

Distribution of Myxobacters in Aquatic Habitats of an Alkaline Bog

LEONARD A. HOOK†

Department of Biology, Central Michigan University, Mt. Pleasant, Michigan 48859

Received for publication 1 March 1977

Ten species of myxobacteria were identified from samples from an alkaline bog and adjacent soils. The frequency of occurrence and the diversity of species were highest at the margin of the bog and were lowest in the center and bottom of the bog lake.

Recent studies indicate that members of the family *Myxococcaceae* are frequently found in marine and freshwater environments (1-4, 8, 15), although it has been suggested that their presence in these habitats may be a result of runoff from adjacent soils (3; E. R. Brockman, *Bacteriol. Proc.*, p. 52, 1971). An increase in the occurrence of myxobacters has been reported in freshwater that is heavily laden with coliform bacteria (8, 15). This study was undertaken to determine which myxobacterial species were present in an alkaline bog lake and to estimate their relative frequency of occurrence.

The study area, Davis Lake (Vestaburg Bog), is located 1.5 km southeast of Vestaburg, Mich., in the central portion of the state's lower peninsula. The bog lake measures 100 by 80 m and occupies the bottom of a steeply banked, oblong basin measuring 500 by 150 m (5). The bog lake is bordered on three sides by a quaking *Sphagnum* sp. mat dominated by bog leather-leaf, *Chamaedaphne calculata* (5, 7). Near the open water, the mat is a semiwoody Rifle peat that becomes increasingly decomposed near the edge of the basin, forming a moat of standing water (fossa) at the margin of the basin (12). A diagram showing the bog lake and the sampling sites is given in Fig. 1.

Samples were obtained from aquatic habitats on eight dates from 15 September 1975 to 28 April 1976. For comparison, terrestrial regions A and B (Fig. 1), each represented by a composite of 10 subsample sites that circumscribe the bog lake, were sampled on 1 July 1976, and site C (not a composite) was sampled on 15 September 1975. The pH of the water at each sampling site was determined in the field with a Corning model 6 portable pH meter (Corning Scientific Instruments, Medfield, Mass.). The dissolved oxygen and temperature were measured in situ with a YSI model 51A dissolved-oxygen meter

(Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio).

Alkaline pH values observed in Davis Lake (Table 1) are atypical as compared with those commonly seen in acid peat bogs. The pH range observed in the various habitats of Davis Lake would not preclude the growth of myxobacteria (16). On a given sampling date, dissolved-oxy-

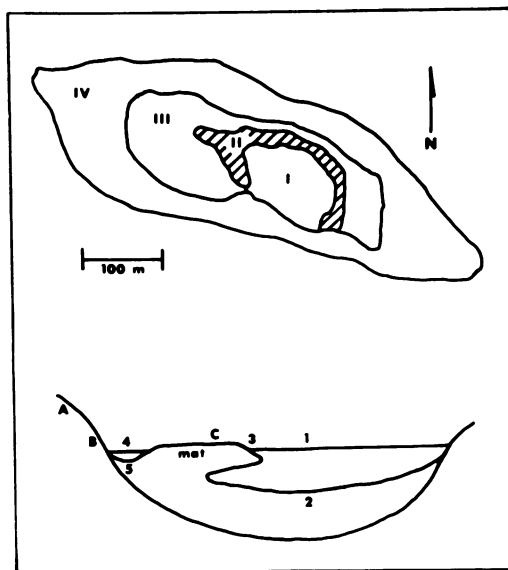


FIG. 1. Overhead (top) and cross-sectional (bottom) diagrams of Davis Lake, showing topography and sampling sites. (Overhead diagram redrawn from map by John Worthington and adapted from Gilliam et al. [7].) The cross-sectional diagram is not drawn to scale so that the sampling sites may be clearly indicated. I, Open water; II, quaking *Sphagnum* mat; III, fossa forest; IV, basin. Aquatic sampling sites: 1, bog lake surface water; 2, bog lake bottom sediment; 3, bog mat; 4, fossa surface water; 5, fossa bottom sediment. Terrestrial sampling sites: A, upland soil; B, fossa soil; C, *Sphagnum* sp. from the mat.

† Present address: Department of Microbiology, Louisiana State University, Baton Rouge, LA 70803.

gen levels were nearly uniform throughout the bog habitats with the exception of the bottom sediments (site 2), where the environment was nearly anaerobic. It was at this site that the lowest total myxobacterial frequency was observed (see below).

The primary detection medium for non-cellulolytic myxobacters consisted of a mineral salt agar base (17) onto which was streaked autoclaved, rehydrated bakers' yeast (Red Star Active Dry Yeast, Milwaukee, Wis.). Cellulolytic and non-cellulolytic myxobacters were detected on mineral salt agar overlaid with sterile Whatman no. 1 filter paper disks (13). Cycloheximide (Acti-dione; ICN Pharmaceuticals, Inc., Cleveland, Ohio; 25 mg/liter of medium) was added to retard the growth of contaminating fungi according to the method of Brockman

and Boyd (4). Solid particulate inocula were obtained from aquatic samples either by centrifugation (10 min at 4,000 × g) or by membrane filtration (Millipore Corp., Bedford, Mass.; 0.45-μm pore size) and then scraping off the filtered debris for use. The 0.3- to 40-mg inoculum from each sample was spotted onto five places on each of 40 primary detection plates (20 yeast and 20 filter paper plates). On a few occasions, fewer than 40 plates were made from certain samples. The plates were incubated at room temperature and, after 3 and 6 weeks of incubation, were examined for the presence of myxobacterial fruiting bodies.

Ten species of myxobacters were identified from 39 aquatic samples, a total of 2,764 occurrences from 6,568 inoculations (Table 2). Six species of the family *Myxococcaceae* were identified in the various bog habitats. The most frequently observed was *Myxococcus fulvus*, which was dominant in all aquatic sampling sites, comprising 54 to 90% of all observations. *M. disciformis* was observed in relative abundance in the fossa, but its incidence was low in other portions of the bog, and it was not detected in the soils surrounding the bog lake. This may indicate a positive response to certain growth factors present in the fossa but absent in other portions of the bog. This organism has been detected on previous occasions from soils

TABLE 1. Ranges of pH, dissolved oxygen, and temperature at aquatic sites 1 to 5, measured on eight sampling dates from 15 September 1975 to 28 April 1976

Sampling site	pH	Dissolved oxygen (ppm)	Temp (°C)
1	6.0-8.7	7.0-14.0	-2.0-15.0
2	8.1-8.2	0.6-1.3	5.5-9.5
3	6.0-8.7	3.2-14.0	-2.0-15.0
4	6.0-7.7	7.0-9.2	-2.0-11.0
5	7.9-8.1	7.0-7.8	-2.0-14.0

TABLE 2. Distribution of myxobacters in Davis Lake, listed in order of frequency of occurrence

Species identified	Sampling site															
	Aquatic										Terrestrial					
	1		2		3		4		5		A		B		C	
	1,520 ^a		1,400		1,600		748		1,200		200		200		200	
	+ ^b	% ^c	+	%	+	%	+	%	+	%	+	%	+	%	+	%
<i>Myxococcus fulvus</i>	321	21.1	190	13.6	635	39.7	449	60.0	394	32.8	29	14.5	83	41.5	0	0.0
<i>M. disciformis</i>	2	0.1	4	0.3	25	1.6	26	3.5	260	21.7	0	0.0	0	0.0	0	0.0
<i>M. stipitatus</i>	25	1.6	115	8.2	10	0.6	21	2.8	13	1.1	0	0.0	0	0.0	0	0.0
<i>M. coralloides</i>	6	0.3	0	0.0	108	6.8	24	3.2	39	3.3	34	17.0	168	84.0	0	0.0
<i>Polyangium sorediatum</i>	0	0.0	0	0.0	29	1.8	38	5.1	20	1.7	0	0.0	0	0.0	0	0.0
<i>Archangium geographyra</i>	0	0.0	0	0.0	1	0.06	0	0.0	2	0.2	1	0.5	1	0.5	0	0.0
<i>Myxococcus virescens</i>	1	0.05	1	0.07	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<i>Myxococcus xanthus</i>	0	0.0	0	0.0	1	0.06	1	0.1	0	0.0	129	64.5	0	0.0	0	0.0
<i>Melittangium lichenicola</i>	0	0.0	0	0.0	0	0.0	0	0.0	2	0.2	0	0.0	Present ^d	Present	0	0.0
<i>Polyangium cellulosum</i>	0	0.0	1	0.1	0	0.0	0	0.0	0	0.0	2	2.0	0	0.0	0	0.0
Sum of all species	355		311		809		559		730		195		253		0	

^a Number of inoculations.

^b +, Number of inoculation spots showing the presence of fruiting bodies.

^c %, Percentage of positive inoculation spots.

^d Observed (according to the method of Gilbert and Martin [6]) growing on tree bark.

and high-peat bogs (hochmoors) characterized by the growth of *Sphagnum* sp. (9, 10), but has rarely been identified from other sources (14). Likewise, the occurrence of *M. stipitatus* and *Polyangium sorediatum* in aquatic bog habitats, although not detected in the surrounding soils, may be present in the bog as a result of favorable growth factors. However, these and other myxobacters have been isolated from soils from numerous regions (11). No myxobacters were cultivated from fragments of living *Sphagnum* sp. (site C). *M. xanthus* was predominant in the upland soil (regional site A), and *M. coralloides* was predominant in the fossa soils (regional site B), each comprising 66% of the total observations. The occurrences of *P. cellulolum*, *Archangium gephyra*, *Melittangium lichenicola*, and *M. virescens* followed no discernible pattern.

The frequency of occurrence and diversity of species of myxobacters in aquatic bog habitats were highest in the sampling sites closely associated with the upland soils surrounding the bog lake (sites 4 and 5) and were lowest in the center and bottom of the bog lake. Members of the family *Myxococcaceae* were the most common myxobacters. It was evident that the topography of the bog and its basin facilitated soil runoff, which washed nutrients and soil microorganisms into the bog. This is reflected in the fact that all species of myxobacteria encountered in the soil samples were also present in aquatic samples. The results of this study indicate differing survivabilities among certain myxobacters in Davis Lake and suggest that *Myxococcus disciformis*, *M. stipitatus*, and *Polyangium sorediatum* multiplied in aquatic habitats. A more detailed study of the physical and chemical parameters of the bog environment may help to determine which criteria are conducive to the growth of certain myxobacters and which are not.

This study was supported by the Central Michigan University School of Graduate Studies Student Research Grant.

I wish to thank Ellis R. Brockman for his assistance throughout the study and John M. Larkin for reviewing the manuscript.

LITERATURE CITED

1. Brauss, F. W., I. Heyne-Katzenberger, H. Pech, and H. Barth. 1967. Beitrage zur Mikrobiologie von Binnengewassern (I.). Arch Hyg. Bakteriol. 150:716-724.
2. Brockman, E. R. 1967. Fruiting myxobacteria from the South Carolina coast. J. Bacteriol. 94:1253-1254.
3. Brockman, E. R. 1973. Isolation of myxobacteria from marine habitats in the U.S. Virgin Islands, p. 45-52. In L. H. Stevenson and R. R. Colwell (ed.), Estuarine microbial ecology. University of South Carolina Press, Columbia.
4. Brockman, E. R., and W. L. Boyd. 1963. Myxobacteria from soils in the Alaskan and Canadian Arctic. J. Bacteriol. 86:605-606.
5. Currier, P. J., and R. O. Kapp. 1974. Local and regional pollen rain components at Davis Lake, Montcalm County, Michigan. Pap. Mich. Acad. Sci. Arts Lett. 7:211-225.
6. Gilbert, H. D., and G. W. Martin. 1933. Myxomycetes found on the bark of living trees. Stud. Nat. Hist. Iowa Univ. 15:3.
7. Gilliam, J. A., R. O. Kapp, and R. D. Bogue. 1967. A post-Wisconsin pollen sequence from Vestaburg Bog, Montcalm County, Michigan. Pap. Mich. Acad. Sci. Arts Lett. 52:3-17.
8. Graf, W. 1975. Myxobacteria of the *Myxococcus* group as indirect indicators of fecal matter in surface water. I. Communication. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe B 160:28-39.
9. Krzemieniewska, H., and S. Krzemieniewski. 1926. Die Myxobakterien von Polen. Acta Soc. Bot. Pol. 4:1-54.
10. Krzemieniewska, H., and S. Krzemieniewski. 1927. Rozsiedlenie miksobakteryj. Acta Soc. Bot. Pol. 5:102-139.
11. McCurdy, H. D. 1974. The gliding bacteria, p. 76-127. In R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams and Wilkins Co., Baltimore.
12. MacDonald, R. L., P. A. Delcourt, and R. O. Kapp. 1971. Plant communities of the MacCurdy Ecological Tract, Montcalm County, Michigan. Mich. Acad. 4:17-28.
13. Peterson, J. E. 1969. Isolation, cultivation and maintenance of the myxobacteria, p. 185-210. In J. F. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 3B. Academic Press Inc., New York.
14. Peterson, J. E., and J. C. McDonald. 1966. The demise of the myxobacterial genus *Angiococcus*. Mycologia 58:962-965.
15. Raverdy, J. 1973. Sur l'isolement et l'activite bacteriolytique de quelques myxobacteries isolees de l'eau. Water Res. 7:687-693.
16. Singh, B. N. 1947. Myxobacteria in soils and compost: their distribution, number and lytic action on bacteria. J. Gen. Microbiol. 1:1-12.
17. Stanier, R. Y. 1942. The *Cytophaga* group: a contribution to the biology of the myxobacteria. Bacteriol. Rev. 6:143-156.