge of microbial diversity, a particular effort is made to emphasize that perhaps only 10-15% of the microbial world is currently represented in culture and understood, and that Nature holds a vast treasure of organisms from which we still have much to learn. Therefore, an additional function of lectures is to indicate how various, diverse microbes can serve as important models to answer fundamental questions in biology (eg. sensory phenomena; mechanisms of cell differentiation) and to solve practical problems (eg. detoxification of environmental pollutants; conversion of biomass to food, fuels, or chemical feedstocks).

B. Laboratory: The organization and intended function of laboratories is presented above in item II.B. A list of laboratory exercises and independent projects is presented in Appendix III and IV, respectively.

Appendix I. Instructional Staff

Dr. John A. Breznak, Co-Director	}	Department of Microbiology Michigan State University East Lansing, Michigan 48824-1101
Stefan Wagener, Postdoctoral TA		
Dr. Martin Dworkin, Co-Director	}	Department of Microbiology Medical School Box 196 UMHC University of Minnesota Minneapolis, MN 55455-0312
S. Courtney Frasch, Graduate TA Trainee		
Dan Smith, Graduate TA Trainee		
Dr. Hans G. Trüper, Invited Faculty	}	Institut für Mikrobiologie & Biotechnologie Rheinische Friedrich-Wilhelms-Universität Meckenheimer Allee 168 D-5300 Bonn 1 Federal Republic of Germany
Christiane Dahl, Graduate TA		
Dr. Pamela R. Contag, Course Coordinator		Dept. of Microbiology & Immunology Stanford University School of Medicine Stanford, California 94305-5402
Dr. Joseph Calabrese		Dept. of Plant Pathology & Environmental Microbiology West Virginia University Morgantown, WV 26506-6057

Appendix II. Lecture Schedule for 1991 MBL Summer Course

MICROBIAL DIVERSITY

[Unless indicated otherwise, lectures were held in Loeb 208 at 8:30 am and 10:00 am, with a break period from 9:30-10:00.]

<u>Lect.</u>	<u>Topic</u>	<u>Lecturer</u>
1	Introduction/Theory of Enrichment Culture	Dworkin
2	Nutrition & Cultivation of Microbes	Breznak
3 (2:00 pm)	Ecology of the Great Sippewissett Salt Marsh	Goehringer*
4 (7:30 pm)	Microscopy	Wagener
5&6 (Candle House)	Introduction to Woods Hole; & Deep Sea Hydrothermal Vent Communities [with afternoon tour of the <i>Oceanus</i> (tentative)]	Jannasch*
7&8	Anaerobic Microbial Food Webs: Fermenters	Breznak
9	Natures Mixed Cultures: Biochemical & Genetic Interactions	Dworkin
10	Microbial Motility & Tactic Phenomena	Dworkin
11&12 (Candle House)	Microbial Evolution & Phylogeny	Woese*
13	Sulfur-Oxidizing Symbionts of Marine Invertebrates	Cavanaugh*
14 (10:30 am; Candle House)	Origins of Life (Joint with the Parasitology Course)	Sogin*
15	Anaerobic Food Webs (cont.): Methanogens	Breznak
16	: Sulfate & Sulfur-Reducing Anaerobes	Breznak
17	: Acetogens	Breznak
18	: Obligate & Facultative Syntrophs	Breznak
19&20	Oxygenic Phototrophs: Cyanobacteria	Waterbury*
21&22	Anoxygenic Phototrophs I & II	Trüper
23	Optical Trapping in Microbiology & Cell Biology (Joint with the Physiology Course)	Block*
(Candle House)	Mini-Symposium: Adhesion	Invited Speakers
24	Anoxygenic Phototrophs III	Trüper
25	Relationship of Cell Envelope Components to Gliding Motility in <i>Cytophaga</i>	Leadbetter*
26&27	Basic Genetics & Molecular Biology I & II	Contag
28&29	Prokaryotic Development (Series includes General Principles; Endospores; <i>Caulobacter</i> ; <i>Anabaena</i> ;	Dworkin

Myxobacteria; and the Actinomycetes)

[4th of July Class Picnic]

30-32	Prokaryotic Development (cont.)	Dworkin
33	<i>Bdellovibrio</i>	Dworkin
34	Cellulose Structure, Biosynthesis, and Degradation	Breznak
35	Symbiotic Cellulose Digestion: Ruminants <i>et al.</i>	Breznak
36&37	Luminescent Bacteria: Marine I & II	Dunlap*
38	Luminescent Bacteria: Terrestrial (<i>Xenorhabdus</i>)	Contag
39&40	Autotrophy and Autotrophs I & II	Trüper
41	Microbial Reduction of Fe & Mn	Calabrese
42	Extreme Environments & Extremophiles	Trüper
43&44	Halophily and Halophiles I & II	Trüper
45	Hyperthermophiles	Trüper
46	<i>Rhizobium</i> and <i>Azotobacter</i>	Dworkin
47	N ₂ Fixation (Biochemistry)	Breznak
48-50	Physiological Ecology of Phototrophic Bacteria I-III	van Gemerden*
(Candle House) Mini-Symposium: Microbial Bioremediation		Invited Speakers
51	Spirochetes	Breznak
52	Eukaryotic-Prokaryotic Motility Symbioses	Tamm*
53	Flagellar Motors of Bacteria	Khan*
54	Anaerobic Protozoa	Wagener
(Candle House) Mini-Symposia: (am) Microbial Positioning Mechanisms; (pm) Biopolymer Decomposition		Invited Speakers

*Guest lecture by non-resident instructor of the following affiliation:

- P. Dunlap, D. Goehringer, H. Jannasch and J. Waterbury: WHOI
- S. Khan, M. Sogin and S. Tamm: MBL
- S. Block: Rowland Institute, Worcester, MA.
- E. R. Leadbetter: U. Conn
- C. Cavanaugh: Harvard Univ.
- C. Woese: Univ. of Illinois
- H. van Gemerden: Univ. of Groningen, Wageningen, Netherlands

Appendix III. Laboratory Exercises for the 1991 MBL Summer Course

MICROBIAL DIVERSITY

[Note: Instructor(s) primarily responsible for the exercise is given in parentheses]

<u>Date</u>	<u>Laboratory Exercise</u>
6/17	1:00 pm Principles & Purposes of the Laboratory Exercises (Dworkin/Breznak) 2:00 pm Ecology of the Great Sippewissett Salt Marsh (Goehringer) 3:00 pm Field Trip to the Great Sippewissett Salt Marsh 7:30 pm Microscopy Lecture and Lab Rotations on Microscopes (Wagener)
6/18	Tour of the <i>Oceanus</i> Research Vessel (Jannasch) Introduction to Anaerobic Techniques (Breznak) Enrichment & Isolation of Lactic Acid Bacteria and Clostridia (Breznak) (eve) The "Volta Experiment" (all)
6/19	Enrichment & Isolation of Methanogens and CO ₂ -Reducing Acetogens (Breznak/Wagener)
6/20	Enrichment & Isolation of Sulfate and Sulfur-Reducers (Trüper/Dahl)
6/21	Enrichment & Isolation of Sulfur and Nonsulfur Phototrophs (Trüper/Dahl)
6/24	Enrichment & Isolation of <i>Beggiatoa</i> and <i>Gallionella</i> (Dworkin/Calabrese) Operation of Anaerobic Glove Box (Breznak/Wagener)
6/25	Enrichment & Isolation of <i>Caulobacter</i> and Myxobacteria (Dworkin/Calabrese/Frasch/Smith) Introduction to Analytical Techniques (Breznak/Wagener/Calabrese)
6/26	Flex Day - Examine experiments in progress; transfer cultures as necessary; make relevant observations and measurements (all)
6/27	Enrichment & Isolation of Cyanobacteria (Waterbury)
6/28	Isolation of Spirochetes (Breznak/Wagener) Examination of Termite Hindgut Microbial Community (Breznak/Wagener) Video Microscopy (Wagener)
7/1	Magnetotactic Bacteria (Dworkin/Calabrese) Isolation of <i>Bdellovibrio</i> (Dworkin/Calabrese)
7/2	Constructing Phylogenetic Trees from Nucleotide Sequences (Sogin) (eve) Discussion of Student Independent Projects (all)
7/3	N ₂ -Fixing Bacteria: <i>Azotobacter</i> & <i>Rhizobium</i> (Dworkin/Calabrese) Spectroscopy Rotation (Trüper/Dahl)
7/5	Flex Day (all) Students Clarify, Revise, and Initiate Independent Projects (all)

- 7/8 Isolation of Marine Luminescent Bacteria (Dunlap)
Isolation of *Xenorhabdus* (Contag)
- 7/9-29 Students Finish Formal Laboratory Enrichments & Isolations and
Independent Projects (all)
- 7/24 Use of Fluorescent Oligonucleotide Probes for Examining Microbial
Communities (Wallner/DeLong)
- 7/30-31 Student Presentations of Results of Independent Projects (all)

Appendix IV. Student Independent Projects (List followed by abstracts; complete reports on file in course library/reading room).

Boldt, Y.

- Isolation of aromatic degrading *Arthrobacter* sp.
- Characteristics of H_2/CO_2 acetogenesis by the hindgut microbiota of *Cryptotermes cavifrons* termites (with G. Karsten).
- SEM of "*Rubberneckia*" (a devescovinid protozoan from the hindgut of *C. cavifrons*) and its episybiotic bacteria (with G. Karsten).

Breitung, J.

- *Prosthecochloris*, a green sulfur bacterium isolated from a Winogradsky column.
- CO-oxidizing sulfate-reducing bacteria.
- Studies on a methanesulfonate-degrading, methano- and sulfuridogenic enrichment culture.
- Attempted isolation of an anaerobic methane-oxidizing bacterium.
- Anaerobic respiration of luminescent bacteria with trimethylamine-N-oxide as electron acceptor (with M. Kern).

Budavari, A. (with M. Larsen)

- Enrichment of novel phototrophs.
- Enrichment of spore-forming phototrophs.
- Comparative study of gliding motility by reflective interference microscopy.
- Enrichment for biopolymer-degrading sulfate-reducing bacteria.
- Acidophilic methanogens from Cedar Swamp.

Caspi, R.

- Attempted isolation of Fe & Mn-reducing bacteria.

Edgar, B.

- Attempted sequencing of 16S rRNA of the bacterial episybiont of *Kentrophorus* (with Mohanraju, R., below).

Fent, K.

- Isolation of organotin-degrading bacteria under aerobic and anaerobic conditions.

Hsing, W.

- Attempted isolation of spirochetes and cellulolytic bacteria from termite guts.
- Isolation of long filamentous gliding bacteria.

Jäger, H.

- Isolation of acid-tolerant myxobacteria.
- Analysis of red photosynthetic "berries" (with C. Frasch).

Johnson, Mark D.

- Isolation of cyanobacteria from green, photosynthetic "berries".
- Estimation of the contribution of cyanobacterial N_2 fixation to N economy of green photosynthetic berries.
- Enrichment and isolation of autotrophic sulfate-reducing bacteria.

Karsten, G. R.

- Studies on "*Rubberneckia*" and acetogenesis in *C. cavifrons* (with Y. Boldt).

Kern, M.

- Enrichment & isolation of *Heliobacteriaceae*.
- Anaerobic growth of bioluminescent bacteria by TMAO respiration (with J. Breitung).

Larsen, M. (see Budavari, above).

Li, R.

- Isolation and preliminary characterization of cyanophages.

Lin, C.

- Attempted isolation of a chitin-degrading, sulfate-reducing bacterium from salt marsh sediments.

Mohanraju, R.

- Isolation of methanogenic bacteria from cedar swamp sediments.
- Attempted sequencing of 16S rRNA from the bacterial episymbiont of *Kentrophorus* ciliates (with Edgar, B., above).

Ostroumov, S. A.

- Effects of a non-ionic surfactant on marine cyanobacteria.
- Improvement of the method of enrichment of dissimilatory sulfur-reducing bacteria.

Pitta, T.

- Sulfonate-reducing bacteria.

Schnell, S. S.

- Phototrophic Fe⁺²-oxidizing bacteria.
- Fe & Mn-reducing, aromatic compound-oxidizing bacteria.
- Isolation of aerobic thermophilic spore-forming bacteria.

Slater, S. C.

- Attempted isolation of bacteriophage for diverse bacteria.
- Transposon mutagenesis of *Xenorhabdus luminescens*.

Tratnyek, P. G.

- Enrichments with a core sampler from a surfactant contaminated aquifer.

Wallner, G. A.

- Characterization of magnetotactic bacteria.
- Fluorescence spectroscopy of phototrophic bacteria.

Appendix V. Mini-Symposia (topics & speakers)

I. Mechanisms and Consequences of Microbial Adhesion

- Mechanisms: Madilyn Fletcher, Univ. of Maryland
- Biofloculants and dispersants: Eugene Rosenberg, Tel Aviv University
- Lateral flagella synthesis in *Vibrio*: Linda McCarter, Agouron Inst.

II. Prospects and Potential for Microbial Bioremediation

- Transformations by aquifer microbes: Joseph Suflita, Univ. of Oklahoma
- Enzymology: Larry Wackett, Grey Freshwater Inst. (U. Minn.)
- Principles and prospects: Lily Young, NYU

III. Microbial Positioning Mechanisms

- Chemotaxis in *E. coli*: Howard Berg, Harvard Univ.
- Magnetic positioning: Dick Blakemore, Univ. of New Hampshire
- Positioning by gas vesicles: Tony Walsby, Univ. of Bristol, UK

IV. Biopolymer Degradation

- Cellulose degradation by anaerobic bacteria: E. Canale-Parola, U. Mass.
- Biochemistry & genetics of xylan degradation: R. Hespell, USDA, Peoria
- Lignin degradation by white rot fungi: C. A. Reddy, Michigan State Univ.

14-CO₂ Fixation to Acetate in the *Cryptotermes cavifrons* Hindgut.

H₂ dependent CO₂ fixation in the hindgut of *C. cavifrons* was studied using a 14-CO₂ fixation assay. Four hindgut equivalents were incubated for 5 hours with 14-CO₂ under H₂. Control vials were incubated under Argon. H₂ dependent 14-CO₂ fixation to soluble products was determined as follows:

$$\text{H}_2 \text{ dependent cpm} = \text{mean H}_2 \text{ cpm} - \text{mean Ar cpm}$$

The hindguts of three *C. cavifrons* test groups were assayed. In the first group, termites were obtained directly from wood. In the second group, termites were fed antibiotics for 5 days prior to the assay. In the third group, termites were incubated under 15% H₂.

Mean H₂ cpm for Group 1 was 65,300 while mean Ar cpm was only 4,400. This demonstrates that significant H₂ dependent 14-CO₂ fixation occurred. In Group 2, the antibiotic fed termites, H₂ dependent cpm were decreased to insignificant levels. Incubation under 15% H₂ had no effect on 14-CO₂ fixation levels suggesting that CO₂ fixation in the *C. cavifrons* hindgut is not H₂ limited.

SEM of the *Cryptotermes cavifrons* hindgut protozoa "Rubberneckia".

Pools of Rubberneckia were collected from *C. cavifrons* hindguts using micropipettes under a dissecting scope. Two fixation attempts were made. The first fixation procedure included a 30 minute incubation in 2% glutaraldehyde at room temperature. This was followed by a second fixation in 1% OsO₄ for 30 minutes at room temperature. SEM micrographs revealed well shaped cells with filamentous material covering the surface of Rubberneckia. The flagellated rods and fusiform bacteria known to cover the surface of Rubberneckia were not clearly visible except on the axostyle, where the fusiform bacteria could be seen. In an attempt to improve the fixation, a modified fixation protocol was followed. Modifications included a 1 hour incubation on ice in a solution containing both 2% glutaraldehyde and 1% OsO₄. Fixation of Rubberneckia improved significantly, with both the flagellated rods and fusiforms becoming visible. The filamentous material observed in the first fixation was revealed to be bacterial flagella of the rod shaped episymbionts.

ABSTRACTS of independent projects by Juergen Breitung

Microbial Diversity Summer Course 1991

A) Green phototrophic bacteria isolated from a Winogradsky column

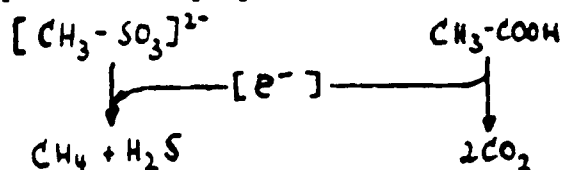
A traditional Winogradsky column was set up containing mud from the Sippewissett marsh and seawater. After three to four weeks of incubation a bloom of green phototrophic bacteria was observed. In order to get a pure culture of these bacteria, agar dilution shakes were made from which after one and a half week green colonies were picked and transferred in a liquid medium. Three pure cultures of *Prosthecochloris* were obtained which was the most abundant organism in the column.

B) Carbonmonoxide oxidizing sulfate reducing bacteria

Taking advantage of lactate oxidizing sulfate reducer enrichments of the course it was possible to highly enrich a carbonmonoxide oxidizing sulfate reducer which uses this compound as electron donor and acetate as carbon source. The bacteria were vibrioshaped and it is postulated that a *Desulfovibrio* species carries out the oxidation of CO to CO₂ and uses the electrons to reduce sulfate to H₂S.

C) Methane sulfonic acids (MSA) oxidizing or reducing anaerobic bacteria

Anaerobic enrichments on this compound showed that a MSA reduction to CH₄ and H₂S occurs only in the presence of acetate. The following working hypothesis is proposed:



Whether the reactions are carried out by one organism or whether it is an interspecies hydrogen transfer should be worked out in the future.

D) Enrichment of an anaerobic methane oxidizing bacterium

Several freshwater samples were checked in anaerobic enrichments for the ability to oxidize CH₄ to CO₂ with either NO₃⁻ or Fe³⁺ as electron acceptors. Thermodynamically the overall reactions are exergonic. No methane consumption could be detected under these conditions over a period of four weeks.

Abstract: the isolation of Mn and Fe reducing bacteria

Primary Project: Isolation of iron and manganese reducing bacteria, which use acetate, phenol or benzoate as carbon source, and iron and/or manganese oxides as electron acceptors.

Procedure

Two different approaches were used to detect reduction:

- a. Growing the cultures in soft agar containing a suspension of metal oxide particles, and observing the clearance of the suspension.
- b. Growing the enrichments in liquid media, and measuring the concentration of the different species of the metals by spectrophotometric methods.

Soft Agars

50 ml serum bottles were gassed with N_2/CO_2 , and inoculated with ~15 ml of mud. The mud was mixed with 1% agar media and let cool until solidified. The rest of the space was filled with 0.75% agar media containing 2mM Mn or Fe oxides. Acetate was added as a carbon source. Clear zones were detected in the agar after 2 to 5 days. Small aliquots from the clear zones were transferred to agar tubes containing the same medium with 0.75% agar. After several days different morphologies of clear zones appeared in the tubes.

Liquid Media

50 ml serum bottles were gassed with N_2/CO_2 , filled with anaerobic media under N_2 , and inoculated with ~15 ml of mud. Carbon sources were acetate, phenol, or benzoate at the respective concentrations of 15mM, 1mM and 2mM. Mn and Fe oxides were added as the sole electron acceptor. Subsamples were taken every few days, and analyzed for concentrations of Mn^{2+} , Fe^{2+} , and total iron. Fe^{3+} was calculated as total iron - Fe^{2+} . When growth was observed, samples were transferred to agar tubes. As a control, mud samples were autoclaved, and used to inoculate bottles under the same conditions.

Plates

Colonies were transferred onto agar plates, which contained a top layer of soft agar with 2 mM Mn oxides.

The plates were incubated in the anaerobic chamber, and after 4 days colonies began to appear, with clear zones around few of them. The bacteria that formed these colonies were very small non-motile short rods.

An interesting phenomena was the presence of large (~5 um) bone shaped highly refractile objects in many of the plates. It was not clear if these objects were living organisms or crystals of some source. Acridine orange staining caused the objects to fluoresce. Probing the objects with fluorescent probes for archaea, eukarya and eubacteria gave negative results. I assume that these are rodochrosite ($MnCO_3$) crystals.

ISOLATION OF ORGANOTIN DEGRADING BACTERIA UNDER AEROBIC AND ANAEROBIC CONDITIONS

Karl Fent

EAWAG, CH-6047 Kastanienbaum, Switzerland
MBL Summer Course, Microbial Diversity, 1991

Tributyltin (TBT) is used as a biocide in antifouling paints on ship hulls and in the materials protection, and triphenyltin (TPT) is employed as a pesticide in agriculture. In anoxic sediments TBT is preserved for long periods of time, whereas TPT may undergo degradation. In this study, an attempt has been made to isolate microorganisms under aerobic and anaerobic conditions able to use these organotin compounds as the sole source of carbon and energy.

Surface sediment samples from Eel Pond were incubated under aerobic conditions in presence or absence of about 1 mM TBT chloride and 1 mM TPT chloride. Two liquid enrichments were made, and microorganisms from the first enrichment were streaked out on agar plates containing either no organic carbon source (controls), or about 1 mM of TBT or TPT as the carbon source. In the original flasks with sediment, in the enrichment cultures, and on agar plates a slow growth of fast moving rods, motile spirilli, non-motile rods, bent rods, and cocci were identified in the presence of TPT. In presence of TBT, motile and non-motile rods and non-motile cocci were observed. On the agar plates, small grey and transparent colonies developed in the presence of TPT and TBT, whereas in controls they were only very small, transparent, and slow growing. Non-motile small cocci and rods were identified in control plates. Streaking out of single colonies yielded very small new colonies after two days of incubation in presence of TBT and TPT, but not in controls.

Attempts to isolate sulfate reducing bacteria under anaerobic conditions from Eel Pond and Quissett harbor sediments able to use TPT and TBT as a sole carbon source were unsuccessful.

This experiment indicates that the colonies in the aerobic enrichment may be able to use TBT and TPT as a sole source of carbon and energy. Further isolation steps must be undertaken to isolate the different species and to assess their ability to growth on organotins. Only chemical analysis can prove, however, if true TPT and TBT degrading bacteria have been enriched.

Heike Jager
Courtney Frasch
Microbial Diversity
Woods Hole, 1991

Abstract: Attempts to characterize the "red berries"

Red aggregates, referred to as berries, were collected from tidal pools from the Great Sippewissett Salt Marsh. We were interested in determining the major component of the red berries as well as the other organisms present and the organization of the berries. To analyze the composition and structure of the berries we used the following methods:

- 1) Agar shakes of disrupted berries in chromatiaceae medium and sulfate reducer medium
- 2) Spectral analysis of pigments and sulfur
- 3) Use of fluorescent labeled oligonucleotides directed against 16s rRNA to probe whole berries
- 4) Transmission Electron Microscopy of whole berries

Results

We observed only slight growth in agar shakes. This could be due to slow growth rate, unfavorable conditions or nonviable cells. Spectral analysis of pigments and sulfur extracted with MeOH from whole berries revealed an absorption of 263 nm, which is characteristic of sulfur, and 772 nm which indicates presence of bacteriochlorophyll a. We also observed a weak reaction with sulfate reducer 16s rRNA oligonucleotide probes suggesting the presence of sulfate reducers in the berries. TEM studies on whole fixed berries revealed the presence of at least two types of organisms 1) oval-shaped rod and 2) long rods in a ratio of 1 rod to 4 oval-shaped rods. Dead cells also occurred in a ratio of 20:1. Both organisms have a typical Gram negative membrane. The oval-shaped rods have a large slime layer surrounding the cell and sulfur globules are also apparent within the cell.

COLONIAL CYANOBACTERIAL "BERRIES" FROM SALT MARSH POOLS

MARK D. JOHNSON

The Great Sippewissett Salt Marsh bordering Buzzards Bay on Cape Cod (Mass) contains a colonial form of cyanobacteria "berries" in and on the surface layers of the bethos. The berries are composed of mucilage and cyanobacterial cells which are usually crusted with diatoms. The mucilage is extremely viscous and foiled all attempts made so far to isolate the cyanobacteria into axenic cultures. Attempts included the use of lysozyme, homogenization, dark incubation followed by phenol killing of heterotrophs as per Carmichael and Gorham (1974). Various media were tried. All to no avail. Photospectrometric analysis showed all 6 individual berries sampled to be of the same organism. Confocal laser microscopy was used to try and determine if non-cyanobacterial organisms co-existed internally with the dominant organisms.

Nitrogen fixation was checked for over an 8 hour sampling period with separate treatments for constant light, constant darkness, switching from light to dark and dark to light. Under none of these conditions was nitrogen fixation detected by gas chromatography, using the acetylene reduction method.

The presumed existence of gas vesicles in the berry cells (shown in TEM micrographs of last year) was disproven using one of Anthony Walsby's pressure cells.

Aside from various tests possible on berry cells in pure culture, worthwhile future experiments could include nitrogen starvation stimulation to turn on the dinitrogenase complex and PCR and sequencing from extracted cells (where contaminants would be a minor problem).

PEFS

Carmichael, W. W. and P. R. Gorham. 1974. An improved methods for obtaining axenic clones of planktonic blue-green algae. *J. Phycol.* 10:236-240.

Scott, W. E. 1974. The isolation of *Microcystis*. *So. African J. of Sci.* 70:179.

Shirai, M. et al. 1989. Development of a solid medium for growth and isolation of axenic *Microcystis* strains. *App. Env. Micro.* 55:2569-2571.

Waterbury, J. Cyanobacteria chapter in The Prokaryotes.

Microbial Diversity course
MBL, Woods Hole 1991
Abstracts of independent projects

Monika Kern

Enrichment and Isolation of Heliobacteriaceae

Members of the family Heliobacteriaceae are anoxygenic phototrophic bacteria which can be isolated from dry surface soils. They are abundant in paddy soils and contain bacteriochlorophyll *g* with an in vivo absorption maximum at 790 nm. Heliobacterium can form endospores which are resistant to high temperatures and dryness. They are also adapted to high light intensities. To enrich for Heliobacteria dry surface soil and freshwater samples were taken. Different carbon sources (pyruvate, acetate, malate, lactate) and nitrogen sources (ammonia or dinitrogen) were used. Enrichments were inoculated with pasteurized and non pasteurized samples and incubated at 35 C and 8000 lux light intensity. No growth of Heliobacterium could be observed in any of the enrichments. From non pasteurized garden soil a good enrichment of Rhodomicrobium vannielii could be obtained. The ability of Rhodomicrobium vannielii to form endospore-like cysts may explain its survival in dry soil.

Monika Kern/Juergen Breitung

Anaerobic growth of bioluminescent bacteria with trimethylamine N-oxide as a terminal electron acceptor: TMAO respiration

TMAO and TMA (trimethylamine) are waste products of protein metabolism. Both compounds are abundant in marine environments. The ability of several newly isolated bioluminescent bacteria and Vibrio fischerii as well as Rhodobacterium leiognathi to use TMAO as a terminal electron acceptor during anaerobic growth was investigated. Anaerobic growth of V. fischerii, R. leiognathi and four isolates could be enhanced by TMAO. Growth was accompanied by an increase in pH, which was probably caused by the production of TMA. Like for example in E. coli the TMAO reducing activity of bioluminescent bacteria seems to be dependent on molybdenum. Tungsten, which is known to inhibit Mo-transport and incorporation of Mo in proteins competitively, inhibited TMAO reduction by bioluminescent bacteria.

Isolation and preliminary characterization of cyanophages

Introduction

Phages have been found for many species of cyanobacteria. They are phages of different shape, size and host specificity. Recent studies on cyanophages of marine *Synechococcus* suggested that the phages are important in regulating the dynamics of bacterial populations although they are unimportant in determining the host cell abundances. In order to have better understanding of cyanophages, especially for the later study of their genetics, several unicellular species of cyanobacteria were tested for phage infection and preliminary characterization of the infecting phages was carried out by electron microscopy.

Materials and Methods

Host cells used were: *Synechococcus* PCC7942 (fresh water stain), *S.* PCC8015, *S.* PCC8016, *Synechocystis* PCC7335, *S.* PCC7336, *S.* PCC6906 and a new isolate from Florida WH8901. The cells were collected in log phase by spinning at 5,000 rpm for 10 min and the pellet was resuspended in 5 ml of medium.

Phage samples used were either fresh water from ice house pond for *Synechococcus* PCC7942 or sea water from the dock across the street of Redfield building for the marine species. 30 ml of water samples were centrifuged at 6,500 rpm for 15 min and the upper 4 ml were used for the infection.

The host cells and phages from different serial dilutions were mixed in microtiter plate wells for 2 hrs for adsorption. One ml of growth medium was then added to each well and the plates were incubated at room temperature under light. Lysed cells were observed after 5-10 days of infection and samples from both lysed wells and control wells were taken for negative staining (1% uranyl acetate) for electron microscopy. A second infection was also set up for further isolation and identification of the phages.

Results and Discussion

Besides *Synechocystis* PCC7335 and WH8901, lysed wells were found in all five strains of cyanobacteria tested. Samples from only 3 species were prepared for EM study because of the time limit, and phages were found in 2 of them. The phages for *Synechocystis* PCC6906 were tail-less, small and polyhedral (belonging to Podoviridae), and phages found in *Synechococcus* PCC8015 have both tail-less and long noncontractile tailed types (Styloviridae). It seems that at least during this time of the year, cyanophage titres of Woods Hole water samples are relatively high.

Conclusions

For the first time phages were found in the cyanobacterium *Synechocystis*. Cyanophages found in *Synechocystis* PCC6906 are small Podoviridae and phages infecting *Synechococcus* PCC8015 are Podoviridae and Styloviridae.

Isolation and Characterization of *Vibrio alginolyticus*

Chuzhao Lin

The goal of my project was to isolate chitin degrading sulfate reducers from Sippewissett marsh. The fact that chitin is one of the most abundant macromolecules in this relatively high sulfate environment justified the feasibility of this project, although it was reported that no such organisms existed.

Three strategies were employed: 1). sulfate reducers were enriched first followed by the isolation of chitin utilizing bacteria from the enrichment culture; 2). chitin degrading bacteria were enriched and their sulfate reducing ability tested; 3). using N-acetylglucosamine (GluNac, monomer of chitin) as sole carbon source for isolation of sulfate reducer. No bacteria were isolated from the first two methods. And only one type of colony was observed from the GluNac sulfate reducer agar shakes. Through series of agar shake dilution, a pure culture was obtained.

The isolated bacteria had following properties:

G(-), oxidase (+), catalase (+) and it is a facultative anaerobe, rod shape motile and produces no H₂S. On solid agar surface, cell swarmed. Among the substrates tested, glucose, acetate, lactate, succinate, citrate, sucrose, fructose, maltose, glycerol, ethanol, urea, lysine, alanine supported the growth, while lactose, adonitol, arabinose, sorbitose, sorbitol, cellobiose, orthinine, tartrate did not. The major end product from glucose is formate, lactate, acetate and other minor components. Thus the bacterium is a *Vibrio*. It's ability to use sucrose but not cellobiose made it a *V. alginolyticus*. It is reported that *V. alginolyticus* can also be isolated from seafood, estuarine, neritic, and brackish water.

Although no chitin degrading sulfate reducer were found, *V. alginolyticus* was successfully isolated. The appearance of only one type of colony in the very first agar shake dilution series suggested that N-acetylglucosamine was a very selective substrate for *V. alginolyticus* and other vibrios in general.

ABSTRACT

Isolation of Methanogenic bacteria from the sediments of Cedar swamp

Primary Project: Isolation of methanogenic bacteria from the sediments of an acidophilic swamp with H_2 CO_2 as the substrate.

Methodology: The anerobic techniques as outlined in the manual was followed with some slight modification in media preparations. Enrichments were made from the Cedar swamp sediments by using different substrates like H_2 CO_2 , Formate, Acetate and Methanol. Enrichment with H_2 CO_2 was selected on the basis of methane production. The medium composition is as per the manual with the only addition of streptomycin to a final concentration of 2.5ug/ml so as to get rid of other contaminants. The Roux bottles as described by Hermann et.al.(1985) was used . Streking was carried out inside the glove bag and the headspace of the bottles were flushed and filled with H_2 CO_2 and incubated at 30 C in an upright position. Pale yellow colonies showed their appearance, these colonies were picked observed for fluorescence. These colonies were then restreaked onto fresh plates. The headspace of the bottles were analysed for the presence of methane. Microscopic observation of the cells showed long rods which were motile. Pure colonies were then transformed into liquid cultures for growth and characterization is to be carried out.

Tom P. HA

Sulfonate Reducing Bacteria

Enrichment culture for sulfonate Reducing Bacteria was set up using the sulfate Reducing medium with all sulfate deleted. Taurine, Isethionate and cysteate were provided (30mM) as an electron acceptor and lactate, Ethanol and acetate were provided as an oxidizable substrate and carbon source. 100 ml of school street marsh water was added as inoculum and the enrichments were incubated under anaerobic conditions at 28°C ~~at~~ in the dark. 3 of the enrichments showed growth on Isethionate + Lactate, Isethionate + Ethanol, and Taurine and Lactate. Both Isethionate enrichments produced H₂S indicating the sulfonate sulfur was being reduced. The Taurine enrichments did not produce H₂S ~~and~~ indicating ~~the~~ there were no sulfonate Reducers present but rather ~~the~~ Lactate ~~the~~ Fermenters were present. The Isethionate + Lactate enrichment used the lactate and produced acetate as determined by HPLC analysis. H₂S production was monitored by the method of Elime. The utilization of Isethionate could not be measured by HPLC because it co-migrated with some unknown compound in the medium. No pure cultures have been isolated at this point.

Abstract

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home address: Tsurjupy street 7-2-31, Moscow 117418).

Effects of non-ionogenic surfactant on marine cyanobacteria

Marine cyanobacteria were cultivated in axenic flask cultures with and without additions of non-ionogenic surfactant Triton X-100 (TX100). Two strains of *Synechococcus* have been used, No. 7805 (non-motile) and No.8103 (with unusual type of motility discovered by J.Waterbury in 1985). The strains studied have different sensitivity to TX100. The strain 7805 was more sensitive than the strain 8103. The surfactant in concentration 5 mg/l inhibited the growth of the strain 7805, but not the strain 8103. Thus, optical density of the culture of the strain 7805 at 680 nm (5 mg/l TX100) was only 21.2% of optical density of the control culture. Optical density in the absorption minimum of the spectrum (where contribution of turbidity is maximalized, and those of pigments is minimal) also was about 20% of control. TX100 (5 mg/l) did not damage the motility of the strain 8103. Low concentration of TX100 (0.5 mg/l) stimulated the growth of the both strains. The strain 8103 had higher cell density also in the presence of higher concentration of TX100 (5 mg/l). In conclusion, first evidence of surfactant toxicity for marine cyanobacteria was obtained and differential sensitivity of the two strains was demonstrated.

Abstract No.2. Improvement of the methods of enrichment of dissimilatory sulfur-reducing bacteria.

In order to make sulfur more available for the bacteria, the following mixture for sulfur suspension preparation is proposed in this study: per 15 ml of cultivation medium, add 0.5 g sulfur. after this (order of additions is important) add 0.325 ml of Triton X-100 (stock solution 250 mg/l), mercaptoethanol 33mM, cystine 15 mg. ;sonication must be conducted under argon. After this the suspension must be autoclaved which increase amount of S in colloidal form.

Acknowledgements. The author is grateful to Prof. M.Dworkin, Prof.J.Breznak, Prof.H.Trueper, Prof.J.Waterbury for consultations and advices, all TA and students of the course for help and creative atmosphere.

1. Phage Isolation. Attempts were made to isolate bacteriophage capable of growth on *Xenorhabdus luminescens*, *Rhodospirillum rubrum*, *Clostridium mysambii*, two newly isolated *Clostridium* species, and three newly isolated luminescent *Vibrio* species. The inocula were homogenized, centrifuged in a microfuge to remove particulate matter, and filtered through a 0.45 micron filter prior to dilution and plating. In general, inocula were initially added to log-phase cultures of each test organism and grown for 1-2 days in an attempt to amplify phage originally present at low titer. The samples screened for phage capable of infecting test organisms were sea water from garbage beach, fresh water from numerous small ponds in the Woods Hole area, and soil samples. The *Clostridium* species were additionally inoculated with gut extracts of the North American termites *Cryptotermes cavitans* and *Reticulitermes flavipes*, and with filter-sterilized supernatant solution of each of the other *Clostridium* species. Both the termite guts and the *Clostridium* species serving as inoculum were derived from cultures grown in media with or without 0.1 mg/ml mitomycin C in an attempt to induce any lysogenic phage present in the hosts. No plaques were ever detected in the inocula from the termite guts. Similarly, none of the procedures described above yielded any identifiable phage as monitored by plaque formation on any of the other tested strains.

2. Transposon Mutagenesis of *Xenorhabdus luminescens*: As a first step toward isolation mutant of *Xenorhabdus* with altered developmental regulation, an attempt was made to introduce transposons into *X. luminescens* using three separate vectors harbored in *E. coli*. These systems included phage P1 carrying Tn5, F+ carrying Tn5, and a plasmid encoded defective, chloramphenicol-resistant Tn10 with transposase expressed from the same plasmid. Attempts to introduce these transposons were made using protocols for P1 transduction, *E. coli* mating, and transformation, respectively. *Xenorhabdus* was susceptible to both kanamycin and chloramphenicol, although spontaneous Kan resistant colonies arose at a rate of approximately one in 10^{-7} . No spontaneously cam-resistant strains were detected, and no confirmed transposon hops were ever achieved using these techniques. Attempts will be made in the future to introduce transposons via electroporation.

**Production of Methane and Acetate from Five Varied Sediments
when Cultured Under Conditions which Promote Methanogenesis
and Acetogenesis and while Using Methanol as the Major
Substrate.**

Dan Smith
Woods Hole Microbial Diversity Project
Summer, 1991

ABSTRACT

Five sediments from the Woods Hole area were used as inocula in media which would promote both acetogenesis and methanogenesis and given 4mM methanol as the sole substrate. The question was asked, which type of bacterium would predominate under such conditions since it is implied that when TMB is used as an alternative substrate for acetogens (methanogens cannot utilize TMB) that there is a methanol intermediate? The demethoxylation of TMB should liberate methanol and the question of what happens to this methanol in the environment was asked by simply supplying methanol directly to the sediments and asking if methane or acetate or a combination thereof was produced. Results show that only methanogenesis is detected in the experimental methanol substrate vial since up to 10% methane was detected in some samples and no acetate was detected in those samples where methanol was the substrate. This suggests that the de-methoxylation of an alternative substrate such as TMB by acetogens to methanol must require the methanol to be sequestered in the cell since if the methanol was released into the surrounding environment, methanogens would utilize it and out compete the acetogens. Similar results have been reported by other investigators.

For references, please see original paper bibliography.

References:

- 1) Orelmend, (1982) *App. Env. Microb.* 43:462
- 2) Lovely et. al, (1983) *App. Env. Microb.* 45:187-192
- 3) Ward et.al, (1985) *Adv. Aquat. Microb.* 3:141-179
- 4) *J. Bacter.* (1983) 155: 1208-1218
- 5) *App. Env. Microb.* (1983) 45: 1310-1315
- 6) *J. Bact.* (1983) 153: 1415-1423
- 7) *Arch. Microb.* (1984) 138: 360-364
- 8) Bache, R. (1981) *Arch. Microb.* 130: 255-261
- 9) Zehnder, (1988) Biology of Anaerobic Microorganisms, Ch. 13
Biochemistry of Methane Production Vogels et. al, John Wiley and Sons Inc

Enrichments with a Core Sample from a Surfactant Contaminated Aquifer

A section of core was obtained from the sewage contaminated aquifer originating from the Otis Air Force Base, Cape Cod, MA. An important component of the contamination of this site is detergent so the core material was used as an inoculum for a variety of enrichments involving surfactants.

Liquid enrichments for microorganisms that degrade and/or are tolerant to various surfactants were made under aerobic conditions. Some growth was observed in 50 mM sodium dodecylbenzenesulfonate (with and without glucose) but these cultures developed too slowly for further enrichment or isolation. Abundant growth was observed in 50 mM Triton X-100 (with and without glucose) and three enrichments were eventually obtained. The first was an apparently pure yeast culture that, in the end, appeared to be a contaminant. The other two isolates were rod-shaped bacteria, distinguishable from one another by colony morphology and pattern of cell division.

The surfactants contaminating the aquifer at Otis AFB are mostly alkyl benzene sulfonates so several anaerobic enrichments were prepared to select for organisms that can use sulfonate functional groups to accept electrons during metabolism of lactate. The cultures containing taurine and isoethionite showed growth with production of sulfide, but those containing cysteate and sodium dodecylbenzenesulphonate (SDS) gave no growth.

Guenter Wallner

1. Characterization of Magnetotactic Bacteria

Magnetotactic bacteria were isolated from an enrichment jar that was set up in 1988 with sediment and water from School Street Marsh, Woods Hole, by taking advantage of their magnetotaxis: with a small magnet they were attracted to the glass wall of the jar. The visible cell aggregation was sucked off with a pulled out pasteur pipette and transferred to a small tube containing sterile buffer. By repeating the "magnetotactic washing" in several tubes contaminant bacteria were diluted out and the retained cell suspension was highly enriched for magnetotactic bacteria.

Characterization of the isolated magnetotactic bacteria:

- composed of three morphological subpopulations
 - all of them: Gram negative; magnetotaxis towards north pole of magnet.
 - reaction to oxygen: microaerotolerant (or microaerophilic)
 - hybridization with rRNA targeted fluorescent oligonucleotide probes: all positive signal with "Eubacterial" probe; one subpopulation also weak signal with "Sulfate Reducing Bacteria" probe.
 - transmission electron microscopy: in samples prepared by thin sectioning only a spherical cell (gram-negative cell wall type) without a magnetosome and a pair of magnetosomes (two parallel chains of square subunits) surrounded by cell debris were found.
- Cultivation attempt: anaerobic and microaerobic agar dilution series with acetate, lactate or succinate as substrates were not successful.

2. Fluorescence Spectrophotometry of Phototrophic Bacteria

Fluorescence excitation and emission spectra of suspensions of untreated living cells of phototrophic bacteria were run on a PTI "ALPHASCAN" fluorescence spectrophotometer. The purpose of these spectra is to find out if there are characteristic peaks ("colors") of emitted fluorescence for different groups of phototrophic bacteria, that could be used to differentiate them at a single cell level in a fluorescence microscope or a flow cytometer.

For none of more than ten purple nonsulfur bacteria fluorescence spectra could be obtained, not even after many variations in cell concentration and excitation or emission wavelength (poor energy transfer?).

For one green and two purple sulfur bacteria it could be shown that excitation light targeted against carotenoid pigments is capable of inducing fluorescence emission in chlorophyll molecules by energy transfer. The wavelength of the emitted light depends not only on the type of chlorophyll but also on the association with proteins in the light harvesting systems or reaction centers. Therefore these are not enough data to see if "signature peaks" for different groups of phototrophic bacteria exist. But at least for part of them it should be possible to distinguish them from non-phototrophic bacteria by their infrared fluorescence after excitation with wavelengths that are available from common light sources and are absorbed by carotenoid pigments.

Appendix VI. Students in the 1991 Course: Microbial Diversity

Boldt, Yvonne R.	Grad Student	Microbiology, Univ. Minnesota
Breitung, Jürgen	Grad Student	Microbiology, Philipps Univ., Marburg, FRG
Budavari, Adriane I.	Recent B.A. (Biology)	from Swarthmore College, PA.
Caspi, Ron	Grad Student	Marine Biology, Scripps Inst.
Edgar, Blake D.	Asst. Editor, <i>Pacific Discovery Magazine</i> , Cal. Acad. Sci., San Francisco (MBL Science Writing Fellow)	
Fent, Karl Dr.	Env. Toxicologist, EAWAG/ETH, Switzerland (currently on sabbatical at WHOI)	
Hsing, Weihong	Grad Student	Microbiology, U. Massachusetts, Amherst
Jäger, Heike	Grad Student	Microbiology, Univ. Osnabruck, FRG
Johnson, Mark D.	Grad Student	Biology, Univ. Alabama
Karsten, Gudrun R.	Grad Student	Microbiology, Ruhr-Univ. Bochum, FRG
Kern, Monika	Grad Student	Microbiology, Univ. Bonn, FRG
Larsen, Mark A.	Recent B.S. (Biology)	from Univ. Puget Sound, WA.
Li, Rixin	Grad Student	Biology, Texas A&M Univ.
Lin, Chuzhao	Grad Student	Vet. Pathology, Univ. of Illinois
Mohanraju, R.	Grad Student	Marine Biology, Portonovo, India
Ostroumov, Sergei A.	Dr. Env. Toxicology, Dept. of Hydrobiology, Univ. Moscow, USSR; currently visiting scientist at Marine Sci. Res. Ctr., SUNY, Stony Brook, NY	
Pitta, Thomas P.	Grad Student	Microbiology, U. Conn.
Schnell, Sylvia S.	Grad Student	Microbiology, Univ. Tübingen, FRG
Slater, Steven C.	Grad Student	Microbiology, Case Western Reserve, OH
Tratnyek, Paul G. Dr.	Postdoc	Env. Sci., EAWAG, Switzerland
Wallner, Günter A.	Grad Student	Microbiology, Tech. Univ. München, FRG