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MiniReview

Uncultured giant sulfur bacteria of the genus *Achromatium*

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Abstract

Achromatium is a genus of large unicellular sulfur bacteria. Despite being first described in the late 19th century, no *Achromatium* spp. have yet been isolated in culture, and for over 100 years, knowledge of their ecology, physiology and relationships to other bacteria has been scant. In recent years, the application of culture-independent techniques combined with in situ process measurements and single-cell activity measurements in sediments harbouring large *Achromatium* populations, has expanded our knowledge of these bacteria. Aspects of carbon and sulfur metabolism in *Achromatium* are now better understood, but their preferred electron acceptor(s) remain unknown. Unexpected diversity has been uncovered in *Achromatium* populations and it is now clear that the organism routinely described as *Achromatium oxaliferum* actually comprises several distinct *Achromatium* spp. © 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

1. Introduction

One of the greatest achievements that the application of molecular biological techniques has brought to microbial ecology is an appreciation of the huge extent of bacterial diversity. This is based primarily on the discovery of deeply branching bacterial taxa recognised solely from ribosomal RNA sequences recovered from environmental samples [1]. In addition to the revelation of the existence of a whole new dimension to the bacterial world, culture-independent approaches have kindled renewed interest in bacterial taxa that have long been recognised by their striking morphology (e.g. *Achromatium* [2], *Beggiatoa* [3], *Nevskia* [4], *Thioploca* [5]) and prompted studies of more recently discovered morphologically striking bacteria [6–8]. A continuing inability to culture most of these organisms in the laboratory has meant that until recently their relationships with other bacteria have remained enigmatic.

Uncultured bacteria from the genus *Achromatium* have recently been the focus of research in our laboratories and a combination of molecular biological approaches and more traditional analyses have been used to investigate the phylogenetic relationships and potential ecological role of *Achromatium* in freshwater sediments. Some recent advances in our understanding of the ecology and phylogenetic relationships of bacteria from the genus *Achromatium* are discussed in this article.

2. The genus *Achromatium*

There are two validly described *Achromatium* species (*A. oxaliferum* and *A. volutans*) and a third species '*Candidatus Achromatium* minus' has recently been proposed ([9] see Section 5).

A. volutans was first described in 1903 and was originally named *Thiophysa volutans* [10]. It was discovered in organic rich muds from the Bay of Naples and was characterised by its large cells (7–18 × 18–29 µm) and the presence of abundant intracellular elemental sulfur (Fig. 1A). A second larger species *Thiophysa macrophysa* (21–40 µm in diameter) which flourished only in sediments of lower salinity (ca. 0.5% NaCl) was subsequently described by Nadson [11]. The taxonomy of morphologically conspicuous sulfur bacteria was extensively revised in 1944 by C.B. van Neil who placed both previously described *Thiophysa* species in the genus *Achromatium* as a single species *A. volutans* [12]. Since the early descriptions of *Thiophysa* spp., there have been few reports of *Achromatium* (*Thiophysa*) *volutans* in the literature [11,13–16]. With one

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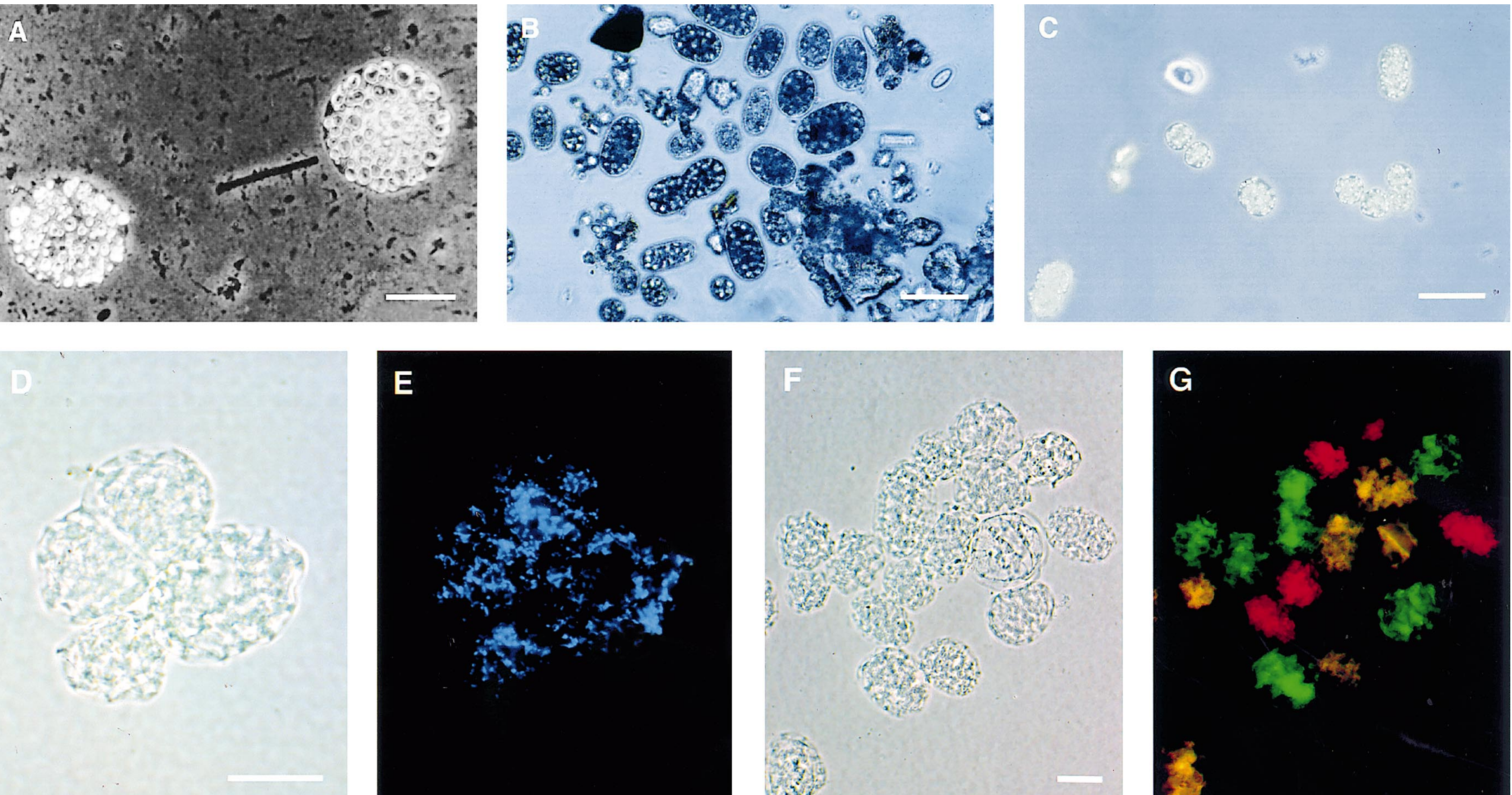

Fig. 1. Photomicrographs of *Achromatium* spp. A: *A. volutans*. B: *A. oxaliferum* from sediments of Rydal Water, Cumbria, UK. The large granular inclusions of calcium carbonate can be seen clearly within the cells. C: *A. oxaliferum* cells from Rydal Water sediments that have been treated with 0.1 M HCl to dissolve the calcium carbonate crystals revealing intracellular elemental sulfur globules. D and E: Paraformaldehyde-fixed *A. oxaliferum* cells from Lake Stechlin, Germany, stained with DAPI, phase contrast (D) and epifluorescence (E) micrographs of the same microscopic field are shown. The heterogeneous distribution of the DAPI-stained DNA within the cells is evident. F and G: Fluorescent in situ hybridisation of *Achromatium* cells from Lake Stechlin, Germany. The cells were simultaneously hybridised with probes AFK192-CT (5′-TACGGTCCCCTGCTTTCCC-3′; red) and AST433-F (5′-TTCCCCCCCGAAAGTGC-3′; green). Cells which bound only a single probe appear either red or green and those which bound both probes appear yellow. It is apparent that *A. oxaliferum* communities in Lake Stechlin comprise at least three distinct genotypes. This is now known to be the case for *Achromatium* populations from three other European freshwater lakes. The scale bars represent 20 μm except for B and C where the scale bar is 40 μm. (A reproduced from [33] with permission, Springer-Verlag, Berlin, Germany).

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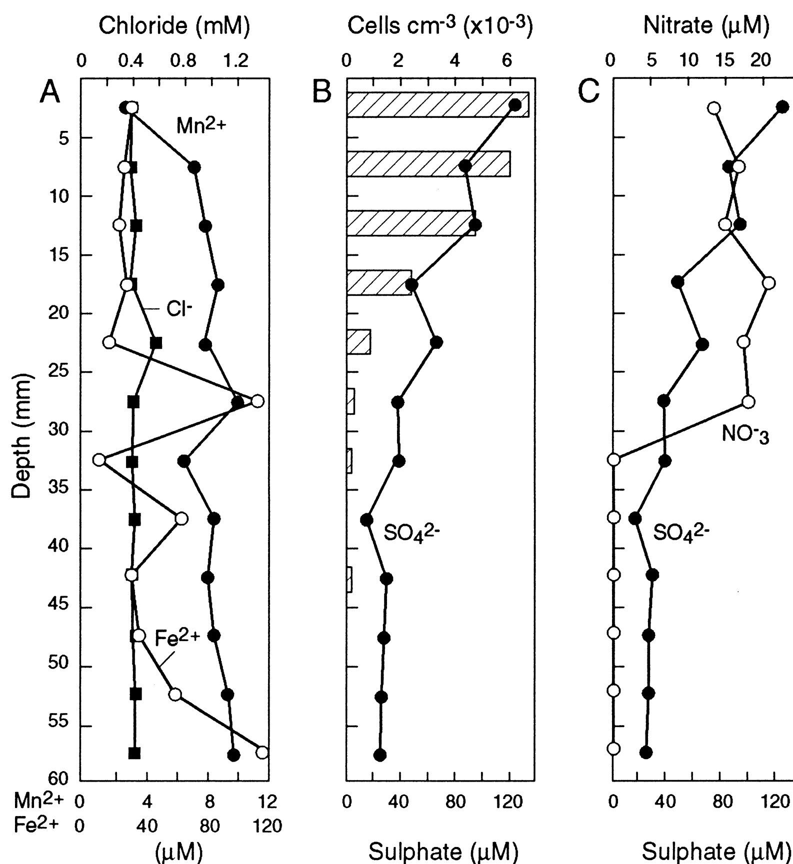

Fig. 2. Depth profiles of *Achromatium* cells (shaded bars in B) and redox sensitive chemical species in Jenny Dam sediments. Large numbers of *Achromatium* cells are present in the manganese reduction zone in this sediment.

exception [16], all reports have been from sulfur springs or marine and estuarine sediments. More recently, *A. volutans* has been reported from shallow submarine hydrothermal vents near the Greek Island of Milos [17]. Our understanding of the ecology and taxonomy of *A. volutans* has progressed little since its original description almost a century ago but the application of molecular biological approaches would undoubtedly shed much light on its relationship to other uncultured sulfur bacteria including the only other validly named member of the genus *Achromatium*, *A. oxaliferum*.

A. oxaliferum, like *A. volutans*, produces extremely large unicells and deposits intracellular elemental sulfur (Fig. 1C). In general, *A. oxaliferum* is larger than *A. volutans* and cells in excess of 100 µm in length have been described. Uniquely among the bacteria, *A. oxaliferum* precipitates intracellular calcium carbonate crystals [18] (Fig. 1B). Although reference to organisms similar to *A. oxaliferum* probably appeared as early as 1875 [19], it was formally described in 1893 [20] and has subsequently been identified in freshwater and brackish habitats from around the world. Reports from the UK [18], Africa, Austria, Belgium, Czechoslovakia, Denmark, France, Germany, Ireland, Russia [21,22], The Netherlands [23], Scandinavia [24] and the USA [13] suggest that organisms morphologically identifiable as *A. oxaliferum* are globally distributed.

3. Ultrastructure of *A. oxaliferum*

The intracellular structure of *A. oxaliferum* is somewhat

Table 1
Classification of different physiological types of colourless sulfur bacteria [28]

Physiological type	Carbon source		Energy source	
	Inorganic	Organic	Inorganic	Organic
Obligate chemolithotroph	+	−	+	−
Facultative chemolithotroph	+	+	+	+
Chemolithoheterotroph	−	+	+	+
Chemoorganoheterotroph	−	+	−	+

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unusual. Although the volume of individual cells can be in excess of 3×10^4 μm^3, greater than 70% of this may be occupied by intracellular inclusions of calcite [2,25]. As with other large sulfur bacteria, this may maximise the ratio of cell surface area to cytoplasmic volume, reducing substrate uptake and transport limitation in such large cells. In some *Thioploca* and *Beggiatoa* species and '*Thiomargarita namibiensis*', a similar role has been proposed for the large central vacuoles which are also believed to store reserves of nitrate used as an electron acceptor by these bacteria [8,26,27].

Recent observations also suggest that the organisation of DNA in *A. oxaliferum* cells may be unique. Examination of *Achromatium* cells hybridised with fluorescent oligonucleotide probes and stained with the DNA-specific fluorescent dye 2,4-diamidinophenylindole (DAPI) by epifluorescence microscopy revealed an unexpected pattern of DAPI staining. Rather than homogeneous staining of the bacterial DNA, a heterogeneous distribution of the DAPI stain was noted (Fig. 1D,E). Initially this was interpreted as representing epibiontic bacterial cells on the surface of the *A. oxaliferum* cells. Further investigation, however, demonstrated that they could neither be hybridised with any of a range of bacterial group-specific probes nor were they visible by phase contrast microscopy. We speculate that the DAPI staining observed was due to the presence of discrete accumulations of DNA within the cells. Interestingly, this observation is consistent with the original description of *A. oxaliferum* by Shewiakoff [20]. More or less numerous small bodies were noted at the junctions of the $CaCO_3$ inclusions. These stained blue or violet with haematoxylin and Schewiakoff suggested that these were 'chromatin grains'. The ability of such 'grains' to divide and their occurrence in the nucleus of plant and animal cells examined at this time suggested that chromatin was the hereditary material of cells. It cannot be judged retrospectively if what Schewiakoff had observed was in fact DNA, but haematoxylin is still used in medicine to stain the nucleus of *Eukarya*.

4. Ecophysiology of *A. oxaliferum*

It has always been suggested that *A. oxaliferum* is an aerobic chemolithoautotroph that gains energy from sulfide oxidation and fixes inorganic carbon. This was based principally on the presence of elemental sulfur globules within *A. oxaliferum* cells and their occurrence at the sediment–water interface. However, non-photosynthetic bacteria that deposit intracellular elemental sulfur are known to exhibit diversity in their metabolic capabilities and range from heterotrophic organisms to obligate chemolithoautotrophs ([28], Table 1). Heterotrophic colourless sulfur bacteria are postulated to deposit elemental sulfur as a by product of detoxification mechanisms aimed at reducing sulfide concentrations or removing reactive oxygen species. In this case, the elemental sulfur is apparently oxidised no further. For example, heterotrophic members of the genus *Beggiatoa* (which also includes chemolithoautotrophs, facultative autotrophs and chemolithoheterotrophs) apparently use intracellular elemental sulfur as an electron acceptor during transient periods of anoxia [29]. In contrast, mixotrophic and autotrophic organisms may gain energy and reducing power from sulfide oxidation and oxidise elemental sulfur completely to sulfate [29]. The range of metabolic strategies adopted by colourless sulfur bacteria has focused recent studies on determining the specific use of carbon and sulfur by *A. oxaliferum*.

4.1. Sulfur metabolism in *A. oxaliferum*

Studies of sediments harbouring large *A. oxaliferum* populations and physically enriched samples of cells have clarified some aspects of sulfur metabolism in *A. oxaliferum*. In sediments where *A. oxaliferum* was abundant, dissolved sulfide was either undetectable or present only at very low concentrations [25,30,31]. This was due to a combination of low sulfate reduction rates (resulting from low sulfate concentrations) and high levels of reactive iron in the sediments [25,30]. These conditions present a considerable challenge for an organism that utilises sulfide as an electron donor as it must compete with chemical sinks for a scarce resource. Nonetheless, depth profiles of sulfate concentration and the abundance of *A. oxaliferum* cells in sediment cores were frequently correlated ([30], Fig. 2), suggesting a possible link between sulfate production in the sediments and the magnitude of the *A. oxaliferum* population. This was confirmed by augmenting sediment cores from the margins of Rydal Water, a lake in the English Lake District, with physically enriched samples of *A. oxaliferum* cells. This artificially increased the magnitude of the *A. oxaliferum* population and in the presence of sodium molybdate, an inhibitor of sulfate reduction, the sulfate accumulation rate was positively correlated with the size of the *A. oxaliferum* population [30]. This provided circumstantial evidence that *A. oxaliferum* cells were capable of oxidising a reduced sulfur species to sulfate. The production of sulfate as an end product also implied that *A. oxaliferum* (at least the community present

Table 2
Incorporation of ^{35}S from radiolabelled sulfate by *Achromatium* cells in a freshwater sediment from Rydal Water[a]

Treatment	Proportion of cells labelled with ^{35}S (%)
$^{35}SO_4^{2-}$	73.3 ± 0.9
$^{35}SO_4^{2-}$ cells extracted with methanol	2.0 ± 0.6
$^{35}SO_4^{2-}$+molybdate	0.3 ± 0.3
$^{35}SO_4^{2-}$+formaldehyde (2% v/v)	0

[a]Incubations were performed over 3 h. The percentage of cells assimilating each substrate was determined by analysing 100 randomly selected cells. The data presented are the mean ± S.E.M. ($n=3$).

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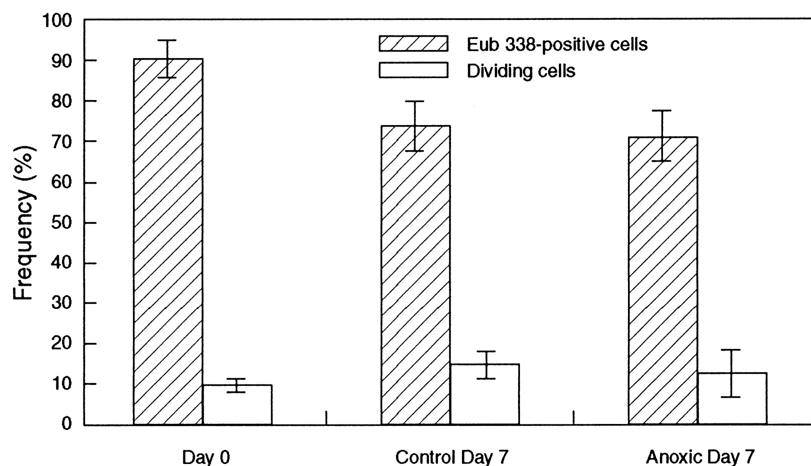

Fig. 3. Effect of incubation of sediments under anoxic conditions on the proportion of Eub338-positive *Achromatim* cells. Nine replicate sediment core microcosms were reconstituted from a bulk sediment sample. Three were sampled immediately, three were sealed and incubated under degassed water with a nitrogen head space (anoxic), and the final three cores were incubated in microcosms open to the atmosphere (oxic control). The oxic and anoxic cores were incubated for 7 days at 15°C. *Achromatium* cells were purified from the sediment cores and subjected to whole cell in situ hybridisation using the general bacterial probe Eub338 (5′-GCTGCCTCCCGTAGGAGT-3′) labelled with tetramethylrhodamine. For each sample, 300–400 cells were counted and scored for hybridisation with the fluorescent oligonucleotide. The data represent the mean of three replicate determinations and the error bars represent 2× the standard error.

in Rydal Water sediments) could conserve energy from reduced sulfur oxidation [29].

The source of sulfate under the conditions of these experiments was most likely intracellular elemental sulfur, however, the primary source of reduced sulfur utilised by *A. oxaliferum* could not be identified. Dissolved sulfide was undetectable at the outset of the experiment and would not have been produced in the sediment cores containing sodium molybdate. Depletion of other pools of reduced sulfur (principally iron monosulfides, pyrite and organic sulfur) could not be determined. These sulfur species were present in much greater abundance in the sediment than sulfate, and the variation in their quantitation was of the same order as the amounts of sulfate produced [30].

Table 3
Uptake of ^{14}C-radiolabelled substrates by *Achromatium* cells in freshwater sediment cores from Rydal Water and Hell Kettles[a]

Substrate	Proportion of cells assimilating substrate (%)	
	Rydal Water	Hell Kettles
^{14}C-Bicarbonate	78.7 ± 1.7[b]	66.0 ± 0.6[e]
^{14}C-Bicarbonate, 24 h incubation	79.7 ± 0.9[b]	75.7 ± 1.3[e]
^{14}C-Acetate	73.0 ± 0.6[b]	59.7 ± 0.6[e]
^{14}C-Acetate	13.3 ± 1.9[c]	62.7 ± 1.8[3f]
^{14}C-Glucose	1.0 ± 0.6[c]	1.3 ± 0.9[3f]
^{14}C-Protein hydrolysate	14.3 ± 0.7[c]	7.3 ± 1.2[3f]
^{14}C-Bicarbonate	53.7 ± 1.2[d]	52.3 ± 1.2[g]
^{14}C-Bicarbonate, HCl-treated	51.3 ± 2.2[d]	0[g]

[a]Incubations were performed over 3 h unless otherwise stated. The percentage of cells assimilating each substrate was determined by analysing 100 randomly selected cells. The data presented are the mean ± S.E.M. (n = 3). All negative control incubations, treated with formaldehyde (2% v/v final concentration), gave no response. Considerable temporal variations were observed in substrate uptake patterns, consequently only data from experiments conducted on the same date should be compared.
[b]13 August 1997.
[c]4 July 1997.
[d]28 April 1998.
[e]26 August 1997.
[f]10 June 1997.
[g]18 March 1998.

The application of microautoradiographic analyses, however, revealed that the most likely source of reduced sulfur utilised by *A. oxaliferum* in Rydal Water sediments was dissolved sulfide and that *A. oxaliferum* was an important component of a tightly coupled sulfur cycle in this sediment [32]. When radiolabelled sulfate was added to sediment cores in the presence or absence of sodium molybdate, radiolabel was deposited as intracellular elemental sulfur only in the absence of molybdate. In sediment cores amended with sodium molybdate, sulfide production is prevented and oxidation of labelled sulfide to intracellular elemental sulfur by *A. oxaliferum* cells cannot occur ([32], Table 2). These data not only support the notion that *A. oxaliferum* in sediments from Rydal Water can oxidise dissolved sulfide to sulfate and obtain energy or reducing power in the process, but also that can compete effectively with chemical sinks of sulfide, most notably dissolved ferrous iron and reactive iron minerals in an environment where dissolved sulfide was never detected. Such tightly coupled cycling of sulfur has implications for the importance of sulfate reduction as a pathway for organic carbon mineralisation in freshwater sediments inhabited by *A. oxaliferum*. In sediments of this type, a greater proportion of energy flow may be directed through sulfate reduction than would be estimated by conventional measures of sulfate reduction (e.g. radiotracer studies) where regeneration of oxidant (sulfate) cannot be measured directly.

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4.2. Carbon metabolism in A. oxaliferum

The ecological role of *A. oxaliferum* is not defined solely by its utilisation of sulfide as an energy source and hence its importance in the recycling of sulfate, but also by its carbon metabolism (Table 1). It has been considered widely that *A. oxaliferum* is an autotroph [33] but until recently no data were available on carbon metabolism in the bacterium. One recent report stated that attempts to detect RuBisCO activity had been unsuccessful [9], but subsequently it has been shown that some *A. oxaliferum* cells present in Rydal Water sediments do have the ability to fix inorganic carbon and probably harbour a gene homologous to *rbcL* encoding the large subunit of RuBisCO [25,32]. Of particular interest however is the observation that *A. oxaliferum* cells abundant in sediments of another small freshwater lake in the North of England (Hell Kettles) did not have the capacity to fix inorganic carbon [32]. Thus, in common with other distinctive sulfur bacteria (e.g. *Beggiatoa* and *Thiothrix*), considerable metabolic diversity exists within the genus *Achromatium*. It is now clear that *Achromatium* from Rydal Water sediments can assimilate inorganic and/or organic carbon (Table 3) and thus are most likely autotrophs or mixotrophs (Table 1). Interestingly, the proportion of cells assimilating radiolabelled bicarbonate was not significantly decreased in the presence of unlabelled acetate (R. Howarth, N.D. Gray and I.M. Head, unpublished data), suggesting that they probably are not facultative chemolithoautotrophs. In contrast, the *Achromatium* cells present in Hell Kettles sediments, which cannot assimilate inorganic carbon into cellular carbon, are either chemoorganoheterotrophs or chemolithoheterotrophs (Table 1).

4.3. Potential electron acceptors used by A. oxaliferum

Unlike the emerging picture of carbon and sulfur metabolism in *A. oxaliferum*, very little is known about the electron acceptors that the bacterium may utilise. There is evidence that *A. oxaliferum* exhibits a tactic response to alterations in oxygen gradients in sediments [30] and *A. oxaliferum* has been observed not to survive seasonal anoxia in the profundal sediments of stratified lakes [22], suggesting that oxygen is its preferred electron acceptor. However, these may be indirect effects of oxygen and it is possible that the primary requirement for oxic conditions by *A. oxaliferum* is for the replenishment of alternative electron acceptors that become depleted by anaerobic respiratory processes. This is supported to some degree by the observation that large numbers of *A. oxaliferum* persist in sediments at depths where oxygen is depleted [2,30,31]. Furthermore, the proportion of *Achromatium* cells that gave a positive signal with a fluorescent oligonucleotide probe specific for the bacterial domain (Eub338) in whole cell hybridisation analysis was the same in sediment cores incubated under oxic or anoxic conditions for

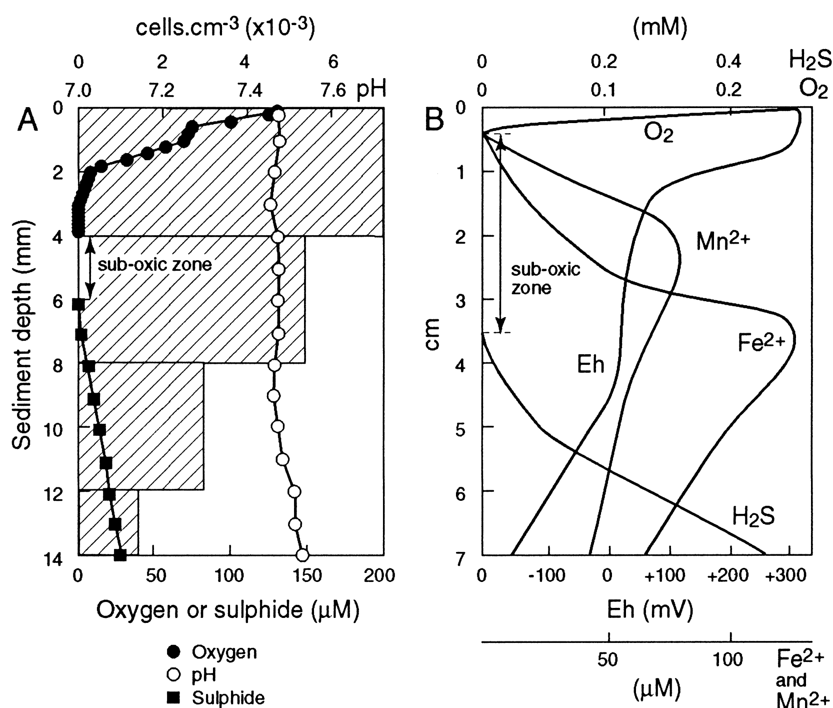

Fig. 4. A: Distribution of *Achromatium* cells (shaded bars), oxygen, dissolved sulfide and pH in sediment from Lake Stechlin, Germany. Redrawn from [31]. B: Stylised representation of oxygen, sulfide, Mn(II) and Fe(II) and redox potential in marine sediments, based on data from [37,38,40]. Note the difference in depth and sulfide concentration scales. In both profiles, the sub-oxic zone where oxygen and sulfide are not detectable is indicated. The sub-oxic zone is associated with iron and manganese reduction and biological and abiotic oxidation of sulfide in the absence of oxygen.

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7 days (Fig. 3). In both cases, the proportion of probe-positive cells after 7 days was slightly lower than that measured at the outset of the experiment. However, although hybridisation with a 16S rRNA targeted oligonucleotide probe indicates that a cell contains high numbers of ribosomes, this does not always correlate with other more specific measures of cellular activity [32]. The use of electron acceptors other than oxygen is well known among sulfur bacteria. A number of nitrate-reducing colourless sulfur bacteria have been described and there is evidence that denitrification and dissimilatory ammonification may both be important catabolic processes in sulfur bacteria [26,27,34,35]. Where nitrate profiles have been measured in *A. oxaliferum*-bearing sediments, it was depleted within the first few millimetres [30] or remained constant at depths where *Achromatium* was most abundant, suggesting that *Achromatium* was not actively reducing nitrate (Fig. 2). It is also conceivable that nitrite may be used as an electron acceptor since some nitrite reducers are unable to reduce nitrate, however there is no evidence to suggest that this is the case in *Achromatium* spp.

Oxidation of dissolved and solid phase sulfides under anoxic conditions has also suggested a role for biologically mediated iron and/or particularly manganese reduction, in the process [36–39]. One line of evidence for anaerobic sulfide oxidation linked to iron or manganese reduction comes from studies of marine sediments which show removal of dissolved sulfide at a depth in the sediment where oxygen does not penetrate ([37,38,40], Fig. 4). A similar profile of oxygen and sulfide has been noted in at least one *Achromatium*-bearing sediment. In this case, a large proportion of the *A. oxaliferum* population occurred in the region of the sediment separating the oxic and sulfidic zones ([31], Fig. 4). Although in the study of Babenzien and Sass [31] iron and manganese profiles were not measured, removal of sulfide in this 'sub-oxic' zone has been associated with corresponding peaks in Fe^{2+} and Mn^{2+} in studies of marine sediments ([37,38,40], Fig. 4). In our laboratory, substantial *A. oxaliferum* populations were detected in regions of a freshwater sediment where iron and manganese reduction were evident [30] and it is therefore not inconceivable that *A. oxaliferum* may have the capacity to oxidise sulfide under anoxic conditions using iron or manganese as oxidants. However, high levels of reduced iron and manganese in the sub-oxic zone of sediments may be explained by several other phenomena. Organic carbon mineralisation linked to iron and manganese reduction is likely to be a significant process particularly in the iron rich sediments (up to 23 μmol cm^{-3} reactive iron) with high TOC (13–20% by weight) where *A. oxaliferum* is typically found [25]. Chemical oxidation of sulfide by iron and manganese minerals also produce Fe^{2+} and Mn^{2+} [36,38], and elemental sulfur disproportionation, which may occur preferentially in the sub-oxic zone of sediments, is only thermodynamically feasible if sulfide is maintained at low levels by reaction with manganese oxides or iron oxyhydroxides [40,41].

The use of exogenous electron acceptors by *A. oxaliferum* need not even be invoked to explain its survival under anoxic conditions. In common with some *Beggiatoa* species [29], *A. oxaliferum* may utilise intracellular elemental sulfur as an endogenous reserve of electron acceptor permitting its survival over short periods of anoxia.

It is clear that currently any suggestions relating to electron acceptor use by *A. oxaliferum* remain speculative, and until studies of the stoichiometry of sulfur oxidation and electron acceptor reduction can be studied in highly purified, sediment-free cell preparations [26], or cultured isolates of the bacterium can be maintained in the laboratory, it is likely to be difficult to elucidate the full metabolic repertoire of *A. oxaliferum*.

5. Phylogeny and diversity in the genus *Achromatium*

The application of molecular approaches to characterise populations of *A. oxaliferum* has revealed unexpected diversity in the bacterium. As long ago as 1913, West and

Table 4
Morphological differentiation of different subpopulations of *Achromatium* spp. from three freshwater sediments

Achromatium subpopulation	Mean cell diameter (μm)[a]	Number of cells analysed
Rydal Water sediment		
R5	14.28 ± 0.58[b]	58
R1	19.85 ± 0.59[c]	50
R8	15.07 ± 0.43[b,d]	70
Hell Kettles sediment		
HK9	21.95 ± 1.22	22
HK13	16.43 ± 0.88[e]	31
HK1/24	25.17 ± 0.63	46
Jenny Dam sediment		
JD2/13	11.67 ± 0.37	66
JD8	18.49 ± 0.60[c]	48
JD1	17.13 ± 1.08[c,d,e]	5

[b,c,d,e]Figures with the same letter are not statistically different ($P > 0.05$; pairwise comparison, minimum significant difference of log_{10}-transformed data).
[a] ± 95% confidence limits.

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Griffiths described two organisms, *Hillhousia mirabilis* and *Hillhousia gigas* [18], which were clearly two morphologically distinct varieties of what previously (and subsequently) was described as *A. oxaliferum* [42]. Nadson and Visloukh [43] further suggested the existence of several morphologically different forms of *A. oxaliferum* which they designated with the additional epithets *minus, medium, majus, elongatum* and *gigas*. This analysis was later refuted [42] and in the majority of subsequent literature, large unicellular bacteria that deposit intracellular sulfur and calcite have been considered as a single species, *A. oxaliferum*. The possible existence of several species of *Achromatium* morphologically similar to *A. oxaliferum* was resurrected with the recovery of several related, yet divergent sequences in clone libraries generated from 16S rRNA gene fragments amplified from highly purified suspensions of *A. oxaliferum* cells from Rydal Water sediments [2]. Nonetheless, it was not until the application of whole cell, fluorescent in situ hybridisation using multiple, fluorochrome-labelled probes targeting 16S rRNA sequences recovered from two different *Achromatium* communities that the co-existence of genetically distinct but morphologically similar achromatia in a single sediment was unequivocally demonstrated ([9], Fig. 1F,G). It was independently reported that multiple 16S rRNA sequences related to the *A. oxaliferum* sequence first identified in sediments from Rydal Water, Cumbria, UK [2], were recovered from other sediments harbouring substantial *Achromatium* populations [44]. Whole cell in situ hybridisation again confirmed that these represented genetically distinct subpopulations within a single *Achromatium* community [44]. More detailed study of the *Achromatium* communities present in freshwater sediments from the North of England revealed that the different subpopulations identified fell within distinct size classes ([44], Table 4), supporting the morphometric characterisation that Nadson and Visloukh had first proposed over 75 years ago. However, in any particular *Achromatium* community, cell dimensions cannot be used to distinguish the different subpopulations present due to the considerable overlap in cell size between the different subpopulations, and analysis by whole cell hybridisation with specific probes is necessary to confidently distinguish the different subpopulations [9,44].

The discovery that *A. oxaliferum* populations appear to comprise several phylogenetically related but distinct subpopulations, that by current benchmarks are different species [44], has important consequences for the interpretation of ecophysiological data obtained with *Achromatium* cells present in situ or purified from sediment samples (Section 3). The clear metabolic differentiation of *Achromatium* cells present in geographically separated lake sediments suggests that in a single *Achromatium* community, physiologically and hence ecologically differentiated populations may co-exist in the same sediment. Adaptation to different redox conditions within a single sediment by di-

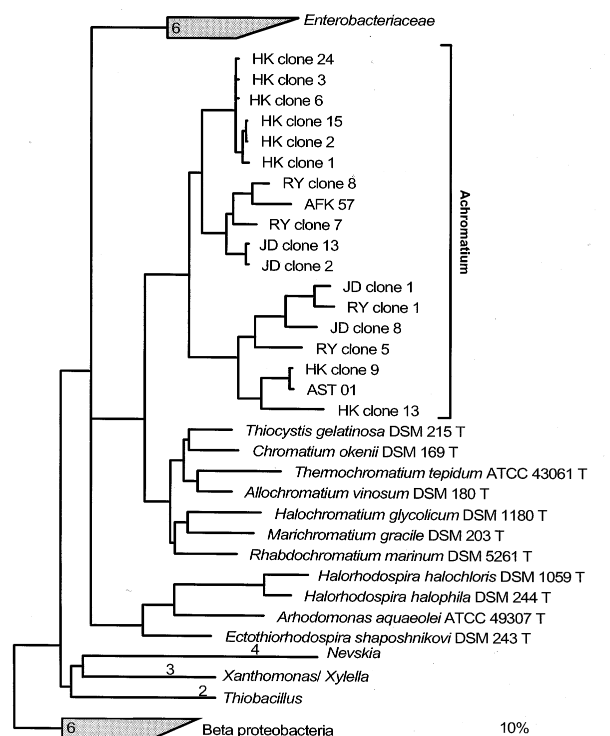

Fig. 5. Phylogenetic tree based on comparative 16S rRNA sequence analysis of all known *Achromatium* sequences and related bacteria from the γ subdivision of the class *Proteobacteria*. The sequences are all derived from 16S rRNA gene clone libraries. Sequences obtained from *Achromatium* cells purified from Hell Kettles (Co. Durham, UK), Rydal Water (Cumbria, UK), Jenny Dam (Cumbria, UK), Lake Fuchskuhle (Germany) and Lake Stechlin (Germany) are designated with the suffixes HK, RY, JD, AFK and AST, respectively. It has been proposed that the *Achromatium* sp. present in Lake Fuchskuhle sediments (represented by sequence AFK 57) is a new species '*Candidatus Achromatium* minus'. Data analysis: *Achromatium* sequences were added to the 16S rRNA sequence data base of the Technical University Munich using the program package ARB [50]. The ARB_EDIT tool was used for sequence alignment. The alignment was checked by eye and corrected manually. Tree topologies were evaluated by performing maximum parsimony of the full ARB data set (ca. 13 000 sequences). Distance trees using neighbour joining were inferred using 208 sequences and a maximum likelihood tree was constructed using 71 sequences. The tree is a consensus tree based on a maximum likelihood tree, taking into consideration the maximum parsimony and distance trees. Only at least 90% complete sequences were used for the final tree. Alignment positions at which less than 50% of sequences of the β and γ subclasses of the class *Proteobacteria* shared the same residues were excluded from the calculations. Bifurcations indicate branch points which appeared stable. Multifurcations indicate tree topologies which could not be significantly resolved based on the available data set. Numbering on branches indicates the number of related sequences from the groups indicated that were included in the analysis. The bar indicates 10% estimated sequence divergence.

vergent *Achromatium* species co-existing in Rydal Water sediments has already been implied from changes in their relative abundance in different redox zones [44] but more definitive evidence is required to identify the basis for niche differentiation and how diversity is maintained in this bacterial community.

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6. Concluding remarks

Until recently, our knowledge of the ecology of bacteria from the genus *Achromatium* stemmed largely from their morphological features. Contemporary studies have revealed, however, that the ecological roles of *Achromatium* may be considerably more diverse than originally realised. It is now apparent that members of the genus *Achromatium*, in common with other distinctive sulfur bacteria (e.g. *Beggiatoa* [45] and *Thiothrix* [46]) encompass more than one physiological type. Data from ecophysiological studies, which by their very nature are rarely definitive, have demonstrated that some achromatia are chemolithoautotrophs (obligate or facultative) or mixotrophs whereas others lack the ability to fix inorganic carbon and are most likely chemolithoheterotrophs or chemoorganoheterotrophs.

Achromatium populations from five freshwater lake sediments in North West Europe have now been extensively characterised. These studies have demonstrated that in four of these environments, the *Achromatium* communities comprise at least three genetically distinct subpopulations. Almost full length 16S rRNA sequences have been obtained from the component subpopulations in three of these lakes ([44], Fig. 5). Analysis of these sequences revealed a high degree of sequence divergence ($>8\%$ in some cases) consistent with the different subpopulations identified, representing distinct *Achromatium* species. On the basis of high 16S rRNA sequence divergence and morphological differences, it has been proposed that the *Achromatium* sp. present in Lake Fuchskuhle represents a new species, '*Achromatium minus*', and it is clear that further *Achromatium* species remain to be described.

Although we now have a far greater appreciation of the extent of diversity within the genus *Achromatium*, the relationship, if any, of *A. volutans* to the *A. oxaliferum*-like organisms now characterised remains unknown and similar studies to those described here will be required to establish the phylogenetic position of *A. volutans*.

Perhaps more importantly, the discovery of divergent *Achromatium*, that at the very least represent species level differentiation, co-existing in the same sediment, raises several interesting ecological questions. To co-exist and avoid competitive exclusion of the least fit subpopulations, the different *Achromatium* species must occupy distinct niches and hence not compete directly for the same resources. The fact that differences in carbon metabolism have been noted in geographically separated *Achromatium* communities that are also phylogenetically distinct suggests that this is a plausible scenario [32,44]. Alternatively, the co-existing species may compete for the same resources but do so optimally under different environmental conditions and heterogeneous or fluctuating conditions in the sediment may permit their co-existence. Adaptation of *Achromatium* subpopulations to specific redox conditions in sediments has also been implied from differences in community composition at different depths and redox zones in sediment cores [44]. Despite these interesting observations, the precise mechanism which leads to the co-existence of these ostensibly ecologically similar bacterial species remains to be elucidated. In future, studies of *Achromatium* communities in situ using a combination of single-cell identification by whole cell hybridisation, and single-cell activity measurements [47–49] promise to reveal the physiological mechanisms that underpin the co-existence of closely related but ecologically distinct bacteria and may have implications reaching beyond the ecology of *Achromatium* alone.

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