The Distribution of Some Genera of Filamentous Bacteria in Littoral and Profundal Lake Sediments

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The distribution of several genera of filamentous bacteria was determined in profundal and littoral sediments of three lakes of differing trophic status. The bacteria were tentatively identified on the basis of cytochemical tests and microscopic observations of organisms in wet mount preparations and on agar-coated and uncoated slides incubated in the sediment cores. The population density and the diversity of the filamentous bacteria increased with increasing nutrient content of the lakes. The general observations of higher numbers in the profundal than in the littoral zone and a decrease in population density with sediment depth were confirmed for most genera. Some of the genera may be microaerophiles. An affinity for surfaces was observed. *Pseudanabaena* usually occurred only in littoral samples and *Beggiatoa* only in the two richer lakes. *Vitreoscilla* was found mainly in the two richer lakes and *Leptothrix* was present in large numbers in all three lakes.

INTRODUCTION

Filamentous bacteria comprise a group of organisms which is morphologically distinct but does not necessarily possess a unifying metabolic feature. Although many different genera of filamentous bacteria have been observed in a variety of habitats and over a considerable period of time (Kolk, 1938; Skuja, 1948, 1956, 1958; Pringsheim, 1951; Maier & Murray, 1965; van Veen *et al.*, 1973; Pierson & Castenholz, 1974; Dubinina & Gorlenko, 1975), many of them have never been cultured and some are still incompletely described. Godinho-Orlandi & Jones (1981) made quantitative estimates of the total population of filamentous bacteria in sediments of lakes ranging from eutrophic to oligotrophic. Their survey demonstrated that the total population of filamentous bacteria was positively correlated to the trophic status of the lakes, and that they were more numerous in profundal than in littoral sediments and occurred predominantly in a narrow zone near the sediment–water interface. Although few in number when compared with the total population of bacteria, they were probably of considerable significance in terms of biomass. This paper deals with the vertical distribution of filamentous bacteria in littoral and profundal sediments in three selected lakes of differing degrees of nutrient enrichment. The work had two main aims: first, to provide tentative identification of the bacteria, and second, to determine the depth distribution of the individual groups.

The E_h profiles of the three lakes taken together with the observations of Godinho-Orlandi & Jones (1981) suggested that many filamentous bacteria may occur in zones where oxygen concentrations are extremely low. These observations were, however, based on samples taken at relatively large depth intervals (5 mm). To obtain more information, shallower sampling intervals (2 mm) were employed and the genus composition of the populations was analysed.

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The majority of the genera were not isolated and were tentatively identified on morphological and cytochemical characteristics.

METHODS

Sampling. The three lakes studied were Wastwater, Windermere, South Basin and Blelham Tarn. They were selected from the 17 lakes surveyed by Godinho-Orlandi & Jones (1981) as representative of the oligotrophic, mesotrophic and eutrophic states, respectively. Profundal sediment samples were taken with a Jenkin surface mud corer near the deepest point and littoral sediment samples at the nearest point to the shore with soft sediment, which corresponded to a depth of about 15% of that in the deepest point. All samples were taken during summer stratification, before and after deoxygenation of the profundal sites. The sediment cores were extruded and sub-samples were removed at 2 mm depth intervals.

Counts of filamentous bacteria on agar-coated slides. The procedure was that described by Godinho-Orlandi & Jones (1981). Uncoated glass slides were also incubated in the sediment cores in the same manner.

'Agar sausage' technique. Membrane-filtered lake water was boiled with 0.25% (w/v) agar, and the solution was dispensed into sterile test tubes and allowed to solidify. The agar sausages were removed by heating the closed end of the test tubes. Some agar sausages were inserted into the sediment and others were placed horizontally at the sediment-water interface. They were incubated for 7 d at 15 °C.

Direct counts in wet mounts. The method used was based on that described by Edmondson (1974), the details of which are given by Godinho-Orlandi & Jones (1981).

Cytology and cytochemical tests. Uncoated glass slides incubated in the sediment for periods varying from 7 h to 7 d were used for these tests (the agar coating interfered with many of the staining procedures used). The slides were removed from the sediment, wiped clean on the underside and examined by phase-contrast microscopy at a magnification of $\times 400$. The position of individual filamentous bacteria was recorded by a rough sketch map and the readings on the microscope stage verniers. Both wet mount and dried slides were used. In the latter case the slides were re-examined immediately after drying. The dried slides were gently fixed by heat. Cytological features were examined as follows. Lipid inclusions were stained according to Burdon (1946) and the methods for volutin granules, cell wall stain, spore stain and Gram stain were those described by Norris & Swain (1971). Sulphur inclusions were dispersed by drawing pyridine into a wet mount while the organism was observed under a microscope placed in a fume cupboard. The test for iron deposition was performed on a heat-fixed slide. This was covered with 2% (w/v) $K_4Fe(CN)_6 \cdot 3H_2O$ and left to stand for 15 min. The excess solution was drained off and the slide was dried in air. The slide was then covered with 0.2% (v/v) HCl for 10 s, rinsed with tap water and dried at room temperature. Sheaths containing iron were stained blue. A modification of the technique described by Farquhar & Boyle (1971) was used to demonstrate the presence of a sheath. The area of the heat-fixed slide containing filamentous bacteria was ringed with petroleum jelly and 400 μ l each of lysozyme (125 μ g ml^{-1}) and EDTA (500 μ g ml^{-1}) were then added. The slide was incubated in a high-humidity chamber at 37 °C for 45 min. 800 μ l sodium lauryl sulphate was added to a final concentration of 0.01M and the slide was incubated for a further 30 min at 27 °C. The ring of grease was removed, and the slide was allowed to dry and stained with Hucker's crystal violet. The empty sheath stained pale purple. To test for gas vacuoles the wet mount was examined microscopically, then transferred to a pressure cell similar to that described by Walsby (1974) and exposed to a pressure of 965 kPa. The pressure was raised quite rapidly, within 5 to 10 s, and immediately released at about the same speed. The preparation was re-examined microscopically and the bright refractile granules which disappeared after this treatment were considered to be gas vacuoles. The method for staining flagella was that of Mayfield & Inniss (1977). Chlorophyll fluorescence in the filamentous bacteria was tested under a Leitz Orthoplan microscope equipped with a Ploem illuminator and the filter combination described by Godinho-Orlandi & Jones (1981).

Tentative identification of filamentous bacteria. In addition to the tests listed above, morphological features used in the identification scheme included cell and filament dimensions, motility, the presence or absence of branching, and colour under phase-contrast illumination. Preliminary trials demonstrated that the available keys were inadequate for the characterization, to genus level, of filamentous bacteria in freshwater sediments. This was partly due to instability of features such as sheath formation; this is discussed later. A tentative key was therefore drawn up based on morphological and cytochemical characteristics (Godinho-Orlandi, 1980).

Analysis of data. Replicate cores were taken at each site and a total of 50 fields per sample were counted. In theory, this should ensure that the means of the counts would be normally distributed (central limit theorem). In practice, we found the data to be extremely variable, and attempts to determine the parent distributions of the individual populations and to find a suitable transformation for the data failed. Non-parametric statistical tests were used to test differences between samples. Wilcoxon's signed rank test allowed differences between depth profiles from different sites to be compared.

RESULTS AND DISCUSSION

The initial observations of filamentous bacteria were made on wet mounts of diluted sediment samples examined by phase-contrast microscopy. Preliminary trials were then run to determine the effect of the slide surface coating on the number of filamentous bacteria in order to find a technique which would encourage their growth. Agar coating proved to be the best (Godinho-Orlandi & Jones, 1981). Extensive observations of the filamentous bacteria were made using agar-coated slides incubated in the sediment cores. Several attempts were made to obtain pure cultures of these groups; these were described by Godinho-Orlandi (1980). During the trials for isolation only two genera were isolated, and were tentatively identified as *Cytophaga* and *Pseudanabaena*. The presence of larger numbers of unicellular bacteria which readily outgrew the filamentous forms in the enrichment cultures was a major problem.

Various procedures to reduce the number of unicells, including the use of antibiotics, failed. If viable isolates of filamentous bacteria were to be obtained from sediments, then alternative enrichment techniques, not based on classical clinical procedures, would have to be developed. Among the different techniques used, the agar sausage technique provided particularly good enrichments of *Pelonema*. This, and the results obtained with agar-coated slides, suggested that the various media tried did not reflect the chemical conditions in the sediment, and also that the presence of a surface may be important. In addition, there is increasing evidence that considerable care in controlling oxygen concentration may be necessary if microaerophiles are to be grown successfully (Stouthamer *et al.*, 1979).

The problems encountered in attempting to obtain pure cultures of a significant proportion of the filamentous bacteria compelled us to use cytochemical tests as aids to tentative identification of the filamentous bacteria. The characteristics chosen were those used in *Bergey's Manual* (Buchanan & Gibbons, 1974) and Eikelboom (1975). Some problems were encountered, the major one being concerned with sheath formation. Organisms were observed to migrate out of their sheath and to exhibit gliding motility. Dubinina (1976) reported the migration of filaments of *Leptothrix* out of their sheaths which had become heavily impregnated with iron, impeding the diffusion of nutrient to the cells. Conversely, other gliding filaments which did not possess a sheath when motile were observed with such structures when stationary. Lewin (1970) pointed out that the secreted slime trails of gliding organisms could accumulate and form a sheath and this could, in some cases, impede motility. These properties could lead to confusion in the identification of certain organisms, e.g. *Leptothrix* and *Vitreoscilla*. Such observations raised doubts as to whether sheath formation is a sufficiently stable characteristic (or the condition under which the sheath was produced is sufficiently well understood) for it to be used for identification at this level (Lewin, 1970).

Although there must be reservations about identifying filamentous bacteria by direct observations, an attempt was made to obtain some information about the distribution of individual groups in three lakes – Wastwater (oligotrophic), Windermere, South Basin (mesotrophic) and Blelham Tarn (eutrophic) – during summer stratification. Direct counts in wet mounts provided an estimate of the real distribution of the bacteria within the sediment, and the agar-coated slides, used as an enrichment technique, provided a microclimate to encourage the growth of a greater variety of organisms. The general observation of higher numbers of bacteria in the profundal than in the littoral sediments (Willoughby, 1969; Jones, 1980; Godinho-Orlandi & Jones, 1981) was confirmed for many individual groups. Counts of the majority of filamentous bacteria decreased with increasing sediment depth. Large numbers of empty sheaths encrusted with iron were observed in Wastwater.

The genera of filamentous bacteria most commonly encountered were *Vitreoscilla*, *Leptothrix*, *Flexibacter*, *Beggiatoa*, *Achroonema*, *Cytophaga*, *Pelonema*, *Bactosquilla*, *Peloploca* and *Pseudanabaena*, some of which are illustrated as they appear on agar-coated slides in Fig. 1. Two distinct forms tentatively assigned to the genus *Flexibacter* were

Fig. 1. Examples of the filamentous bacteria of different genera observed on agar-coated slides under phase-contrast illumination: (a, b) *Vitreoscilla* spp.; (c, d) *Leptothrix* spp.; (e, f) *Flexibacter* II – (f) on uncoated glass slide under bright-field illumination; (g) *Flexibacter* I; (h) *Peloploca* (arrowed); (i) *Pelonema* (on agar sausage); (j) *Bactoscilla*; (k) *Achroonema*; (l) *Cytophaga*; (m) *Beggiatoa leptomitiformis*; (n) *Pseudanabaena*. The bar markers represent 10 µm. GV, gas vacuoles; SG, sulphur granule; VG, volutin granule.

Fig. 2. Vertical distribution of *Leptothrix* spp. and *Flexibacter* I in the profundal sediments of Windermere, South Basin, from counts in wet mount preparations and on agar-coated and uncoated slides. The bars indicate 95% confidence limits.

Fig. 3. Vertical distribution of *Flexibacter* I and *Flexibacter* II in the profundal sediments of Wastwater, Windermere, South Basin and Blelham Tarn, from counts on agar-coated slides. The bars indicate 95% confidence limits.

recognized. *Flexibacter* I was lipid positive and moved more rapidly than *Flexibacter* II, which contained refractile granules. Sediment population profiles were obtained for all the genera listed above, but only a few results are presented here to summarize the trends. The counts of *Leptothrix* and *Flexibacter* I in Windermere profundal sediments (Fig. 2) illustrate the differences between wet mounts, agar-coated slides and uncoated slides. Even when the slide counts were corrected to a volume basis they were still lower than those obtained with wet mounts. Counts of the two distinct forms of *Flexibacter* on agar-coated slides illustrate the differences between the three lakes sampled (Fig. 3). The counts in Windermere and

Fig. 4. Vertical distribution of *Leptothrix* spp. in the littoral and profundal sediments of Wastwater, from counts in wet mount preparations. The bars indicate 95 % confidence limits.

Fig. 5. Vertical distribution of *Vitreoscilla* spp., *Leptothrix* spp. and *Flexibacter* I in the littoral and profundal sediments of Windermere, South Basin, from counts in wet mount preparations. The bars indicate 95 % confidence limits.

Blelham Tarn were significantly higher than those in Wastwater ($P < 0.05$) but the organisms had a more restricted depth distribution in Blelham Tarn (the more eutrophic lake). Although *Flexibacter* II was found to depths of 12 mm in Wastwater, the population density was so low that a reliable estimate could not be made from the number of sub-samples counted ($n = 50$). One example of the difference between littoral and profundal sediment populations is shown in Fig. 4: in Wastwater, *Leptothrix* had a more restricted depth range in the profundal sediments, but at depths where it was found the population estimates were significantly higher ($P < 0.05$) than those in the littoral zone. Larger numbers of *Vitreoscilla*, *Leptothrix* and *Flexibacter* I were also found in the profundal than in the littoral samples of Windermere and Blelham Tarn (Figs 5 and 6), although marked differences in depth distribution were not observed in these lakes.

We have tried to summarize the results obtained with wet mounts for all the genera in terms of the highest counts obtained, whether each genus occurred at a greater depth in the littoral zone or occurred at the sediment–water interface (Table 1). In Blelham Tarn the counts for all groups were higher in the profundal zone. In Windermere the counts were also higher at the profundal site, except for *Beggiatoa leptomitiformis*, *Pseudanabaena* and *Flexibacter* II, which were found only in the littoral zone. The organism designated Group 10

Fig. 6. Vertical distribution of *Vitreoscilla* spp. and *Flexibacter* I in the littoral and profundal sediments of Belham Tarn, from counts in wet mount preparations. The bars indicate 95 % confidence limits.

Table 1. Comparison of the vertical distribution of filamentous bacteria (counted in wet mount preparations) in the littoral and profundal sediments of three lakes

Organisms	$10^{-4} \times$ Maximum count of filaments observed in sediment (g dry wt) $^{-1}$					
	Wastwater		Windermere, South Basin		Belham Tarn	
	Littoral	Profundal	Littoral	Profundal	Littoral	Profundal
<i>Vitreoscilla</i> spp.	—	—	5.5 (11.1)*	22.4 (44.6)†	32.6 (32.2)†	146.9 (109.7)
<i>Leptothrix</i> spp.	27.8 (27.8)†	41.6 (51.9)†	13.1 (19.5)†	82.5 (54.9)†	5.4 (11.3)*	19.9 (39.7)
<i>Flexibacter</i> I	—	—	6.5 (13.0)*†	22.4 (44.6)†	16.3 (24.2)†	515.0 (335.5)†
<i>Beggiatoa</i>	—	—	12.2 (24.4)†	—	14.9 (29.4)*†	619.5 (748.4)†
<i>leptomitiformis</i>						
<i>Achroonema</i>	—	—	—	5.1 (10.3)	5.6 (11.6)*	289.8 (189.0)†
<i>Flexibacter</i> II	—	—	9.8 (14.7)	—	—	232.4 (174.3)†
<i>Cytophaga</i>	—	—	—	—	—	465.3 (283.9)†
<i>Pelonema</i>	—	—	—	—	—	515.0 (283.9)†
<i>Bactoscilla</i>	—	—	—	—	5.4 (11.3)*	20.7 (40.9)†
<i>Peloploca</i>	—	—	—	—	—	104.5 (154.8)†
<i>Beggiatoa</i> spp.	—	—	—	56.5 (30.7)	64.5 (44.3)†	79.9 (79.8)
<i>Pseudanabaena</i>	11.2 (16.7)†	—	22.1 (66.9)†	—	—	—
Group 10	—	—	—	9.2 (18.3)†	—	—
Unidentified forms	6.1 (12.1)*	61.9 (49.3)†	24.5 (19.6)*	54.1 (43.4)*	21.9 (22.0)*	494.2 (412.9)†
Empty iron sheaths	10.2 (10.2)	164.9 (62.9)†	—	—	—	—

The 95 % confidence limits are shown in parentheses. —, Not detectable.

* Maximum count was at greater depth in littoral than profundal sediments.

† Organisms peaking within 0 to 6 mm depth of sediment.

(Fig. 7a) did not fit into any of the genera described in *Bergey's Manual* (Buchanan & Gibbons, 1974). It was a slender filament usually found within a sheath encrusted with iron. The filaments were straight, but not consistently so, and non-motile, although a very gentle

Fig. 7. Filamentous bacteria observed on agar-coated slides incubated in sediment cores for 7 d at 8 °C. These bacteria were designated Group 10 (arrowed in *a*) and Group 2c (*b*, *c*, *d*). A filament encrusted in iron is shown in (*d*). No counterparts for these organisms were found in *Bergey's Manual* (Buchanan & Gibbons, 1974) and brief descriptions are provided in the text. The bar markers represent 20 µm. AT, attachment point.

almost imperceptible movement along the filament was observed very rarely in those where the sheath was not encrusted with iron. The filaments were 0.2 to 0.5 µm wide by 12 to 260 µm long. Cytochemical tests showed that the organism was sheathed and iron was usually deposited in this sheath. Results of tests for lipid inclusions, spores, gas vacuoles, Gram stain and chlorophyll fluorescence were all negative.

Counts of filamentous bacteria using agar-coated and uncoated slides were made above and below the sediment–water interface. The counts below the interface again showed that, in most cases, the numbers of filamentous bacteria decreased with increasing depth. The highest counts on that part of the slide exposed to the water were usually between 0 and 4 mm above the sediment–water interface, and the numbers decreased with increasing distance from the sediment surface. Tables 2 and 3 summarize the differences obtained between profundal and littoral sites and also between the counts obtained above and below the sediment surface. The organisms which peaked at or near the sediment surface (between 0 and 6 mm depth) are also shown. The flexibacters (I and II) were usually more numerous at the littoral site. Group 2c organisms (Fig. 7*b*, *c*, *d*), observed mainly in Windermere, South Basin, were another group which did not correspond to any genus described in *Bergey's Manual* (Buchanan & Gibbons, 1974). The filaments (35 to 120 µm long) were attached at one end and contained

Table 2. Comparison of the vertical distribution of filamentous bacteria counted on agar-coated slides incubated in the littoral and profundal sediments of three lakes

Organisms	$10^{-2} \times$ Maximum count of filaments cm^{-2}					
	Wastwater		Windermere, South Basin		Blelham Tarn	
	Littoral	Profundal	Littoral	Profundal	Littoral	Profundal‡
<i>Vitreoscilla</i> spp.	—	0.13 (0.3)	3.1 (2.2)	2.8 (1.4)†	9.6 (3.8)†	0.1 (0.3)†
<i>Leptothrix</i> spp.	—	—	—	3.7 (1.8)†	0.5 (0.7)†	—
<i>Flexibacter</i> I	101 (30)*†	0.9 (0.6)†	1.9 (1.5)†	3.2 (1.6)†	32.9 (10.9)†	4.5 (2.7)†
<i>Beggiatoa</i>	—	—	—	0.3 (0.4)	2.1 (0.9)	0.3 (0.4)†
<i>leptomitiformis</i>	—	—	—	—	—	—
<i>Achroonema</i>	—	—	—	—	0.4 (0.5)	0.3 (0.4)†
<i>Flexibacter</i> II	0.9 (1.4)*†	0.06 (0.13)†	1.9 (0.9)†	0.9 (0.7)	2.4 (1.2)	1.7 (1.2)†
<i>Cytophaga</i>	—	—	—	—	0.1 (0.3)†	2.9 (1.7)†
<i>Pelonema</i>	—	—	—	—	—	0.8 (0.7)†
<i>Bactoscilla</i>	—	—	0.8 (0.6)*†	—	4.8 (1.3)†	—
<i>Peloploca</i>	—	—	—	—	—	—
<i>Beggiatoa</i> spp.	—	—	—	0.3 (0.5)	2.8 (1.3)†	0.1 (0.3)†
<i>Pseudanabaena</i>	8.7 (4.9)*†	0.2 (0.4)*	1.7 (1.0)†	—	1.2 (0.8)	—
Group 10	—	—	—	42.8 (13.4)†	—	—
Group 2c	—	—	0.4 (0.8)†	1.8 (1.3)*†	1.7 (1.2)†	—
Unidentified forms	5.3 (4.0)*†	0.06 (0.2)†	0.5 (0.6)†	2.1 (1.1)†	1.2 (0.8)	0.6 (0.7)†
Empty iron sheaths	—	0.13 (0.3)†	—	—	0.3 (0.4)	—

The 95% confidence limits are shown in parentheses. —, Not detectable.

* Count was higher above the sediment–water interface than below.

† Organisms peaking within 0 to 6 mm depth of sediment.

‡ Counts done below sediment–water interface only.

Table 3. Comparison of the vertical distribution of filamentous bacteria counted on uncoated slides incubated in the littoral and profundal sediments of three lakes

Organisms	$10^{-2} \times$ Maximum count of filaments cm^{-2}					
	Wastwater		Windermere, South Basin		Blelham Tarn	
	Littoral	Profundal	Littoral	Profundal	Littoral	Profundal‡
<i>Vitreoscilla</i> spp.	—	0.06 (0.13)*†	0.4 (0.5)*†	2.4 (2.3)†	6.6 (4.4)†	0.1 (0.3)†
<i>Leptothrix</i> spp.	—	0.06 (0.13)*†	—	1.4 (0.9)†	0.8 (0.7)†	—
<i>Flexibacter</i> I	10.3 (4.9)†	0.3 (0.3)*	3.5 (1.9)*†	1.8 (1.1)†	8.6 (3.4)†	0.5 (0.2)†
<i>Beggiatoa leptomitiformis</i>	—	—	0.1 (0.3)†	0.3 (0.4)†	0.9 (0.6)†	1.5 (1.3)†
<i>Achroonema</i>	—	—	—	—	0.1 (0.3)†	0.9 (0.9)†
<i>Flexibacter</i> II	—	—	2.11 (1.1)*†	0.4 (0.5)†	1.0 (0.9)†	0.5 (0.6)†
<i>Cytophaga</i>	0.4 (0.8)†	0.06 (0.13)†	—	—	—	4.6 (2.6)†
<i>Pelonema</i>	—	—	—	—	—	1.8 (1.3)†
<i>Bactoscilla</i>	—	—	1.5 (0.8)*†	—	2.3 (1.0)*†	—
<i>Peloploca</i>	—	—	—	—	—	—
<i>Beggiatoa</i> spp.	—	—	—	—	4.1 (1.6)†	0.4 (0.5)†
<i>Pseudanabaena</i>	1.3 (0.7)*†	—	2.3 (1.1)*†	—	6.6 (1.8)†	—
Group 10	—	—	0.1 (0.3)†	39.9 (9.4)†	—	—
Group 2c	—	—	0.4 (0.6)*†	0.6 (0.6)†	1.2 (1.2)†	—
Unidentified forms	6.2 (8.9)†	0.3 (0.3)†	0.5 (0.5)†	1.7 (0.9)†	0.3 (0.4)*	3.7 (1.9)†

The 95% confidence limits are shown in parentheses. —, Not detectable.

* Count was higher above the sediment–water interface than below.

† Organisms peaking within 0 to 6 mm depth of sediment.

‡ Counts done above sediment–water interface only.

cells 0.9 μm by 2.0 μm . Cell length could vary along a single filament. The long filaments were characteristically bent, giving the impression that they had been broken, and were, in certain cases, impregnated with iron. The filaments were occasionally observed leaving the

Table 4. *Effect of the counting method on the number of groups of filamentous bacteria observed in sediments of three lakes*

Counting method	Littoral sediment			Profundal sediment		
	Wastwater	Windermere, South Basin	Blelham Tarn	Wastwater	Windermere, South Basin	Blelham Tarn
Wet mount	2	6	8	1	6	12
Agar-coated slides	3	6	12	3	9	8
Uncoated slides	4	9	11	5	9	8

sheath (speed about $0.07 \mu\text{m s}^{-1}$) whereupon they became difficult to distinguish from unsheathed gliders. Cytochemical tests confirmed the presence of cell wall, sheath, volutin granules and iron deposition. Gas vacuoles were sometimes present but tests for sulphur inclusions, spores, flagella, Gram stain and chlorophyll fluorescence were negative.

Some organisms were absent from certain lakes regardless of the technique of enrichment and enumeration used. These were as follows: Wastwater – *Beggiatoa leptomitiformis*, *Achroonema*, *Pelonema*, *Bactoscilla*, *Peloploca*, *Beggiatoa* spp., Groups 10 and 2c; Windermere, South Basin – *Cytophaga*, *Pelonema* and *Peloploca*; Blelham Tarn – Group 10. The increasing diversity of filamentous bacteria with increasing lake trophic status is summarized in Table 4.

Among the particular groups observed, *Pseudanabaena* was usually found only in littoral samples, presumably because of its requirement for light. *Beggiatoa* spp. were found only in Windermere, South Basin and Blelham Tarn, and in Windermere, South Basin the higher numbers occurred at greater depths in the profundal zones. *Beggiatoa* spp. other than *Beggiatoa leptomitiformis* were not found in the littoral zone of Windermere, South Basin. These observations might be explained by the greater organic input in the richer lakes, thus creating a potential for sulphate reduction, generating the sulphide ion used by the *Beggiatoa* (all those observed on the slides contained large numbers of sulphur granules). *Vitreoscilla* spp. were found mainly in the two richer lakes and at a greater depth in the littoral sediment suggesting that these organisms may also have a preference for low oxygen tensions. It was, however, impossible to deduce the physiological characteristics of an organism from the depth at which it occurred in the sediment: although oxidizing E_h values may be observed this is not proof that oxygen is available at a given depth (Revsbech *et al.*, 1980). *Leptothrix* spp., on the other hand, were present in large numbers in Windermere, South Basin and Wastwater possibly suggesting a definite requirement for oxygen.

Filamentous bacteria which deposited iron were observed more consistently in Windermere, South Basin than in the other two lakes. This may reflect the intermediate status of Windermere, South Basin where oxygen concentration is reduced (but not to zero) and the E_h discontinuity layer migrates upward in the profundal sediment (Mortimer, 1942) to an extent sufficient to provide a source of iron. A similar population of iron depositors might be expected to develop in Blelham Tarn during the critical period when oxygen and iron concentrations were suitable. In such a eutrophic lake this period would be very short (Jones, 1975) and could easily be missed with all but the most careful sampling programmes.

Some bacteria peaked only at the surface of the sediment in wet mount, and above the sediment (i.e. in the water column) on slide cultures, suggesting that these organisms were aerobes. There was very little evidence to suggest that some bacteria were obligate anaerobes. However, the fact that, in contrast to the results for the other two lakes, the slides incubated in Blelham Tarn cores showed a lower diversity of filamentous bacteria than the wet mounts (Table 4) suggests that some organisms might have been inhibited by the inevitable introduction of small quantities of oxygen during the handling procedure.

Although we have failed to isolate the majority of the filamentous bacteria observed, this paper provides some systematic information on their population densities and the freshwater

habitats where they may be found. Although low in numbers, biomass estimates (Godinho-Orlandi & Jones, 1981) suggest that they are a significant component of the sediment bacterial flora.

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