APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1976, p. 83-90 Vol. 31, No. 1
 Printed in U.S.A.

Characterization of Anaerobic Heterotrophic Bacteria Isolated from Freshwater Lake Sediments[1]

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Received for publication 25 July 1975

Strict anaerobic culture techniques were used to quantitatively and qualitatively evaluate the anaerobic heterotrophic bacteria present at the sediment-water interface of hypereutrophic Wintergreen Lake (Augusta, Mich.). Anaerobic plate counts remained constant from March through December, 1973, ranging from 2.4×10^6 to 5.7×10^6 organisms/g (dry weight) of sediment. The isolatable bacteria represented a small percentage of the total microbial community, which was shown by direct microscopic counts to be 2.0×10^{11} organisms/g (dry weight) of sediment during June and July. Bacteria of the genus *Clostridium* dominated the isolates obtained, accounting for 71.8% of the 960 isolates examined. A single species, *Clostridium bifermentens*, comprised 47.7% of the total. Additional bacterial groups and the percentage in which they were isolated included: *Streptococcus* sp. (10.8%), unidentified curved rods (9.5%), gram-positive nonsporing rods (5.6%), and motile gram-negative rods (1.9%). Temperature growth studies demonstrated the ability of all the isolates to grow at in situ sediment temperatures. Gas-liquid radiochromatography was used to determine the soluble metabolic end products produced from [U-^{14}C]glucose and a U-^{14}C-labeled amino acid mixture by representative sedimentary clostridial isolates and by natural sediment microbial communities. At in situ temperatures the natural sediment microflora produced soluble fermentative end products characteristic of those elaborated by the clostridial isolates tested. These results are considered strong presumptive evidence that clostridia are actively metabolizing in the sediments of Wintergreen Lake.

Development of methodology for the strict anaerobic cultivation of microorganisms (1, 12) has facilitated the isolation of obligate anaerobic bacteria from a variety of anoxic habitats (11, 12, 15). Although these techniques are standard procedure in investigations concerning methanogenic bacteria in lake sediments (6, 19), they have yet to be widely applied to the isolation of the nonmethanogenic heterotrophic bacteria present in these sediments. Application of the roll tube technique (12) to the enumeration of nonmethanogenic bacteria in sewage sludge has increased viable counts 10 to 100 times over those obtained using aerobic techniques or less stringent anaerobic methods, which fail to maintain anoxic conditions during the entire sampling procedure (15). These results suggest the presence of substantial numbers of obligately anaerobic as well as facultatively anaerobic bacteria in this habitat.

Like sewage sludge, the initial substrates in sediments are complex organic compounds not utilizable by methane bacteria. The diversity of organic inputs to lake sediments suggests the presence of a corresponding, physiologically diverse, heterotrophic microbial community active in the anaerobic dissimilation of these substrates (7).

The present study represents an attempt to quantify and characterize the predominant groups of anaerobic heterotrophic bacteria present at the sediment-water interface of a eutrophic lake, using strict anaerobic culture techniques (1, 12). As a knowledge of the numbers and types of bacteria isolated from a particular habitat provides little information concerning the metabolic activities of these organisms in the natural environment, gas-liquid radiochromatography (13) was used to assess the soluble fermentative end products produced from [U-^{14}C]organic substrates by natural sediment microbial communities and by isolates derived from these sediments. (Portions of this investigation were presented at the 74th Annual Meeting of the American Society for Microbiology, 12-17 May 1974, Chicago, Ill., and at the 75th Annual Meeting of the American

[1] Kellogg Biological Station publication no. 290 and Michigan Agricultural Experiment Station journal article no. 7312.

Society for Microbiology, 27 April-2 May 1975, New York, N.Y.)

MATERIALS AND METHODS

Study site. All investigations were conducted on Wintergreen Lake, a small hardwater basin located within the W. K. Kellogg Bird Sanctuary, Augusta, Mich. The lake is shallow, with a maximum depth of 6.3 m and a mean depth of 3.54 m. Annual mean primary productivity values identify Wintergreen Lake as hypereutrophic, the latter designation based on compression of the trophogenic zone to a point where light rather than available nutrients limit productivity (22). The pelagic zone of Wintergreen Lake is characterized by significant autochthonous organic input, the annual succession of phytoplankton being punctuated in the summer by dense blooms of protein-rich blue-green algae. The hypolimnion of the lake is anaerobic for nearly 7 months of the year, with anoxic conditions extending to 3 m during summer stratification. The development of extensive anoxia bordering on the photic zone insures that the majority of mineralization of particulate organic matter in this basin is sedimentary and anaerobic. All sediment samples secured during the investigation were taken at a depth of 6 m or greater.

Bacteriological sampling procedures. Sediment samples for bacteriological analyses were secured with a Plexiglas gravity corer (8.5 by 100 cm), which preserved the sediment-water interface. Samples were collected at approximately monthly intervals from March through December, 1973. Cores were immediately plugged with air-tight rubber stoppers for transport to the laboratory, where a subsample was taken using a sterile glass subsampler (3 by 23 cm). The subsample was continuously gassed with a mixture of 90% N_2-10% H_2 or 85% N_2-5% CO_2-10% H_2 before being plugged with rubber stoppers, clamped in a press, and transferred to an anaerobic glove box. The glove box atmosphere was initially 90% N_2-10% H_2, but was subsequently altered to 85% N_2-5% CO_2-10% H_2. No significant differences were observed in the types or numbers of bacteria isolated under the two gas atmospheres. The recovery of organisms under a gas atmosphere lacking H_2 was not evaluated.

A 1:10 dilution (vol/vol) of duplicate subsamples of surface sediment (0- to 2-cm layer) was made in prereduced (PR) dilution fluid. The composition of the diluent varied with the gas phase used. Under a N_2-H_2 atmosphere, the diluent contained, in grams per liter: $(NH_4)_2SO_4$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.05; NaCl, 1.0; K_2HPO_4, 6.5; KH_2PO_4, 3.5. Under N_2-CO_2-H_2, the dilution fluid contained, in grams per liter: K_2HPO_4, 6.5; KH_2PO_4, 3.5; Na_2CO_3, 2.5. In addition, each diluent contained 0.05% cysteine-hydrochloride·water and 0.2% of a 0.1% resazurin solution. The final pH of each solution was 6.9.

The initial 1:10 dilutions were either stirred on a magnetic stirrer for 5 min or mixed in a Waring blender for 1 min. Appropriate additional 1:10 and 1:1 dilutions were made in anaerobic dilution fluid and inoculum from six dilutions was each spread on duplicate PR agar plates containing resazurin as an oxidation reduction indicator. Pour versus spread plates were not evaluated. Media inoculated included 1% peptone-yeast extract-glucose (PYG), 0.5% PYG, brain heart infusion (Difco, Detroit, Mich.), sediment extract supplemented with Trypticase, yeast extract, and mineral salts, and Medium 10 of Caldwell and Bryant (5) modified for use under a predominantly N_2 atmosphere. The complete composition of each medium is listed in Table 1.

Inoculated plates were incubated anaerobically at 15 C for 14 days. The plates were placed in Anaerobic Jars (BBL) containing a palladium catalyst (palladium-coated alumina pellets; Engelhardt Industries, East Newark, N.J.). The jars were sealed and removed from the glove box to a 15 C incubator. After 14 days, the jars were returned to the glove box and opened, and the plates were examined. Quantitative counts were made on 1% PR PYG (determined in preliminary experiments to yield the highest number of microorganisms of the four media used). Colonies selected for additional characterization and identification were obtained at bimonthly rather than monthly sampling intervals and were chosen during periods of lake stratification, as well as during spring and fall overturn.

Isolation of sedimentary anaerobes and presumptive identification of strains. Plates of each medium were carefully examined for colony diversity. Fifty to 60 colonies were randomly picked from each medium and transferred to 1% PR PYG plates. Although colonies were selected at random, an effort was made to insure that representatives of all distinct colony types present on the plates were included in the colonies transferred. The PYG plates were incubated at room temperature (23 to 26 C) within the glove box. Resulting colonies were streaked onto 1% PR PYG plates, and isolated colonies were transferred to PR PYG slants. Isolates were stored at 4 C until further characterizations could be carried out.

The isolates were characterized by the methods described in the Virginia Polytechnic Institute (VPI) Anaerobe Laboratory Manual (10). All characterization tests were performed using PR media, except for the determination of oxygen sensitivity when incubation was aerobic. Characterization tests were performed at 25 C except for temperature growth studies. One percent PY was used as the basal medium in all fermentation tests, with the various test substrates being added at a concentration of 1% (wt/vol).

Analysis of soluble and gaseous fermentation end products. Volatile fatty acids formed in glucose-containing media were analyzed as described by Holdeman and Moore (10). Lactic acid was determined by conversion to its propyl ester (boron trifluoride-propanol esterification reagent; Applied Science Laboratories, Inc., State College, Pa.). Both volatile and nonvolatile acids were analyzed on a Packard model 409 gas chromatograph equipped with a flame ionization detector. Samples were separated on a coiled stainless-steel column (2 m by 0.3 cm outer diameter) packed with 10% SP-1200-1% H_3PO_4 on Chromosorb W (AW-DMCS, 80/100 mesh;

TABLE 1. *Composition of media used for isolation of sediment anaerobes*[a]

Component	% in medium[b]				
	1% PYG	0.5% PYG	BHI	Sediment extract	Modified M10
Peptone	1.0	0.5			
Yeast extract	1.0	0.5		0.4	0.1
Glucose	1.0	0.5			0.1
Cellobiose					0.1
Soluble starch					0.1
Trypticase				0.2	0.4
Hemin					0.0002
Volatile fatty acids (vol/vol)					0.31
Salts solution (vol/vol)					
1[c]	90	90	90		90
2[d]				2.0	
$CaCl_2 \cdot 2H_2O$	0.06	0.06	0.06		0.06
Brain heart infusion			3.7		
Sediment extract (vol/vol)				30[e]	
Resazurin	0.0001	0.0001	0.0001	0.0001	0.0001
Cysteine-hydrochloride·water	0.05	0.05	0.05	0.05	0.05
Agar	1.5	1.5	1.5	1.5	1.5

[a] Final pH, 6.8 to 7.0.

[b] Final percent as weight/volume or as indicated. BHI, Brain heart infusion.

[c] Composition, in grams per liter: K_2HPO_4, 0.56; KH_2PO_4, 0.33; Na_2CO_3, 0.22; $MgSO_4 \cdot 7H_2O$, 0.11; $(NH_4)_2SO_4$, 0.22; NaCl, 0.53; $FeCl_3$, 0.005.

[d] Composition, in grams per liter: $(NH_4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.1; NaCl, 2.0; K_2HPO_4, 7.0.

[e] Sterilized separately and added aseptically after autoclaving. Extract consisted of clarified supernatant obtained after autoclaving 1 kg of sediment with 1 liter of distilled water.

Supelco, Inc., Bellefonte, Pa.). Helium was used as carrier gas at a flow rate of 25 ml/min. Chromatographic operating conditions were: inlet temperature, 200 C; oven temperature, 120 C; detector temperature, 180 C.

H_2 and CO_2 produced by individual isolates grown in 1% PYG broth under 100% N_2 were separated on a coiled stainless-steel column (2 m by 0.3 cm outer diameter). The packing material was silica gel (60/80 mesh; Applied Science Laboratories, Inc.). N_2 was used as carrier gas (25 ml/min). Operating conditions were: inlet temperature, 130 C; oven temperature, 50 C; detector temperature, 60 C; filament current, 150 mA.

Metabolism of labeled organic compounds by natural sediment microbial communities. A 1:10 dilution of the initial 2 cm of sediment was made using PR filter-sterilized (0.22-μm pore size membrane filter; Millipore Corp., Bedford, Mass.) interstitial water as diluent. Ten milliliters of diluted sediment was added to replicate flasks containing either [U-^{14}C]glucose (50 μg, 40 μCi; Amersham/Searle, Arlington Heights, Ill.) or a U-^{14}C-labeled amino acid mixture (50 μg, 38 μCi; New England Nuclear Corp., Boston, Mass.), respectively. Unlabeled glucose and amino acid mixture (Sigma Chemical Co., St. Louis, Mo.) were added to the respective flasks to give final substrate concentrations of 120 μg/ml of sediment slurry. Control flasks were killed by addition of 1 ml of 2% $HgCl_2$. The flasks were incubated anaerobically at 10 C for 72 h.

The reactions were subsequently terminated by addition of 0.2 ml of 6 N H_2SO_4 to each flask. Soluble metabolites were determined as described above, except that volatile fatty acids were analyzed by thermal conductivity detection (filament current, 200 mA). Organic components of the chromatographic effluent were combusted by passage through a heated copper oxide tube, and the resulting [^{14}C]-CO_2 was collected by bubbling the effluent gas into a vial containing 14 ml of methanol-ethanolamine (1:2.5) solution. Six milliliters of scintillation fluid (15 g of 2,5-diphenyloxazole, 1 g of *p*-bis-(*O*-methylstyrl)-benzene, toluene to make 1 liter) was added, and radioactivity was determined by liquid scintillation counting (Beckman model LS-150 liquid scintillation spectrometer).

RESULTS

Quantitation of anaerobic bacteria at the sediment-water interface. Figure 1 depicts viable plate counts of anaerobic heterotrophic bacteria at the sediment-water interface of Wintergreen Lake from March through December, 1973. The anaerobic heterotrophic community showed little seasonal variation. The mean viable count obtained on 1% PR PYG during this period was 4.24×10^6 organisms/g (dry weight) of sediment. The viable counts reached a maximum in June, corresponding to the onset of significant oxygen depletion in the hypolimnion. Although fall overturn restored oxic water to the hypolimnion by late October,

the viable counts did not decline significantly, remaining at peak summer levels through November.

Periodic direct microscopic counts were performed on the sediment-water interface of Wintergreen Lake during June and July. Microorganisms were dispersed from the sediment particles by the method of Bohlool and Schmidt (3). Samples were stained with acridine orange and examined by epifluorescence microscopy (9). A mean direct microscopic count of 2.03×10^{11} organisms/g (dry weight) of sediment was obtained. Chaining rods and filaments, present primarily in discrete microcolonies, dominated the morphotypes observed in the samples examined. Lesser numbers of cocci and vibroid-shaped cells were generally evident as well.

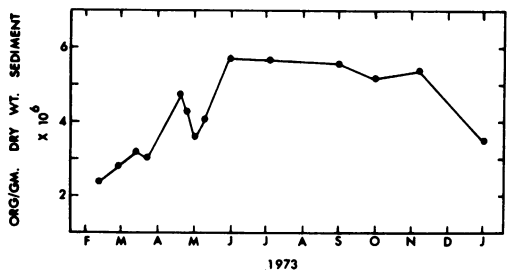

FIG. 1. *Viable plate counts of anaerobic heterotrophic bacteria at the sediment-water interface of Wintergreen Lake from March through December, 1973. Duplicate dilution series were spread on 1% PR PYG plates. Each point represents the mean value obtained from 24 plates.*

Characterization of anaerobic sedimentary isolates. Table 2 summarizes the major groups of facultative and strictly anaerobic bacteria isolated from the sediment-water interface of Wintergreen Lake. Fermentation and additional physiological characteristics of each group are found in Table 3. Group I, tentatively identified as a *Streptococcus* sp., was composed of gram-positive, facultatively anaerobic cocci, 1 to 1.5 μm in diameter. The cells were either spherical or dumbbell shaped and occurred singly, in pairs, or in short chains. No H_2S or indole was produced, and fermentation end products in PYG included major amounts of lactic and acetic acids with trace amounts of propionic, butyric, and isobutyric acids. Strains of group I fermented all sugars tested, produced neither H_2 nor CO_2 from PYG, and grew at 5 C. These isolates comprised 10.8% of the total organisms examined.

Group II, comprising 9.5% of the total isolates, consisted of unidentified, gram-negative, curved rods measuring 0.5 by 3 μm. These organisms were obligately anaerobic, produced H_2S, and were indole negative. Fermentation end products included major quantities of acetic and propionic acids with lesser amounts of butyric, isobutyric, and isovaleric acids. These organisms fermented only maltose among the sugars tested and grew at 10 and 15 C, but not at 5 C.

By far the largest group of isolates obtained,

TABLE 2. *Presumptive identification features and distribution of anaerobic bacteria in Wintergreen Lake sediments*[a]

Morphological group	Isolated strains (%)	Gram reaction	Motility	Oxygen tolerance	Fermentation products	Tentative identification
I Gram-positive cocci, 1 to 1.5 μm	10.8	+	−	F	LApbi-b	*Streptococcus*
II Small curved rods, 0.5 by 3 μm	9.5	−	+	A	APbi-bi-v	Unknown
III Sporeforming rods	71.8					*Clostridium*
a	47.7	+	+	A	Apbi-bi-vi-C	*C. bifermentens*
b	17.8	+	+	A	Apbi-bvi-V	*C. sporogenes*
c	6.3	+	+	A	ApBi-B	*C. butyricum*
IV Gram-positive rods						
a	4.4	+	−	A	AB	*Eubacterium*
b	1.2	+	−	F	ND	Unknown
V Motile gram-negative rods, 0.5 by 2 to 3 μm	1.9	−	+	F	ND	Unknown

[a] Based on a total of 960 isolates. Reactions given are those for a majority of the strains within a group. Symbols and abbreviations: A, Strictly anaerobic; F, facultative; +, positive reaction; −, negative reaction; fermentation products—A, a, acetic; P, p, propionic; B, b, butyric; i-B, i-b, isobutyric; V, v, valeric; i-V, i-v, isovaleric; C, c, caproic; i-C, i-c, isocaproic; L, l, lactic. Uppercase letters refer to major end products; lowercase letters refer to products produced in lesser amounts as described in the VPI *Anaerobe Laboratory Manual* (10). ND, Not determined.

TABLE 3. *Fermentation and additional physiological characteristics of selected sedimentary isolates*[a]

Characteristic	Group no.[b]				
	I	II	III		
			a	b	c[c]
Glucose	a	n	w	w	a
Cellobiose	a	n	n	n	a
Galactose	a	n	n	n	a
Lactose	a	n	n	n	a
Maltose	a	a	w	w	a
Sucrose	a	n	n	n	a
Urease production	ND	ND	−	−	−
Indole production	−	−	+	−	−
H_2S production	−	+	+	+	+
Catalase	−	−	−	−	−
Nitrate reduction	−	−	−	−	−
H_2 production	−	ND	+	+	+
CO_2 production	−	ND	+	+	+
Growth at:[d]					
5 C	+	−	−	+	−
10 C	+	+	+	+	+
15 C	+	+	+	+	+

[a] Abbreviations: a, Acid reaction, terminal pH 5.5 or less; w, weak reaction, terminal pH 5.5 to 6.0; n, no fermentation, terminal pH greater than 6.0; ND, not determined.

[b] Fermentative and additional physiological tests were performed on the first three groups of isolates only. Reactions given are those for a majority of the strains within a group.

[c] Identification of *C. butyricum* is tentative due to the variability of response of these isolates to the biochemical tests performed.

[d] Tubes demonstrating no visible growth after incubation for 30 days were scored negative.

representing 71.8% of the total, group III consisted of obligately anaerobic, sporeforming rods of the genus *Clostridium*. This group could be further subdivided into three subgroups identified on the basis of fermentation end products and additional biochemical reactions as *Clostridium bifermentens*, *C. sporogenes*, and *C. butyricum*. *C. bifermentens* represented the most predominant species obtained, comprising 47.7% of the total isolates.

The predominantly proteolytic nature of these clostridial isolates was substantiated by their inability to ferment a variety of sugars, the sole exception being *C. butyricum*, which fermented all of the sugars tested. All of the clostridial isolates produced hydrogen in PYG broth and grew at environmental temperatures (10 C). *C. sporogenes* was able to grow at 5 C as well.

Groups IV and V represented a minor portion of the isolates, together comprising only 7.5% of the total. Group IV consisted of gram-positive, nonsporing rods and included two subgroups, the first an obligately anaerobic rod, which produced acetic and butyric acids as major fermentation end products and which may be related to a species of *Eubacterium*, and the second an unidentified, facultatively anaerobic rod. Group V consisted of unidentified, motile, gram-negative rods comprising less than 2% of the total isolates.

Table 4 illustrates the hydrolytic capabilities of each group of isolates towards a number of complex organic substrates. No cellulolytic activity was demonstrable, as shown by the inability of all of the isolates to hydrolyze either ball-milled cellulose or carboxymethyl-cellulose. Moreover, only the presumptive *Eubacterium*, the group V isolates, and one subgroup of clostridia, *C. butyricum*, fermented starch. Only two groups, the group V isolates and the presumptive *Eubacterium*, exhibited lipase activity as demonstrated by the ability to hydrolyze tributyrin.

The majority of the isolates demonstrated proteolytic capabilities. The proteolytic nature of the clostridia was evident from the ability of *C. bifermentens* and *C. sporogenes* to hydrolyze both casein and gelatin. Only *C. butyricum* failed to attack these substrates. The remaining strictly anaerobic isolates, the group II curved rods and the group IV *Eubacterium*, also demonstrated proteolytic capabilities, whereas among the facultative isolates only motile gram-negative rods of group V were able to liquify gelatin.

Metabolism of labeled organic compounds by natural sediment microbial communities. Table 5 illustrates the labeled volatile fatty acids produced by the natural sediment microbial community when incubated anaerobically at 10 C for 72 h in the presence of [U-^{14}C]glucose or a U-^{14}C-labeled amino acid mixture, respectively. Although the specific activity of each individual labeled acid is unknown, it is nonetheless evident that ^{14}C was incorporated into an array of soluble metabolites by the natural sediment microflora. Of particular interest is the appearance of label in those regions corresponding to isovaleric, valeric, isocaproic, and caproic acids. These metabolites are highly characteristic of clostridial fermentations, particularly those carried out by *C. bifermentens* and *C. sporogenes* (10, 18).

DISCUSSION

The viable plate counts of anaerobic heterotrophic bacteria reported in this study are similar to those reported in the sediments of other lakes of similar trophic level as Wintergreen Lake. Boylen and Brock (4) reported anaerobic

TABLE 4. *Hydrolytic characteristics of anaerobic isolates*[a]

Group	Casein digestion	Gelatin liquefaction	Starch hydrolysis	Carboxymethylcellulose digestion	Cellulose digestion	Tributyrin hydrolysis
I Facultative cocci	−	−	−	−	−	−
II Anaerobic curved rods	±	+	−	−	−	−
III *Clostridium*						
a	+	+	−	−	−	−
b	+	+	−	−	−	−
c[b]	−	−	+	−	−	−
IV Gram-positive rods						
Anaerobic	−	+	+	−	−	+
Facultative	−	−	−	−	−	−
V Gram-negative facultative rods	−	+	+	−	−	+

[a] Symbols: +, Positive reaction; −, negative reaction. Reactions given are those for a majority of the strains within a group.

[b] Identification of *C. butyricum* is tentative due to the variability of response of these isolates to the biochemical tests performed.

TABLE 5. *Metabolism of [U-^{14}C]glucose and U-^{14}C-labeled amino acid mixture by natural sediment microbial communities*[a]

Volatile fatty acid	Substrate (counts/min)	
	[U-^{14}C]glucose	U-^{14}C-labeled amino acids
Acetic	1,473	653
Propionic	1,230	380
Isobutyric	1,168	412
Butyric	869	384
Isovaleric	1,106	464
Valeric	2,121	818
Isocaproic	1,977	1,111
Caproic	1,620	740

[a] Incubated anaerobically with shaking at 10 C for 72 h.

heterotrophic microbial communities of 4.4×10^5 to 2.8×10^6 organisms/g (wet weight) of sediment in Lake Wingra, Wisconsin, during the winter. Surface sediment heterotrophic bacterial densities in the lower Great Lakes have consistently ranged between 10^6 and 10^7 organisms/g (dry weight) of sediment, regardless of the medium used or the time of sampling (2, 7, 21). Similarly, no significant differences were noted in the quantitative or qualitative recovery of microorganisms on the four media used in the present investigation. The large discrepancy observed between the viable and direct microscopic counts of the bacterial community of Wintergreen Lake sediments reflects the inadequacy of most media used to enumerate sediment microorganisms, as well as the difficulties associated with the enumeration of organisms adhering to a particulate surface, such as sediment or soil. The high percentage of obligate anaerobes isolated in the present study by strict anaerobic culture techniques suggests that aerobic or less stringent anaerobic culture techniques may not be adequate to enumerate substantial portions of the sediment microflora. Additional bacteriological investigations of natural sediments and anoxic waters which use strenuous anaerobic methodology are required to determine the relative numbers and metabolic activities of obligate as well as facultative anaerobes in these habitats.

The most striking feature of the bacterial strains isolated from Wintergreen Lake sediments is the predominance of clostridia among the organisms examined. The genus *Clostridium* accounts for 71.8% of the total isolates. Greater than 50% of the clostridial strains, in turn, were identified as *C. bifermentens* and *C. sporogenes* (47.7 and 17.8%, respectively). Although toxicity tests were not performed, *C. sporogenes* was nevertheless identifiable due to its characteristic "Medusa-head" colonial morphology. A saccharolytic isolate tentatively identified as *C. butyricum* represented a minor portion of the clostridial isolates (6.3%). This organism could not be identified with certainty due to the small number of isolates obtained and the variability of response of these isolates to the biochemical tests performed.

The largely proteolytic nature of the clostridial isolates obtained is likely due to the predominance of nitrogenous organic substrates in Wintergreen Lake sediments. As indicated, this proteinaceous material is derived primarily from extensive blooms of blue-green algae which dominate the phytoplankton of the lake. The presence of a large ammonify-

ing bacterial population in eutrophic Lake Erie sediments (7) similarly suggests the availability of a ready source of complex nitrogenous substances in enriched lake sediments.

Although clostridia are generally held to be widely distributed in nature (20), very little is known concerning their ability to actively grow and metabolize in natural habitats. Studies of their occurrence in freshwater and marine sediments have for the most part been limited to considerations of these habitats as reservoirs for clostridial spores pathogenic to humans and other animals (14, 17). Matches and Liston (16) examined the sediments of Puget Sound for the presence of clostridia. They reported anaerobic plate counts ranging from 0.73×10^4 to 23.5×10^4 cells/ml of sediment-water slurry. Approximately 30% of these organisms were determined to be clostridia, the three species isolated in greatest numbers being *C. perfringens*, *C. bifermentens*, and *C. novyi*. These workers postulated that the mesophilic clostridial population of Puget Sound sediments is passively accumulated from terrestrial sources and is not derived from active growth of a metabolizing resident population. They based their findings largely on an inability of most clostridia to grow at in situ sediment temperatures (16).

The three clostridial isolates obtained from Wintergreen Lake were able to grow at 10 C. *C. sporogenes* also grew at 5 C, the lowest temperature tested. Since the temperature of the sediment-water interface of Wintergreen Lake exceeded 10 C for nearly 6 months of 1973, reaching a maximum of 13.3 C, clostridia could be actively metabolizing in these sediments during this period. Finne and Matches (8) have recently reported the isolation of low-temperature growing clostridia from marine sediments that can grow at 5 C or less, well within the in situ temperatures of most marine and freshwater sediments.

The production of characteristic clostridial fermentation end products from [U-^{14}C]glucose and a U-^{14}C-labeled amino acid mixture by natural sediment microbial communities provides additional presumptive evidence that clostridia, and possibly *C. bifermentens* and *C. sporogenes* in particular, are active in the degradation of organic substrates in Wintergreen Lake sediments. *C. bifermentens* and *C. sporogenes* elaborate an array of characteristic volatile metabolites, including the distinctive valeric and caproic series of fatty acids. With the exception of a few species of anaerobic cocci (*Peptostreptococcus* sp.) and a small number of *Bacteroides* sp. and *Eubacterium* sp., the production of valeric and isovaleric acids as fermentative end products is generally limited to the genus *Clostridium* (10, 18). Furthermore, production of major quantities of caproic and isocaproic acids as fermentative metabolites is almost exclusively restricted to the clostridia (10, 18).

These results support the hypothesis that a resident clostridial community is actively metabolizing in Wintergreen Lake sediments, particularly during periods in the summer and early fall when sediment temperatures are most favorable for growth of the clostridia. The ability of these organisms to form spores would enable them to withstand periods less favorable for growth, such as during lake turnover and mixing or during periods of reduced sediment temperature. Clearly, the role of clostridial species in the processing of organic matter in natural environments must be more thoroughly examined. The diverse modes of anaerobic energy-generating metabolism displayed by members of this genus, their demonstrated ability to grow at environmental temperatures, and their ability to form dormant spores whenever environmental conditions become unsuitable for growth should afford the clostridia a definite advantage in organically enriched anoxic habitats such as lake sediments.

ACKNOWLEDGMENTS

This investigation was supported by financial assistance provided by the Agricultural Experiment Station of Michigan State University, and by National Science Foundation grants GB-36069X and BO-15665 (Coherent Areas Program for Investigation of Freshwater Ecosystems).

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