

Magnetotactic Bacteria from Freshwater

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Magnetotactic Bacteria, Light and Electron Microscopy

Four species of magnetotactic bacteria have been detected in alkaline waters with pH 7.4–8.7. They were of coccoid to rod-like shape. Each of the species showed different patterns of movement. Two various types of magnetosomes, a chain-like arrangement and a scattered distributional pattern and a lateral polytrich flagellated type could be demonstrated with the coccoid species.

Introduction

In the course of routine investigations of sea water mud samples Blakemore discovered bacteria, which surprisingly showed a magnetotactic behaviour, *i.e.*, they were moving within a magnetic field towards a magnetic pole [1, 2]. From fresh water he isolated a type of spirillum, which he called *Aquaspirillum magnetotacticum*. Furthermore he developed a chemically-defined medium for the cultivation of this type of bacteria. Investigations showed that on the northern hemisphere the magnetotactic bacteria were moving towards the earth's geomagnetic North Pole, on the southern hemisphere (New Zealand) they were moving towards the geomagnetic South Pole. At the equator both types of bacteria were found in almost equal amounts. Moench found coccoid types, the magnetococci [3]. The magnetotaxis is enabled by special organelles, the so-called magnetosomes, which turned out to be crystals consisting of magnetite (Fe_3O_4) with a parallelepiped or an orthohexagonal prismatic structure [4, 5, 8, 9]. In this paper the occurrence of magnetotactic bacteria in fresh water has been examined in ponds with special regard to alkaline waters.

Material and Methods

Bacteria

The magnetotactic bacteria were found in ponds in southwest Germany where 12 out of 60 samples were

positive. Sources of discovery (see Table I):* muddy shore regions with deceasing plants, water surfaces grown with reed, weed and algae on the water surface. For enrichment of the magnetotactic bacteria magnetic stirring bars commonly used in laboratories were applied.

Chemicals

were received, if not mentioned otherwise from Merck, Darmstadt, FRG. The media were obtained from Oxoid.

Gram-assay and potassiumhydroxide-assay see [6].

Poly- β -hydroxybutyric acid PHBA (Burdon)

30 mg of Sudan Black are dissolved in 10 ml of 70% ethanol (= 0.3%) and 50 mg of safranin in 10 ml of water (= 0.5%). A heat-fixed bacterial sample was treated on a permanent slide for 10 min with the Sudan Black solution. Then the solution was removed and dipped into xylene, until no further colour clouds developed. After drying by air a counterstaining was carried out using the safranin solution.

Determinations of oxygen and metal ions

The oxygen content of the water samples was determined with a Clark oxygen electrode YSI-4004. Determinations of the calcium and iron content were obtained by Atomic Absorption Spectroscopy (AAS).

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* Sources of discovery can be imparted on request.



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Light microscopy

was performed with a Zeiss standard microscope. The Zeiss Microflash II served as a light source for microphotography of the fast moving bacteria.

Electron microscopy

was performed with a Siemens Elmiskop 1a. For embedding in Epon 812 samples of bacteria were fixed with 2.5% glutaraldehyde and postfixed with 1% osmium tetroxide. Staining of thin sections was performed with uranyl acetate (UA) and lead citrate and negative staining with 1% UA pH 4.3.

Electron beam X-ray micro analysis was performed with a JEOL Scanning Microscope, Type JXA 50 A, Tokyo.

Videography

The behaviour of chains of magnetotactic bacteria in an artificial magnetic field was recorded on a Sony U-Matic Low Band tape. Photographs were taken from a Blaupunkt Monitor and a Sony Video Tape Recorder with a single picture reproduction facility.

Results

1. Light-microscopic studies of magnetotactic bacteria

An ordinary mud sample was placed under a light microscope taken in order to demonstrate the magnetotactical behaviour of the bacteria. For photographic documentation an exposure time of 1 sec was applied in dark field. As shown in Fig. 1a short and almost linear lanes are observed, if no additional magnetic field influences the cells: the bacteria are moving non-directedly under the 0.5 Gauß earth's magnetic field (Fig. 1a). By switching on a 6 Gauß artificial magnetic field, generated by a pair of Helmholtz coils, the cells straightened spontaneously to the magnetic South Pole and were swimming towards it (Fig. 1b). Now the lanes strictly paralleled the magnetic field lines. When changing the polarity of the magnetic field one could observe an immediate reaction of the bacteria without any deceleration. The bacteria were now swimming in the opposite direction. Magnetotactic bacteria, which have lost motility, are not attracted by a magnetic field, they are merely orientating towards the magnetic South Pole, a movement towards a magnetic pole did not

take place. This phenomenon can also be demonstrated with chains of magnetotactic bacteria (Fig. 2a–2f), which consist of single nonactive cells. This chain was build up accidentally by Brown's molecular movement. When changing the magnetic field's polarity, the complete chain acts like a single dipole. It turns with one end towards the magnetic South Pole during the very short period of mean 0.14 sec, but is not attracted by it. Thus, the chain of magnetotactic bacteria shows a behaviour comparable to a compass needle.

During an artificial magnetic field's impact on cells a macroscopic spot with 5 mm in diameter is generated on the glass walls within a period of 10 min. This spot mainly consists of coccoid-shaped bacteria, single cocci as well as diplococci (Fig. 3a). Fig. 3b shows a single coccoid-shaped magnetotactic bacterium. The highly refractile inclusion body in the cell is a remarkable detail (arrow, also compare Fig. 2a–2f and 3c). According to the staining results this inclusion body consists of poly- β -hydroxybutyrate. This type of bacteria shows a rotational movement and is moving ahead very quickly. Rod-shaped magnetotactic bacteria could only be seen sporadically. They show a rotational movement around the body axis (Fig. 3c), and they move considerably slower than coccoid forms. For a long-shaped bacterium with bright areas (Fig. 3d, arrows) an undulating motility is characteristic which is also reduced in comparison to the coccoid-shaped bacteria. A still larger, rod-shaped bacterium of similar velocity was only observed once. The cell is surrounded by a low contrast layer (arrow), possibly consisting of a mucous material (Fig. 3e). In this case an undulating motility was observed, but when changing the polarity a jerky and undulating movement appeared. Perhaps this modified motility depends on the flagella's construction and arrangement. Fig. 3f shows a ultrathin section of a rod-shaped magnetotactic bacteria.

2. Environmental investigations of the magnetotactic bacteria (Table I)

The water samples containing magnetotactic bacteria showed significant alkalinity (pH-values from 7.4 to 8.7). This can be explained by the composition of the sediment, mainly soils of high lime content. Atomic absorption spectroscopy revealed an average concentration of dissolved calcium of 96 mg/l. In

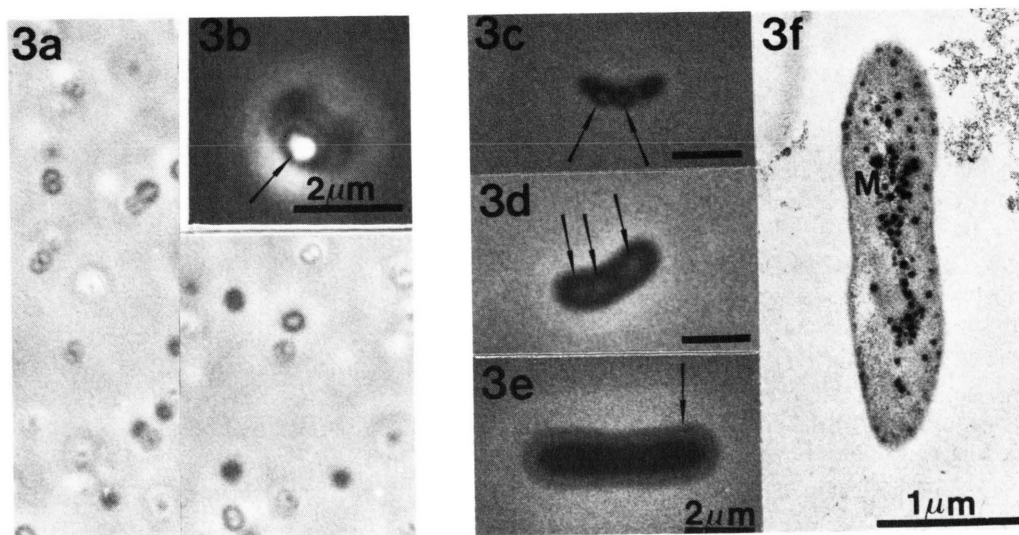
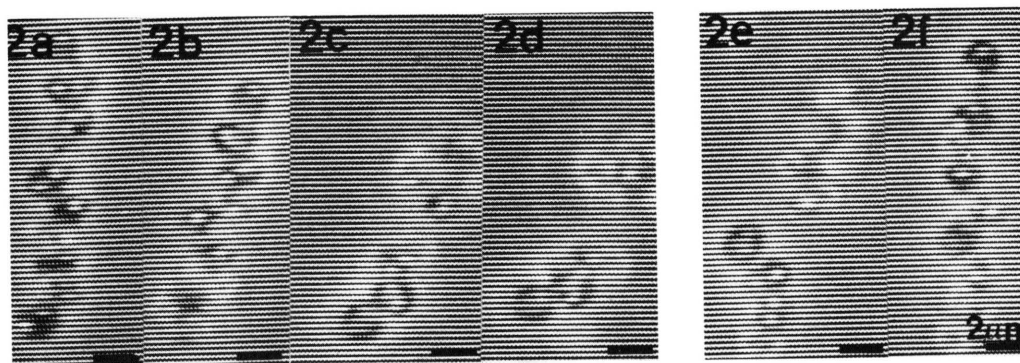
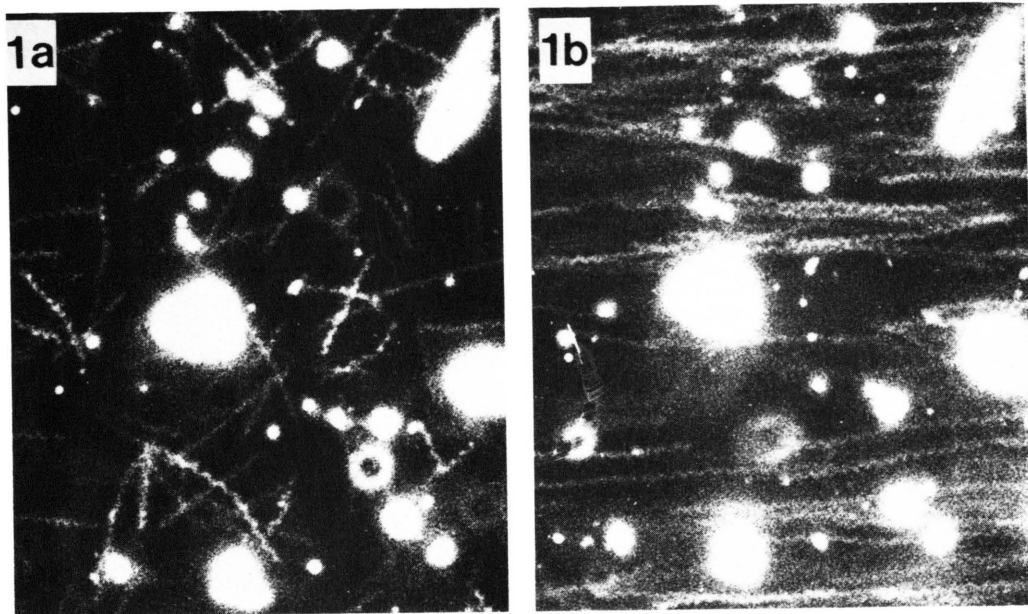


Table I. Comparison between the coccoid-shaped magnetotactic bacteria and the magnetococci (Moench).

Environmental conditions	Moench	Own investigations
pH	6.1–7.9	7.4 – 8.7
Fe	?	0.01 – 0.8 mg/l
Ca	?	82 – 110 mg/l
O ₂	?	0.028– 0.233 µmol/ml
<i>Flagellation</i>	two bundles in basal discs	
<i>Inclusions</i>		
Magnetosomes	chains or dispersed magnetosomes	
PHBA-granules	presumed	+ according to staining experiments with Sudan Black
Polyphosphates	?	dark aggregates in thin section. Confirmation by EBM*
<i>Gram-staining</i>	negative cell wall	negative cell wall. Can also be seen in electron microscopic photographs (Fig. 4d and 4e)
<i>Morphology</i>	coccus	coccoid-shaped

* EBM, electron beam microprobe.

most cases the coccoid type of magnetotactic bacteria was found and only the water sample with pH 7.8 and a high calcium but low iron content harboured a greater variety of morphological types (Fig. 3). Considering the correlation between the oxygen content of a certain water sample and the occurrence of the coccoid-shaped magnetotactic bacteria in this sample, a high oxygen tolerance is apparent. The conditions under which these organisms can exist and even propagate are ranging from oxygen contents of 0.028 µmol/ml to 0.233 µmol/ml (at 21 °C). An oxygen saturation is reached at 0.29 µmol/ml.

3. Electronmicroscopic studies of coccoid-shaped magnetotactic bacteria

According to Gram stain and potassium hydroxide assay the coccoid-shaped magnetotactic bacteria turned out to be Gram-negative. This finding is also confirmed by the structure of cell walls as found in ultrathin sections (Fig. 4a and 4f). There are also some morphological varieties of these magnetotactic bacteria (Fig. 4a–g). In ultrathin sections crystals can be recognized [4] which resemble those described by both, Blakemore [2] and Towe and Moench [8]. These authors characterized the crystals

Fig. 1–3. Light microscopic studies of magnetotactic bacteria.

1a. Non directed movement of bacteria in the earth's magnetic field.

1b. Directed movement in an artificial magnetic field of 6 Gauß.

2a–f. Chain of magnetotactic bacteria. The reorientation in an artificial magnetic field is demonstrated in a picture sequence from left to right. During the reorientation process the chain decays into two parts because of the strong turning movement, whereas the two fragments act as magnetic dipoles separately (2c–2e). Finally, the two parts recombine again to a complete chain (2f).

3a. Sample of magnetotactic bacteria enriched from pond water by a magnetic stirring bar. Inserted photograph (Fig. 3b) shows a single coccoid-shaped magnetotactic bacteria. Arrow indicates the highly refractile inclusion body. Magnification: 7200×.

3c–e. Single rod-shaped magnetotactic bacteria. Arrows indicate inclusion bodies or low contrast layer (3e). Magnifications: 4500× (Fig. 3c–3e). All bars represent 2 µm.

3f. Ultrathin section of a rod-shaped magnetotactic bacteria with scattered magnetosomes. For comparison see Fig. 4 and 5. M, Magnetosomes. Magnification: 20000×. Bar represents 1 µm.

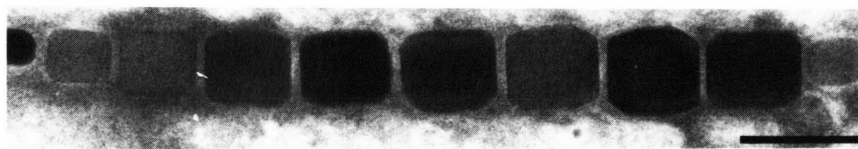
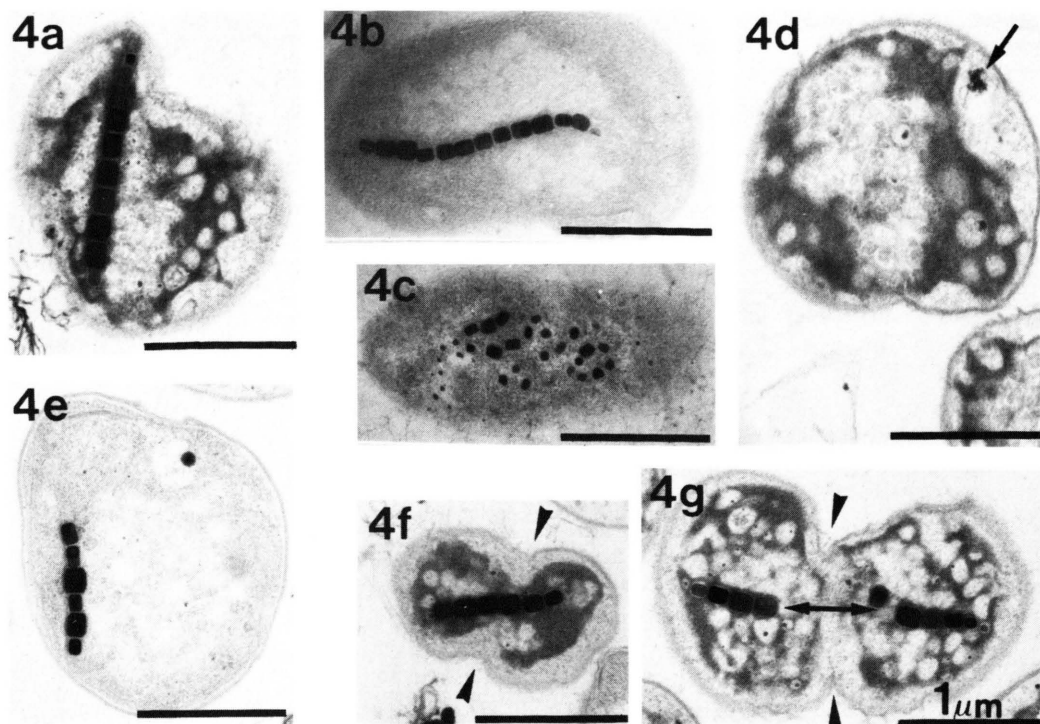


Fig. 5a

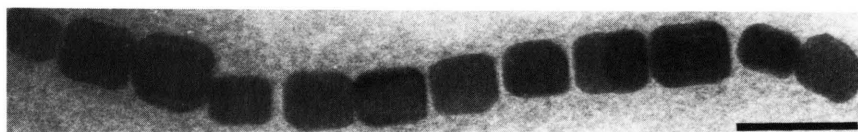
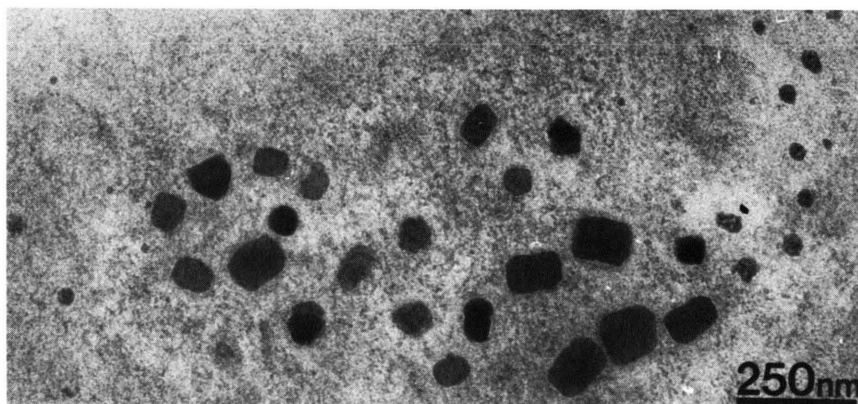


Fig. 5 b



250nm Fig. 5 c

as magnetite, Fe_3O_4 . The laser microprobe mass analyser LAMMA revealed a high iron content in cells of magnetotactic bacteria. Since by AAS in water samples containing magnetotactic bacteria very low iron concentrations of non measurable amounts to 0.01–0.8 mg/l were found, a remarkable iron enrichment in the cells must have occurred. The magnetite containing organelles were designated by Blakemore as magnetosomes which may be responsible for the magnetotaxis of these bacteria. Fig. 4f and 4g represent two stages of cell division in magnetotactic bacteria. The cell division starts with an equatorial lacing (Fig. 4f, arrows), followed by an equal distribution of the magnetosome's chain on the daughter-cells (Fig. 4g). Electronmicroscopically two different arrangements of magnetosomes can be observed: The first type represents chain-like arrangements of magnetosomes (Fig. 5a, b), whereas the second type shows a scattered distributional pattern (Fig. 5c). Among the first type one can distinguish between relatively large crystals with an average width of 154 nm and an average length of 178 nm (Fig. 5a) and remarkably long chains of magnetosomes with 12–13 particles (Fig. 5b). The particles shown in Fig. 5b have average dimensions of 106 nm × 147 nm. In contrast to the chain-like magnetosomes, the scattered type harbours great differences in the size of the magnetite grains: the largest possess dimensions of 109 nm × 125 nm, whereas the smallest detectable particles merely are 30 nm × 30 nm in size (Fig. 5c). In most cases the coccoid magnetotactic bacteria were found to possess two flagellar bundles (FB) in basal discs (B) (Fig. 6a and b).

Investigation of complete cells of the coccoid magnetotactic bacteria by the electron beam X-ray micro analysis revealed high amounts of phosphorous, as shown in Fig. 7. The phosphorous peak may be due to electronopaque inclusions, which possibly are polyphosphates. The black area visible in Fig. 4d (arrow) may be aggregates of polyphosphate.

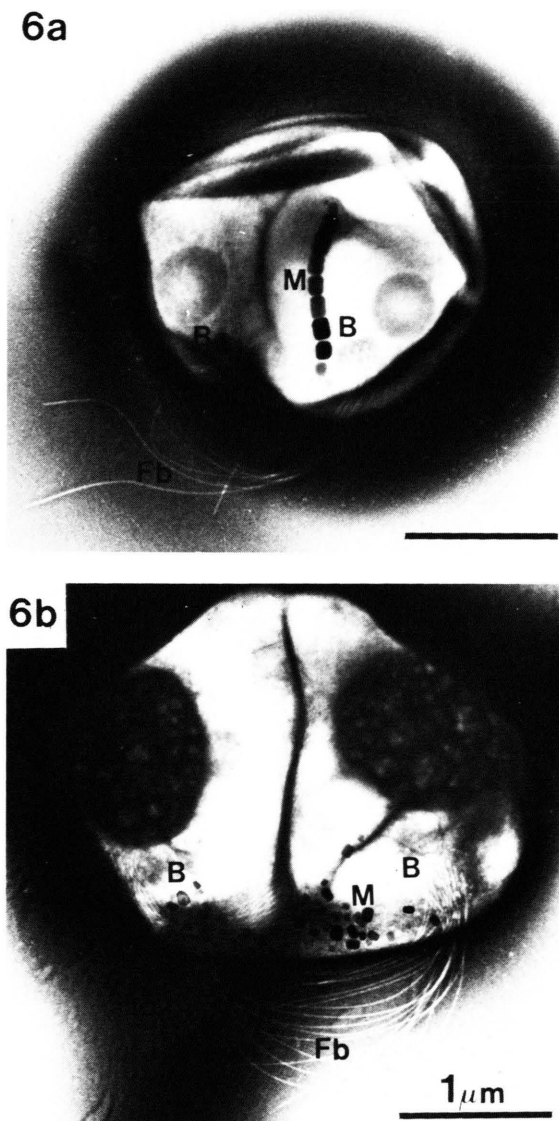


Fig. 6. Single coccoid-shaped magnetotactic bacteria (Negative staining).

6a. Cell with a chain-like arrangement of magnetosomes. 6b. Cell with a scattered distributional pattern of magnetosomes. B, basal discs; FB, flagellar bundles; M, Magnetosomes. Magnification: 23000×. Bars represent 1 μm.

Fig. 4a–g. Electron microscopic studies of the coccoid-shaped magnetotactic bacteria.

4a–g. Ultrathin sections of coccoid magnetotactic bacteria 4a, 4b, 4e, 4f, 4g cells with a chain-like arrangement of magnetosomes, 4c with a scattered distributional pattern.

4f and g. Stages of cell division of magnetotactic bacteria: the process starts with an equatorial lacing (4f) and is followed by an equal distribution of the magnetosomes on the daughter cells (arrows). Magnifications: 20000×. Bars represent 1 μm.

Fig. 5a–c. Configurations of magnetosomes in coccoid-shaped magnetotactic bacteria.

5a. Chain-like arrangement: large grains, mean dimensions 154 nm × 178 nm, enlarged from Fig. 4a.

5b. Chain-like arrangement: long chain with 12–13 particles, mean dimensions 106 nm × 147 nm, enlarged from Fig. 4b.

5c. Scattered Pattern: varying dimensions of 109 nm × 125 nm to 30 nm × 30 nm, enlarged from Fig. 4c. Magnifications: 64000×. Bars represent 250 nm.

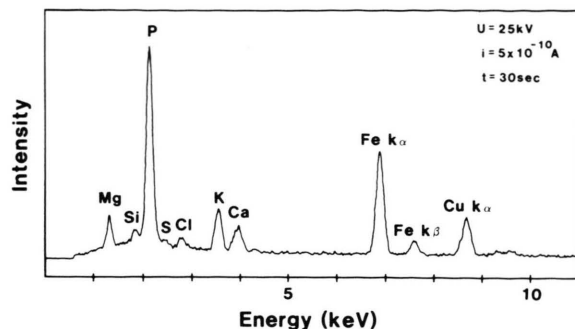


Fig. 7. Electron beam X-ray micro analysis.

Discussion

Magnetotactic bacteria described in this study have been found in biotops with pH 7.4–8.7 with a lime containing underground with 96 mg/l Ca^{2+} and 0.8 mg/l iron. The aerotolerance of the coccoid-shaped species is relatively high: they are able to exist and even to propagate in water with oxygen contents of 0.028–0.233 $\mu\text{mol/ml}$. The discovered bacteria are characterized by a great morphological variety, mainly cocci of different size and few rods.

Obviously many morphological types possess magnetotactic behaviour and the question arises, how the ability for magnetotaxis was acquired. It has not yet been clarified, whether polymorphism plays a role or whether one is dealing with a convergence phenomenon. The fact that the different forms have been found in the same sample rather supports the polymorphism hypothesis. All coccoid cells possess the same arrangement of flagella, but two different configurations of magnetosomes: 1) a chain-like arrangement (Fig. 5a and 5b), and 2) a scattered arrangement (Fig. 5c). The magnetosomes are positioned adjacent to the flagellar bundles. In cells with a chain-like arrangement of the magnetosomes, the magnetite grains are located between the flagellar

bundles (Fig. 6a) and in cells with the scattered distributional pattern they are located close to the flagellar bundles (Fig. 6b). Fig. 5a and b show magnetosomes which appear different in their electron density.

This fact can possibly be explained by the process of cutting for ultrathin sections, which may either produce magnetosomes of varying thickness due to their wave-like arrangement within the cell, or, they deviate in thickness, or perhaps a combination of both.

Magnetite represents a physiologically favourable way of storing high amounts of iron. Moench found 3–4% iron of dry weight in the cells of magnetotactic bacteria. That is an enormous amount, if one considers that the highest amount of iron ever found was 0.27% in *Desulfotomaculum orientis*, while *E. coli* merely contains 0.014% iron.

According to the orientation hypothesis [2] magnetosomes enable the magnetotactic bacteria to orientate along the earth's magnetic field lines. Because of the inclination of earth's magnetic they arrive at deeper and more oxygen reduced sediment layers, where their conditions of life are optimal. This orientation hypothesis is also confirmed by following observation: the enriched bacteria, which have been concentrated in a spot by a magnetic stirring bar are moving instantly downward into the sediment layers after a removal of the stirring bar. The orientation hypothesis has been extended by experiments of Spormann and Wolfe [7]. The bacterium's cell is able to locate its best living conditions by an interaction of the magnetotaxis and an aerotaxis, which finally leads the bacterium in a suitable micro-aerophilic environment.

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