SOME OBSERVATIONS ON THE ABSENCE OF BACTERIA FROM ACID WATERS IN NORTHWEST ENGLAND

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Introduction

The effect of acidity is most marked in nutrient-poor waters and this is reflected in its impact on the biota at all levels. The effect may be direct, for example on the physiology of fish and invertebrates, may involve mobilization of the toxic element aluminium, may reduce primary production by altering the community structure of the algae, or may be indirect through its impact on the processing of organic carbon. We do not intend to provide a major review of the acid rain literature, as this information is readily available elsewhere (Norton et al. 1989) as are reports of detailed case studies (Kerekes 1989). In this brief report we shall concentrate on the effect of low pH on the initial stages of decomposition and the conditioning of incoming particulate carbon or detritus by microbes, particularly certain genera of filamentous bacteria.

In nutrient-rich waters, primary production and the sedimentation of the remains of the primary producers result in the development of an organic-rich benthic layer. This, in turn, permits the development of anaerobic zones; indeed, the depth to which aerobic decomposition occurs rarely exceeds a few millimetres. In the presence of suitable electron acceptors, particularly nitrate and sulphate, populations of denitrifying and sulphate-reducing bacteria develop, and the end-products of their metabolism result in a net generation of alkalinity (Hanselman 1986; Kelly et al. 1982; Schindler et al. 1986; Davison & Woof 1990). These processes have been put to good effect in the recovery and management of extremely acid waters (Davison 1990; Davison et al. 1989).

The effects of acidification are therefore most likely to be observed in well-oxygenated, nutrient-poor waters, in which there is less scope for the full range of bacterial metabolism to be expressed. This is not to imply that the inhibition of anaerobic metabolism is the sole controlling factor; there is an even greater wealth of evidence for the direct impact of low pH and aluminium toxicity on the biota of such waters. However, with a few exceptions (Gahnstrom & Fleischer 1985; Hoeniger 1985), in which the methods used were either environmentally irrelevant or of

insufficient sensitivity, most studies have shown that decomposition is slower in such susceptible, nutrient-poor acid waters (Hoeniger 1986; Hildrew et al. 1984; Burton et al. 1985; Mackay & Kersey 1985; Rao et al. 1984; Rimes & Goulder 1986; Traaen 1980). In some of these investigations, low pH apparently had no effect on the numbers of bacteria present, but.activity per unit cell number and/or the mean cell volume were both reduced under acid conditions.

Although bacterial decomposition processes have provided the focus for attention in many of the above reports, little attention has been given to the composition of the bacterial community and the role of its component parts, particularly in nutrient-poor waters which are provided with sources *of* organic carbon and reducing power in the form of poor quality detritus.

The study sites

Previous long-term research conducted at Windermere, particularly that of Carrick & Sutcliffe (1983) and Sutcliffe & Carrick (1983, 1986) provided a wealth of background information which permitted the selection of sites of widely ranging pH and alkalinity in Cumbria, north-west England. The subjective assessment of many researchers had been that detritus decomposed more slowly in the acid waters and that this was associated with changes in the invertebrate fauna. The purpose of our brief investigation was to determine whether any differences in the bacterial flora might account for these observations. We decided to examine the aerobic bacterial populations of detritus in fast-flowing, well-oxygenated streams. These would be the most susceptible to acid conditions as their metabolic end-products would not generate any acid-neutralizing capacity.

The sites were chosen to include the major underlying geological strata found in the region (Fig. 1). A preliminary study was undertaken in the upper basin of the River Duddon (on bedrocks of the Borrowdale Volcanic Series). Two streams, designated 6 and *f* in Fig. 1, were approximately 1 km apart and drained the same side of the valley (Figs 2, 3). These are small streams ranging from 30 cm to 1 m in width, with fast-flowing well-aerated water. The water in stream *b* had a mean annual pH of 6.7 whereas that in stream /was 4.9. The vegetation on the banks of both streams and on the surrounding land was largely composed of *Nardus stricta* L. (moor mat grass) but mixed swards of other grasses were also present. The main input of detritus into the streams occurred during the winter in the form of dead leaves and stems of *N. stricta,* particularly after snowfall and ensuing freeze-thaw cycles. This material and the water in the streams were sampled.

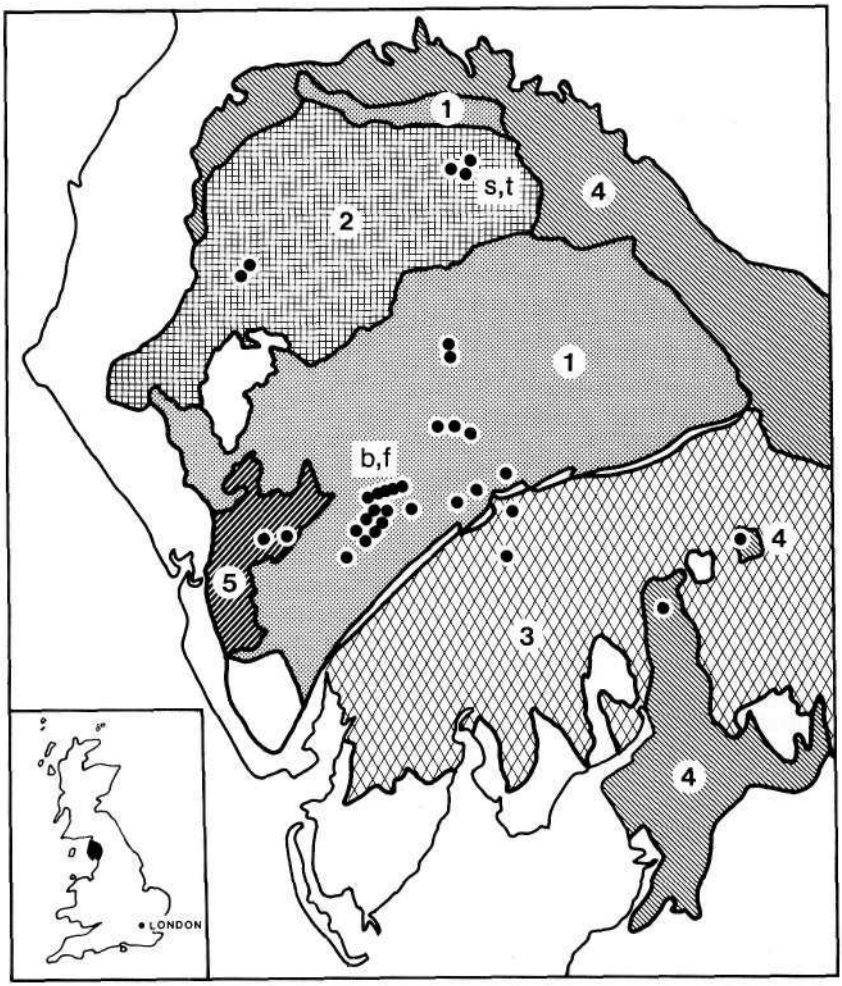

FIG. 1. Map of the sampling sites in Cumbria used in this study. Underlying geology is numbered as follows: 1, Borrowdale Volcanics; 2, Skiddaw Slates; 3, Silurian Slates; 4, Carboniferous series; 5, igneous intrusions. Inset is a map indicating the position of Cumbria in northwest England.

FIG. 2. A small circumneutral side-stream (*b*) in the upper Duddon basin.

FIG. 3. The upper basin of the River Duddon, viewed from Wrynose Pass, with roadway (centre left) and the stony bed of the River Duddon (centre right), small side-streams *b* and *f* (see Figs 1, 2) drain the slopes in the foreground on the left-hand side of the picture.

The bacterial population and its activity

Although it is recognized that dilution plating of natural samples onto artificial media will not provide quantitative information about the bacterial population, the technique may provide an early indication of gross differences in the composition of the microflora. In this instance the samples were diluted and spread on casein-protein-starch agar (Jones 1970). A cursory examination of the agar plates showed immediately that there was a marked difference in the bacterial populations which had developed. The bacterial colonies from the acid stream /were small and mostly white or colourless. The bacteria from the circumneutral stream *b,* on the other hand, often produced spreading, pigmented colonies, most of which were yellow. The colonies are shown in the colour plate on the front cover of this issue of the Forum (with a legend on the inside front cover at the bottom of the page). This provides only a qualitative assessment and therefore both water and detritus were examined in more detail. Direct counts were performed by epifluorescence microscopy (Jones & Simon 1975) and microbial activity was measured as oxygen uptake, carbon dioxide evolution and tetrazolium dye reduction. The results (Table 1) show that, in spite of

Table 1. The chemistry and microbiology of streams *b* and *f* in the upper Duddon valley, Cumbria, northwest England. The term "*Cytophaga*" is used to describe bacteria which form distinctive yellow spreading colonies on the agar medium (see colour plate on the front cover). The reasons for assigning them tentatively to this genus are given in the text. Ranges were obtained over 2 years, single determinations were made for a few variables.

Variables	*Stream b*	*Stream f*
WATER		
pH	6.3 – 7.1	4.6 – 5.1
Alkalinity (μequivalents l^{-1})	102 – 164	-11 – -15
Bacteria per ml (direct counts)	1×10^5	1.1×10^5
Bacteria per ml (plate counts)	$2.6 – 9.7 \times 10^3$	$2.2 – 14.0 \times 10^3$
"*Cytophaga*" spp. per ml	8 – 64	0 – 8
DETRITUS*		
Bacteria per g (direct counts)	$0.8 – 9.7 \times 10^{10}$	$0.7 – 2.9 \times 10^{10}$
Bacteria per g (plate counts)	$3.6 – 7.6 \times 10^8$	$0.6 – 1.9 \times 10^8$
"*Cytophaga*" spp. per g	$3.3 – 4.9 \times 10^7$	0
Oxygen uptake (μmol g^{-1} d^{-1})	490	200
CO_2 evolution (μmol g^{-1} d^{-1})	241 – 270	131 – 156
Tetrazolium dye reduction (μmol g^{-1} d^{-1})	4.2	1.2

*Values given are per gram dry weight of detritus.

FIG. 4. Stream *s*, an acid stream on Skiddaw Slates in the north of the English Lake District.

the marked difference in pH and alkalinity between the two streams, there was little difference in the total bacterial population of the water and only a slightly smaller bacterial count under acid conditions. However, all measurements of activity indicated that decomposition was slower at the lower pH.

As mentioned above, one of the most marked differences was in the absence of yellow spreading colonies on plates inoculated with detritus from the more acidic waters. These colonies are distinctive and are often found when freshwater samples are examined. However, the organisms responsible belong to a taxonomic group which is currently the subject of some debate. We have tentatively assigned them to the genus *Cytophaga* and give our reasons for doing so later in this report.

The above results were obtained from streams on bedrocks of the Borrowdale Volcanic Series. However, almost identical findings were obtained from an acid stream, pH 4.7 (Fig. 4), and a circumneutral stream, pH 6.6, which drain Skiddaw Slates further north in Cumbria (sites s and *t* in Fig. 1). Direct counts of bacteria on the detritus were 2.2 x 10'° and 2.0 x 10^{10} per g dry wt respectively, and carbon

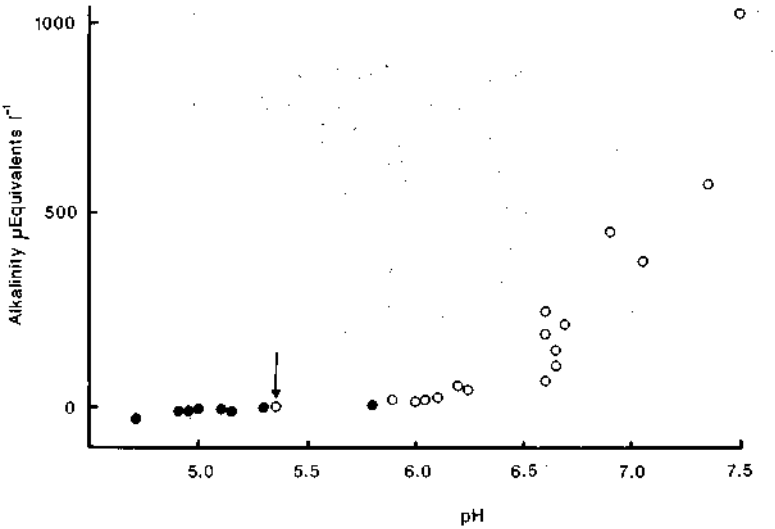

FIG. 5. The presence (O) and absence (●) of bacteria designated *Cytophaga* spp. on decomposing grass stems in Cumbrian streams. The points are plotted in relation to pH and alkalinity of the water. The result marked with an arrow was obtained when the stream was in flood, with attendant soil erosion. These bacteria were consistently absent from detritus placed in acid streams, although they were occasionally observed in the water, particularly under conditions of high flow. Their source remains to be determined.

mineralization was approximately halved in the acid stream. *Cytophaga* spp. were undetectable in the detritus of the acid stream yet colony counts were the equivalent of 7.8 x 10^7 g^{-1} in the other, circumneutral stream. Clearly, it was necessary to compare a wider range of sites. After consultation with Dr D. W. Sutcliffe, 25 streams were examined. To reduce the variability caused by sampling "detritus" of uncertain age, dead stems of *N. stricta* were introduced into the streams and examined after 1 month. The results (Fig. 5) show clearly that the *Cytophaga* spp. were absent from the more acid waters. On the occasions when a few *Cytophaga* isolates were obtained this usually coincided with streams being in spate, or collapse of the banks onto the introduced grass stems. We have yet to determine the source of these bacteria. Similar results were obtained when the same procedure was applied to 10 lakes and tarns of varying pH.

Further evidence for the poor pH tolerance of the bacteria was obtained with the steady loss of *Cytophaga* spp. when detritus from a neutral stream was transferred to an acid site. The bacteria also recolonized detritus moved from acid to neutral water. Addition of limestone chips to an acid stream resulted in the rapid appearance of *Cytophaga* spp. In a study of two lakes in Canada, Hoeniger (1986) reported that bacteria which resembled *Cytophaga* were restricted in their activity at pH values < 5.

The taxonomic status of the bacteria

Although we have tentatively assigned the bacteria which produced the yellow spreading colonies to the genus *Cytophaga* we accept that further study will be required before positive identification can be confirmed. In this instance we considered that it was more important to determine how similar the organisms were than to assign them to a particular genus.

To this end 450 isolates were obtained. Of these, 291 had been specifically selected because of their characteristic spreading growth, although we must record that other, less numerous, colonial morphotypes show a similar, but less marked, site distribution. We chose 34 of these isolates from a wide range of sites and characterized them on the basis of 57 tests. These were conventional phenotypic tests which defined growth characteristics (colour, spreading growth, motility, gliding, flexirubin pigment, Gram stain, growth at 30°C and 37°C), physiology and substrate utilization (oxidation and fermentation of glucose, pectin, aesculin, gelatin and cellulose hydrolysis, $N0_3$ reduction to $N0_2$ and N_2, indole production, assimilation of glucose, arabinose, mannose, mannitol, n-acetyl-glucosamine, maltose, gluconate, caprate, adipate, malate, citrate and phenyl-acetate), possession of certain enzymes (arginine dihydrolase, urease, oxidase, catalase, acid and alkaline phosphatase, esterase, lipase, leucine-, valine- and cystine-arylamidase, trypsin, chymotrypsin, naphthol-as-B1-phosphorylase, α and p galactosidase, (3 glucuronidase, α and p glucosidase, n-acetyl-β-glucosamidase,, α mannosidase, α frucosidase), and sensitivity to selected antibiotics (ampicillin, oxytetracycline, kanomycin, chloramphenicol, streptomycin, erythromycin and 2, 4-diamino-6, 7-di-iso-propylpteridine phosphate (0/129)).

All the isolates responded in an identical manner to 38 of the tests and all those tested were incapable of growth below pH 5.5 on laboratory media. These results indicate a high degree of relatedness and raise the possibility that bacteria which are very similar in function are sensitive to acid conditions in geographically widely separated sites. The bacteria

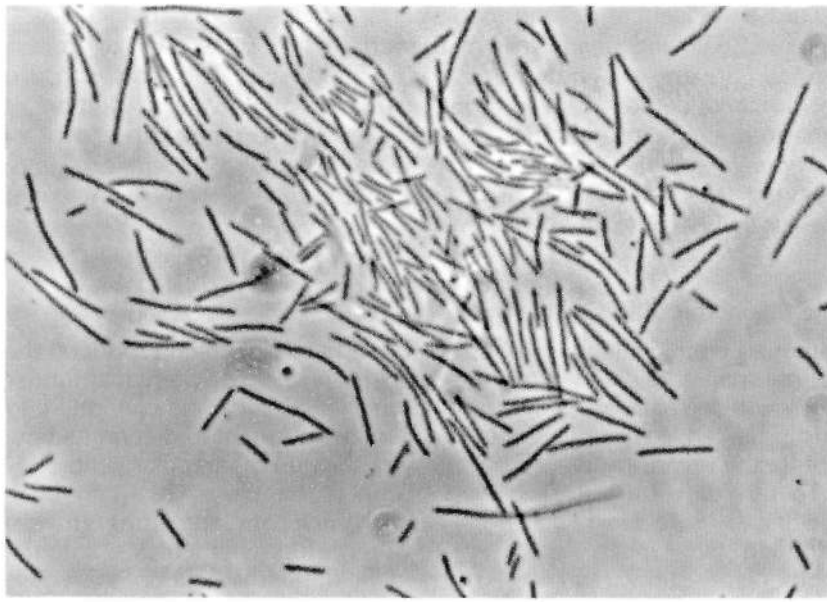

FIG. 6. Phase contrast micrograph of a strain of the gliding bacteria, assigned to the genus *Cytophaga,* which were consistently absent from grass detritus placed in acid waters. The bacteria are 3 – 50 µm in length.

were tentatively assigned to the genus *Cytophaga* on the basis of the test results but particularly because of their yellow pigment, gliding motility and growth on glucose mineral salts medium which contained ammonium as the sole source of nitrogen (Hayes et al. 1979). Individual cells were usually filamentous, being 3 - 5 0 (µm long (Fig. 6) and active gliding was observed at the edges of colonies. The isolates obtained were clearly very similar, but in the case of such a taxonomically "difficult" group as the genus *Cytophaga,* further characterization is required. Small differences in colonial morphology (surface texture, depth of colour) often corresponded to differences in response to the remaining 19 tests upon which there was not complete agreement. We are currently applying the methods of numerical taxonomy to a wider range of isolates while, at the same time, comparing the chromosomal DNA restriction fragments of some isolates from geographically widely separated sites. We thus hope to provide a genetic and phenotypic

analysis of a group of bacteria (tentatively assigned to the genus *Cytophaga)* which consistently exhibit sensitivity to acidic conditions.

The role of the bacteria

Given the taxonomic uncertainty of the isolates and the degree of heterogeneity observed we can only speculate about their role in freshwater systems. The order Cytophagales contains gliding filamentous bacteria, usually growing or isolated at neutral pH. Many members of the group are characterized by their ability to degrade natural polymers and we noted that, of our isolates, all were capable of degrading pectin and 68% also degraded cellulose, and therefore could play an important role in plant polymer decomposition in the streams. This is not to imply that bacteria were the only microbes which might contribute to the differences in decomposition observed, although some fungi are reportedly absent from grass stems in streams of this region with pH < 5.5 (Chamier 1987). The role of microbes in invertebrate nutrition under such circumstances is worthy of further study, but we must now also consider their ability to accumulate elements such as aluminium under acidic conditions (Chamier et al. 1989).

While certain aquatic animals are clearly more sensitive to pH than many microorganisms, perhaps the lower level of detritus "conditioning" due to the absence of these bacteria and other microorganisms in some acid waters also contributes to a reduction in invertebrates of intermediate sensitivity. Detritus "conditioning" generally refers to partial decomposition, with an attendant increase in nitrogen content associated with microbial growth. It was of interest to note that the C:N ratio of detritus in acid streams averaged 46 whereas that in circumneutral streams was 34, and this presumably contributed to their degree of "palatability".

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