The Population Ecology of Iron Bacteria (Genus *Ochrobium*) in a Stratified Eutrophic Lake

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Bacteria assigned to the genus *Ochrobium* have not yet been isolated in pure culture, but have been implicated in the deposition of ferric iron. These organisms have been observed in a number of lakes but the conditions under which active growth occurred have not been defined. A population of *Ochrobium* sp. developed in the anoxic hypolimnion of Esthwaite Water, a eutrophic lake in the English Lake District. Direct counts and experimental evidence obtained with this population suggested that the organism was capable of anaerobic growth. Inhibition of growth by chloramphenicol provided further evidence of its prokaryotic organization. The population overwintered in the sediment and at the onset of summer stratification, with deoxygenation of the hypolimnion, migrated into the water column. There was insufficient evidence to implicate *Ochrobium* sp. in ferric iron deposition.

INTRODUCTION

A few bacteria, including *Thiobacillus ferrooxidans*, obtain energy from ferrous ion oxidation, but a wide variety of bacteria exist which may deposit ferric iron without necessarily obtaining energy from the process. A number of possible mechanisms have been proposed (Glathe & Ottow, 1972) including participation of peroxidases (Dubinina, 1978) and deposition associated with extracellular polymers (Ghiorse & Hirsch, 1979). Many of these bacteria have been shown to be heterotrophs (Cullimore & McCann, 1977; Kullmann & Schweisfurth, 1978) but they include a number of morphologically distinct groups: budding and appendaged bacteria (Ghiorse & Hirsch, 1977; Hirsch, 1968; Kucera & Wolfe, 1957), filamentous bacteria (Dondero, 1975) and encapsulated bacteria of the family *Siderocapsaceae* (Zavarzin, 1974). The taxonomic position of the last group, particularly the genera *Siderocapsa*, *Ochrobium* and *Naumanniella*, is uncertain. Examination of authoritative works, including Beger (1949), Dorff (1934), Skuja (1956) and several later papers, suggests that some confusion might have arisen in their identification. Zavarzin (1974) questioned the validity of the family and considered *Ochrobium* to be a genus *incertae sedis*, possibly even comprising eukaryotic algae of the genus *Pteromonas*. More recently, Dubinina & Zhdanov (1975) have proposed that the genus *Siderocapsa* is indistinguishable from the genus *Arthrobacter*. The original genus has been retained by some authors with species distinguished on morphological features (Svorcova, 1975, 1979). Organisms identified as *Ochrobium* sp. were reported to be prokaryotes containing gas vacuoles (Dubinina & Kuznetsov, 1976) but have yet to be isolated in pure culture (Dubinina, 1976).

Organisms tentatively assigned to the genus *Ochrobium* have been observed in stratified lakes (Dubinina & Kuznetsov, 1976; Drabkova, 1971; Jones, 1978) but details of their population ecology have not been investigated. Earlier observations (Jones, 1978) suggested that these organisms may be capable of anaerobic growth, but the sampling techniques were inadequate to confirm this. The present study examined the population ecology of *Ochrobium*

sp. in more detail, to determine more precisely the depth at which the organism grew in the lake, and to provide more information on its physiological characteristics.

METHODS

Sampling site and procedure. Samples were taken over the deepest point (about 15.5 m) of Esthwaite Water (English Lake District: 54° 22' N, 2° 59' W), a eutrophic lake with a surface area of 1 km² and a mean depth of 6.4 m. The hypolimnion of the lake becomes deoxygenated during summer stratification. Water samples were taken initially with a Friedinger water bottle and subsampled into sterile glass bottles which were flushed with three times their own volume of water before immediate closure. When more precise depth sampling was required a peristaltic pump sampler was used (Cunningham & Davison, 1980). Dissolved O₂ concentration and temperature were measured in the field with a combination thermister-O₂ electrode (Mackereth, 1964). A pressure transducer and a Schenk transparency meter of 0.5 m path length (Sauberer, 1958) were lowered simultaneously through the water column providing a plot of turbidity against depth. The turbidity profiles were used to determine sampling points. All measurements were made relative to the sediment-water interface. Sediment samples were taken with a Jenkin surface mud corer.

Chemical analyses. Total iron concentration was determined according to Mackereth *et al.* (1978) and CO₂ by the head-space gas chromatographic technique described by Jones & Simon (1980).

Direct counts of Ochrobium sp. Water samples were filtered through 25 mm diameter (0.45 µm pore size) Millipore membrane filters. These were dried at 60 °C and cleared with cedarwood oil, and the organisms were counted at a magnification of ×320 under bright-field illumination. A total of 400 organisms were counted, and since these were randomly distributed on the surface of the membrane (Jones, 1975) the 95% confidence limits of the count were ±10% of the mean. Filaments of *Leptothrix* sp. in the samples were counted at the same time. It was impossible to obtain reliable quantitative estimates of *Ochrobium* cells in the sediment but their distribution was determined on agar-coated glass slides. The slides were heated in a bunsen flame, allowed to cool and dipped in 2% (w/v) agar in distilled water held at 60 °C. Excess agar was drained off in the vertical position and the slides were stored horizontally in a high-humidity chamber until required. They were inserted into core samples of sediment so that 2 cm remained above the surface, and incubated at 8 °C for 24 h. The position of the sediment-water interface was marked, the slides were removed and the *Ochrobium* cells were counted along transects measured with a microscope stage vernier. The effect of inhibitors on the growth of *Ochrobium* sp. on the slides was determined by pretreating the overlying water, and allowing the inhibitors to diffuse into the sediment for 24 h, before inserting the slides. A second addition of inhibitor was made as the slides were inserted.

Growth experiments. A 5 l container was filled and continuously flushed with hypolimnetic water in the boat, using the peristaltic pump sampler. A universal bottle, previously flushed with N₂ and with a butyl rubber insert in the cap, was then opened, flushed and sealed under water in the container. This ensured that the sample was not contaminated with O₂. Rapid deposition of ferric iron occurred in contaminated samples. The samples were incubated in the laboratory at 20 °C. Control samples were counted at zero time and the effect of O₂ was determined by the introduction of 1 ml air into selected bottles.

ATP analyses. A sensitive modification of the luciferin-luciferase procedure (Jones & Simon, 1977) was used, with the samples injected directly into boiling Tris buffer pH 7.8. Internal standards were analysed with each sample.

RESULTS AND DISCUSSION

When pure cultures of bacteria are not available, identification based solely on morphological characteristics must be considered to be tentative. *Ochrobium* is one of several genera of encapsulated and otherwise morphologically distinct bacteria which are identified in this way. The organisms described in this study (Fig. 1) and by Jones (1978) are morphologically similar to those assigned to the genus *Ochrobium* by Dubinina & Kuznetsov (1976), although it must be recognized that some confusion might have arisen in the identification of encapsulated bacteria since their original descriptions (see Introduction).

At the onset of summer stratification in Esthwaite Water the release, and immediate oxidation, of ferrous ions from the sediment increased the turbidity in the overlying water. *Ochrobium* sp. capsules and *Leptothrix* sp. filaments were found in the turbid zone, and although their numbers were relatively low (2 orders of magnitude lower than the *Ochrobium* sp. count in August and 4 orders of magnitude lower than the count of total bacteria), their presence suggested either growth *in situ* or an upward migration from the sediment (Fig. 2).

Fig. 1. Photomicrographs of *Ochrobium* sp. capsules from the hypolimnion of Esthwaite Water. Water samples were filtered through a 0.45 μm pore size cellulosic membrane filter which was then air dried and cleared with cedarwood oil: (a) bright-field microscopy; (b) interference contrast microscopy. The bar markers represent 5 μm .

Fig. 2. Distribution of dissolved O_2 (○), turbidity (continuous line), *Ochrobium* sp. (●) and *Leptothrix* sp. (□) with depth in Esthwaite Water during summer stratification (13 July 1978).

Within a month the zone of iron oxidation and the *Ochrobium* sp. and *Leptothrix* sp. populations had moved upward into the water column and peaks were found at depths of 12 to 13 m (Fig. 3). The release of ferrous ions into the anoxic hypolimnion of Esthwaite Water was first reported by Mortimer (1941, 1942) and the use of the transparency meter to determine the zone of iron oxidation and the dynamics of iron transport in the lake has been discussed in detail by Davison *et al.* (1981). The turbidity profiles indicate the depth at which ferrous ions were oxidized and therefore the depth to which O_2 must penetrate periodically, even if it was analytically undetectable (see Fig. 3 and subsequent Figures). Within a week the hypolimnion had become deoxygenated further, CO_2 concentrations had increased and the zone of iron oxidation had developed a sharp peak (Fig. 4a). The *Leptothrix* sp. and *Ochrobium* sp. populations had increased by an order of magnitude and were now separated into two distinct zones, the former above and the latter just below the peak of oxidized iron. This suggested that *Leptothrix* sp. had a greater requirement for O_2 (Jones, 1975, 1978), and although the genus has been observed frequently in the metalimnia of stratified lakes it has

Fig. 3. Distribution of dissolved O₂ (○), turbidity (continuous line), temperature (broken line), *Ochrobium* sp. (●), and *Leptothrix* sp. (□) with depth in Esthwaite Water (9 August 1978).

Fig. 4. Distribution of dissolved O₂ (○), turbidity (continuous line), dissolved CO₂ (broken line), *Ochrobium* sp. (●), *Leptothrix* sp. (□) and ATP concentration (■) with depth in the hypolimnion of Esthwaite Water (August 1978).

also been observed in surface films of ponds (Frolund, 1977) where there was a plentiful supply of O₂. The difference in the depth distribution of *Ochrobium* sp. and *Leptothrix* sp. in Esthwaite Water was even more marked a few days later (Fig. 4b) and during periods of calm it was possible to make rough estimates of the net increases in the population. During the

Fig. 5. Distribution of dissolved O₂ (○), turbidity (continuous line), *Ochrobium* sp. (●) and total bacteria (□) with depth in the hypolimnion of Esthwaite Water (25 July 1980).

Fig. 6. Distribution of dissolved O₂ (○), turbidity (continuous line), *Ochrobium* sp. (●) and *Leptothrix* sp. (□) with depth in Esthwaite Water (7 September 1978).

period of most rapid growth this was equivalent to an apparent generation time for *Ochrobium* sp. of 29 h, but this did not allow for losses due to grazing and population migration. These increases occurred in the apparent absence of O₂. The peak of ATP concentration at these depths coincided exactly with the peak of iron oxidation (Fig. 4c), suggesting that other bacteria were closely associated with this zone and that the population of *Ochrobium* sp. did not make a significant contribution to the total biomass as measured by ATP concentration at this peak region. Further examination of this zone revealed that the total bacterial counts were highest at the peak of iron oxidation (Fig. 5). Bacteria formed the major component of the biomass in the anoxic region of the hypolimnion. This could be attributed to the absence of sufficient light for algal growth and of sufficient O₂ to support a significant ciliate population. The ciliates that did grow in the anoxic water accounted for approximately 10% of the total biomass (B. Finlay, personal communication). The *Ochrobium* sp. population of up to 1.5×10^4 cells ml⁻¹ constituted only a small percentage of the total bacterial population ($> 5 \times 10^6$ ml⁻¹) at these depths (Jones, 1978) and only made a small contribution to the total biomass.

Later in the season, wind-induced turbulence dispersed the zone of iron oxidation and the population of *Ochrobium* sp. (Fig. 6). The cells disappeared from the water column at

Table 1. Counts of *Ochrobium* sp. colonizing agar-coated slides inserted into profundal sediment cores of Esthwaite Water and incubated for 24 h at 8 °C

The results are expressed as means $\pm$ 95 % confidence limits, based on 30 counts.

Depth in sediment (mm)	<i>Ochrobium</i> sp. count (organisms cm ⁻²)	
	January 1980	June 1980
0	ND	107 $\pm$ 89
1.0	ND	38 $\pm$ 58
1.5	472 000 $\pm$ 166 000	0
2.0	120 000 $\pm$ 100 000	19 $\pm$ 38
3.0	4 700 $\pm$ 6 000	11 $\pm$ 13
4.0	400 $\pm$ 420	0

ND, Not detectable (but a heavy deposition of ferric iron hindered microscopic observation).

Fig. 7. Distribution of dissolved O₂ (○), turbidity (continuous line), *Ochrobium* sp. (●) and total iron concentration (□) with depth, at the onset of summer stratification and deoxygenation of the profundal water in Esthwaite Water (9 June 1980).

overturn. During winter, *Ochrobium* sp. cells were found in large numbers a few millimetres below the surface of the sediments in the profundal zone (Table 1), but at the onset of summer stratification, with the reduction of O₂ tension and release of iron, numbers in the sediments decreased dramatically (Table 1) and *Ochrobium* sp. was found in the water column once more (Fig. 7). The decrease in numbers in the sediment, if converted to a volume basis, would be equivalent to a release of 2×10^6 cells ml⁻¹ over the 2 m of water column shown in Fig. 7. The actual numbers observed were much lower than this. There were several possible explanations for this disparity, including: (a) the possibility that not all the cells moved out of the sediment; (b) *Ochrobium* sp. was being actively grazed (ciliates containing large numbers of encapsulated iron bacteria have been observed on the agar-coated slides); (c) the slide technique may have encouraged growth and therefore the population in the sediment was overestimated. It was clear, however, that the *Ochrobium* sp. cells overwintered in the profundal sediments and moved into the water column, presumably assisted by their gas vacuoles (Dubinina & Kuznetsov, 1976), as anoxic conditions developed. The size of this initial inoculum of the water column varied considerably from year to year, being 150 organisms ml⁻¹ in 1978, 3000 organisms ml⁻¹ in 1979 and >5500 organisms ml⁻¹ in 1980.

The development of *Ochrobium* sp. on agar-coated slides incubated in sediment cores was completely inhibited by formaldehyde, suggesting that growth rather than passive attachment

Table 2. Counts of *Ochrobium* sp. colonizing agar-coated slides inserted into profundal sediment cores of Esthwaite Water after 24 h pretreatment with inhibitors

The sediment cores were collected in February 1980. The results are expressed as means $\pm$ 95% confidence limits, based on 30 counts.

Inhibitor (final concentration)	<i>Ochrobium</i> sp. count ($10^{-3} \times$ organisms cm^{-2})	
	Incubation conditions	
	8 °C, 42 h	20 °C, 8 h
None	316 $\pm$ 56	1400 $\pm$ 43
Formaldehyde (4%, v/v)	0	0
Chloramphenicol (100 mg l^{-1})	2.7 $\pm$ 3.2	50 $\pm$ 28
Actidione (100 mg l^{-1})	255 $\pm$ 62	1020 $\pm$ 15

Fig. 8. Distribution of dissolved O_2 (○), turbidity (continuous line), total *Ochrobium* sp. (●) and dividing *Ochrobium* sp. (□) with depth in Esthwaite Water, and net change in numbers of *Ochrobium* sp. after incubation of closed samples for 48 h at 20 °C under anaerobic conditions (■) (28 July 1980).

occurred (Table 2). Chloramphenicol inhibited development almost completely, whereas actidione had no effect (Table 2). These results suggested that the organism was a prokaryote, in agreement with the ultrastructural evidence presented by Dubinina & Kuznetsov (1976).

The conditions under which active growth of *Ochrobium* sp. occurred were less easily defined. Dubinina & Kuznetsov (1976) and Drabkova (1971) have reported *Ochrobium* sp. populations in both oxygenated and deoxygenated water and Dubinina (1976) referred to the growth of encapsulated iron bacteria in water to which O_2 penetrated but was not analytically detectable. The results obtained in Esthwaite Water suggested that *Ochrobium* sp. was capable of anaerobic growth; the absence of O_2 as measured with an electrode in the water column was confirmed by electrochemical analysis (W. Davison, personal communication). The frequency with which dividing cells occur has been shown to be related to growth rates of planktonic bacteria (Hagström *et al.*, 1979) and further examination of samples from the hypolimnion of Esthwaite Water revealed larger numbers of dividing cells in the permanently anoxic water than in the upper hypolimnion, where occasional turbulent penetration of O_2 occurred (Fig. 8). Confirmation of anaerobic growth was obtained when water samples were

incubated in the laboratory. Growth only occurred in those samples that had been collected and incubated in the absence of O_2 (Fig. 8). Introduction of 1 ml air into the vessels resulted in a complete cessation of growth and often a drastic reduction in numbers. The apparent generation times obtained were longer (about 50 h) than those observed in the field. The depth at which the organism grew in the sediments also suggested anaerobic or at least microaerophilic metabolism. The most careful measurements have suggested that dissolved O_2 penetrates only a few millimetres into sediments (Revsbech *et al.*, 1980) even though high E_h values were observed to greater depths.

Planktonic bacteria which appear to be associated with iron and manganese transformations are often difficult to isolate. Gregory *et al.* (1980) concluded that the microbial structures they observed (*Metallogenium* sp.) were not a living form of the organism. In the present study, growth of *Ochrobium* sp. was observed but the physiological characteristics of the organism will not be resolved until it is obtained in pure culture. Until then it cannot be assumed that it actively deposits ferric iron around the capsule. Such a deposition may be a chemical artefact caused by the microscopic examination of *Ochrobium* sp. in the presence of O_2 after removal of cells from anoxic water rich in ferrous ions.

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