

Envelope ultrastructure of uncultured naturally occurring magnetotactic cocci

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Abstract

Magnetotactic bacteria are microorganisms that respond to magnetic fields. We studied the surface ultrastructure of uncultured magnetotactic cocci collected from a marine environment by transmission electron microscopy using freeze-fracture and freeze-etching. All bacteria revealed a Gram-negative cell wall. Many bacteria possessed extensive capsular material and a S-layer formed by particles arranged with hexagonal symmetry. No indication of a metal precipitation on the surface of these microorganisms was observed. Numerous membrane vesicles were observed on the surface of the bacteria. Flagella were organized in bundles originated in a depression on the surface of the cells. Occasionally, a close association of the flagella with the magnetosomes that remained attached to the replica was observed. Capsules and S-layers are common structures in magnetotactic cocci from natural sediments and may be involved in inhibition of metal precipitation on the cell surface or indirectly influence magnetotaxis.

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1. Introduction

Prokaryotes depend on diffusion to survive and have a high surface area to volume ratio, which contributes to their capacity to sorb and precipitate metals, preferentially on their surface [1]. Bacterial cells interact with metal ions present in the environment because of charge characteristics of their surface that contribute to the precipitation and accumulation of metal ions on the cell surface [1]. Negatively charged groups like ionized phosphates and sulfates give the cell surface an overall negative charge that is responsible for the initial mineral precipitation [1]. Magnetotactic bacteria take up large amounts of iron to make the magnetite (Fe₃O₄) or greigite (Fe₃S₄) in their magnetosomes, but no iron minerals have been

found on the cell surface of these bacteria [2]. Thus, the surface characteristics of uncultured magnetotactic bacteria from natural enrichments were studied to understand the interaction of microorganisms with the environment and their role in the mobilization and metabolism of metals.

The cell wall of bacteria in their native habitat is often overlaid by other superficial layers, such as S-layers and capsules, which can be lost under prolonged cultivation [3]. S-layers are molecular assemblies of proteins or glycoproteins, which are weakly acidic, and completely cover the cell in all stages of growth in both Bacteria and Archaea. The S-layer subunits are secreted and, once outside the cell, they interact with each other and with the cell wall surface through non-covalent forces, forming bidimensional crystalline arrays arranged in oblique (1, 2), square (4) or hexagonal (3, 6) symmetry. Most of the functions of S-layers are hypothetical. S-layers are possibly involved in adhesion and surface recognition. They may also function as protective coats, molecular sieves, and as molecule and

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ion trappers [3,4]. Studies on S-layers mineralization are sparse. S-layers are a scaffold for gypsum and calcite mineralization in the cyanobacterium *Synechococcus* GL24 [5]. Furthermore, isolated S-layers from *Bacillus sphaericus* CCM2177 containing added thiol groups have been used as templates for metallic gold deposition [6].

Capsules usually consist of a thick and highly hydrated polymeric repeating oligosaccharide matrix, although proteinaceous capsules have also been described [7]. Possibly, part of these polymers is embedded in the cell wall and the remainder extends roughly linearly into the external milieu. This structure is maintained by a combination of electrostatic and hydrophobic–hydrophilic forces. These polymers must profoundly influence the access of molecules and ions to the cell [7]. Capsules are involved in antigenicity, protection against desiccation, and adhesion to surfaces [7]. Capsules and exopolymeric substances of uncultured microorganisms from biofilms collected in diverse environments are known to mineralize many different minerals, most of them containing large amounts of iron [8–14].

Magnetotactic bacteria are morphologically and phylogenetically diverse and share the ability to orient along magnetic field lines because of organelles called magnetosomes. These bacteria survive in a region in sediment where oxygen and nutrient requirements are available through the use of magneto-aerotaxis [15]. Natural enrichments contain a variety of different morphological types of magnetotactic bacteria, which are fastidious in pure culture. Each morphotype is usually associated with a particular crystalline form of the magnetic mineral. Most magnetotactic bacteria synthesize magnetosomes that contain magnetite crystals (Fe_3O_4) belonging to three main classes: truncated cuboctahedra, pseudo-hexagonal prisms and bullet-shaped crystals [16]. Magnetotactic multicellular aggregates [17] and other unicellular bacteria produce iron sulfide magnetosomes [18] with poorly defined shapes.

We have reported magnetotactic cocci that can produce magnetosomes twice as large as other bacteria [19,20]. The phylogenetic position of these microorganisms has been determined and at least four morphotypes of cocci were described based on phylogenetic analyses of 16S rRNA gene sequences and in situ hybridization at electron microscopy level [20]. Phosphorus-rich granules are a common trait of these marine cocci that are able to naturally incorporate metal ions such as aluminum and iron [21]. The high solubility of these amorphous granules allows the temporary incorporation of foreign ions and possibly the storage of the iron element for magnetosome synthesis [21]. The granules are also the major sites for metal, including heavy metals, accumulation as shown by energy-dispersive X-ray analysis using transmission electron microscopy [22].

As these uncultured magnetotactic cocci presented different cell compartments with different precipitation capabilities, further information on the structure of these mi-

croorganisms is necessary to understand functional aspects of metal accumulation. Few reports are available on the structural characteristics of the surface of magnetotactic bacteria [23], though it is the first gateway for iron uptake. Information on the surface ultrastructure may provide a structural framework for the interpretation of physiological data and interrelations of these bacteria with the environment. Here, we studied the envelope ultrastructure of uncultured magnetotactic cocci present in marine waters from the Itaipu lagoon by transmission electron microscopy using freeze-fracture and freeze-etching. Results indicate that capsule and S-layer are common traits in different morphotypes of magnetotactic cocci. Besides, magnetosomes are in close contact with the surface of most of the cells and also with the flagella.

2. Materials and methods

2.1. Sampling and magnetic collection of magnetotactic bacteria

Samples were collected at Itaipu lagoon (43°04'W, 22°57'S) near Rio de Janeiro. Sediment of the lagoon was stored in bottles and left undisturbed at the laboratory under dim light for several weeks. Periodically, a drop of sediment was placed on a slide and checked for the presence of magnetotactic bacteria using a light microscope and a properly oriented ordinary magnet. Only bottles with a high ratio of magnetotactic bacteria were chosen for the magnetic harvesting of bacteria. For this, specially designed glass chambers [17] filled with lagoon water and sediment were exposed to an aligned magnetic field of a coil. After 20 min of exposure to the field, enriched magnetotactic bacteria were collected with a capillary tube and used in subsequent processing.

2.2. Transmission electron microscopy, freeze-fracture and freeze-etching

For transmission electron microscopy, magnetically concentrated cells were fixed in 2.5% (v/v) glutaraldehyde in sodium cacodylate buffer 0.1 M (pH 7.2) diluted in Millipore-filtered lagoon water at 4°C for 1 h, washed in the same buffer, post-fixed in buffered 1% (w/v) OsO_4 for 1 h, dehydrated through an ethanol series and embedded in PolyBed 812. Ultrathin sections were cut in a Reichert ultramicrotome and stained with 2% (w/v) uranyl acetate for 15 min and lead citrate for 3 min.

For freeze-etching experiments, magnetically enriched bacteria were fixed in a solution containing 2.5% glutaraldehyde in cacodylate buffer 0.1 M for 30 min at room temperature, washed several times in distilled water and placed on cushions of 12.5% (w/v) gelatin attached to a metal support by a piece of filter paper. The support was then quick-frozen by impact against a copper block, pre-

viously cooled by liquid nitrogen [24] or by contact with a liquid nitrogen-cooled sapphire block of a Life Cell CF0100 quick-freezing machine (Research and Manufacturing, Tucson, AZ, USA). Frozen specimens were then transferred to a Balzers freeze-fracture machine and fractured at -115°C . After fracturing, the exposed surface of some of the samples was etched for 1–3 min approximately at -100°C , cooled again to -115°C , then rotary-shadowed with platinum at 15° and carbon at 90° . Replicas were cleaned in sodium hypochlorite, collected on copper grids and examined with a Zeiss EM902 transmission electron microscope at 80 kV. Fast Fourier transform spectra were calculated with Digital Micrograph 2.5 software (Gatan, Pleasanton, CA, USA) on $1024 \times 1024 \times 8$ -bit resolution TIFF images obtained from scanned electron microscope negatives.

3. Results

Samples of magnetotactic bacteria from Itaipu lagoon contained mostly cocci that varied from 1.3 to $4\ \mu\text{m}$ in

diameter. Rarely, we observed rod-shaped bacteria or magnetotactic multicellular aggregates in our samples. Transmission electron microscopy of thin-sectioned magnetotactic cocci showed a cell envelope that contained the typical ultrastructural features of Gram-negative bacteria (Fig. 1a, arrow). The plasma membrane (Fig. 1a, white arrows) was separated from the outer membrane (Fig. 1a, black arrow) by a periplasmic space. The membranes presented a typical racetrack appearance with dimensions compatible with lipid bilayers; both the plasma and the outer membrane were around 7 nm thick. On the top of the outer membrane, a fibrillar coat, 30–70 nm thick, was observed (Fig. 1a, arrowheads) in cells fixed with filtered lagoon water. Magnetosomes and granules were also observed (Fig. 1a) and presented the previously reported characteristics [20,21,25].

Freeze-etching and freeze-fracture experiments revealed the substructure of the cell surface of uncultured magnetotactic cocci. Freeze-fracture images showed an extensive capsule formed by numerous fibrils that extended up to hundreds of nanometers from the cell surface in some cells. The capsular material was anchored on the surface

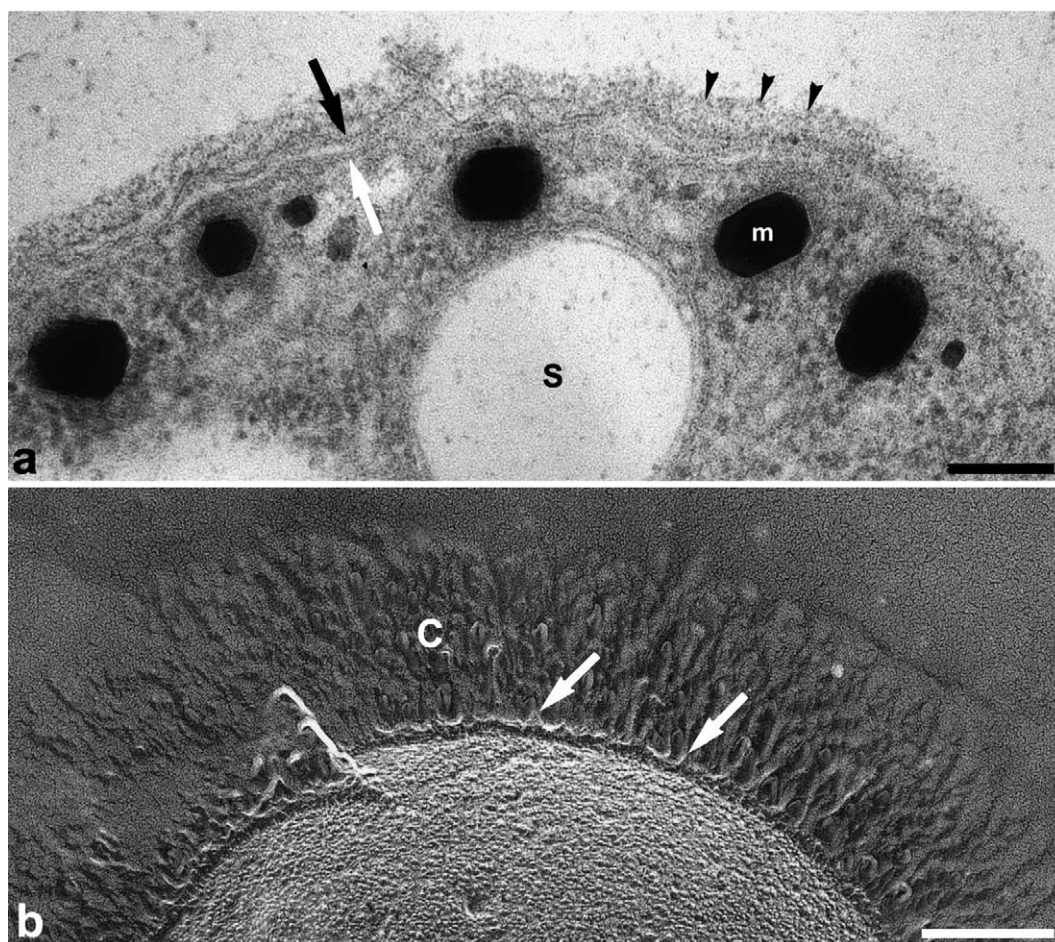


Fig. 1. Envelope structure of magnetotactic cocci. a: Transmission electron microscopy image of a magnetotactic coccus from the Itaipu lagoon showing the plasma membrane (white arrow), the outer membrane (black arrow) and a fibrillar coat (arrowheads). In the cytoplasm, several magnetosomes (m) and a sulfur globule (S) with a typical membrane structure can be observed. b: Freeze-fracture of a magnetotactic coccus showing an extensive capsule (C) formed by fibrils that were connected to the surface by peduncles (arrows). Scale bar indicates 120 nm in a and 400 nm in b.

by peduncles (Fig. 1b, arrows). Freeze-etching of the bacterial outer layers revealed the presence of paracrystalline surface structures (S-layers) distributed in domains on the surface of some cells (Fig. 1a,b). Replicas showed that the outermost layer on most magnetotactic cocci was periodic and corresponded to an S-layer. Fig. 2a shows a magnetotactic bacterium where the magnetosome chains remained attached to the cell surface before the sample was etched and platinum–carbon-replicated. S-layers in these bacteria were seen and were formed by particles (Fig. 2b, arrowheads) periodically organized in hexagonal symmetry, as concluded from image processing of images by fast Fourier filtration (Fig. 2b, inset). We also observed numerous

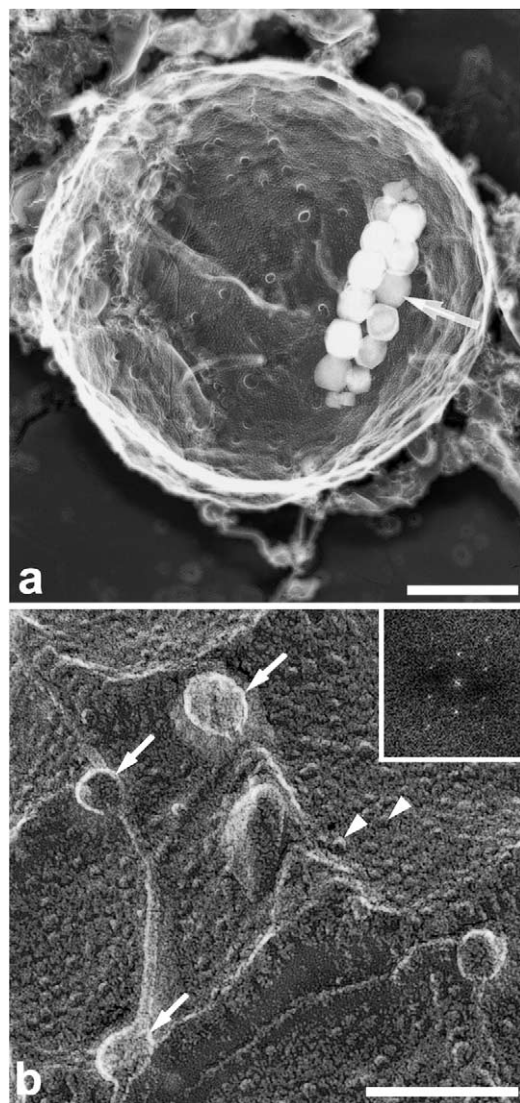


Fig. 2. Freeze etching of uncultured magnetotactic cocci. a: An S-layer formed the outer layer of the surface of magnetotactic coccus. Two chains of magnetosomes (arrow) remained attached to the replica. b: High magnification of the cell surface showing numerous particles from the S-layer (arrowheads) and the presence of membrane vesicles (arrows). Inset shows a fast Fourier transform spectrum of a typical region of a S-layer. Note the hexagonal symmetry of spots in the spectrum. Scale bar indicates 0.5 μm in a and 0.1 μm in b.

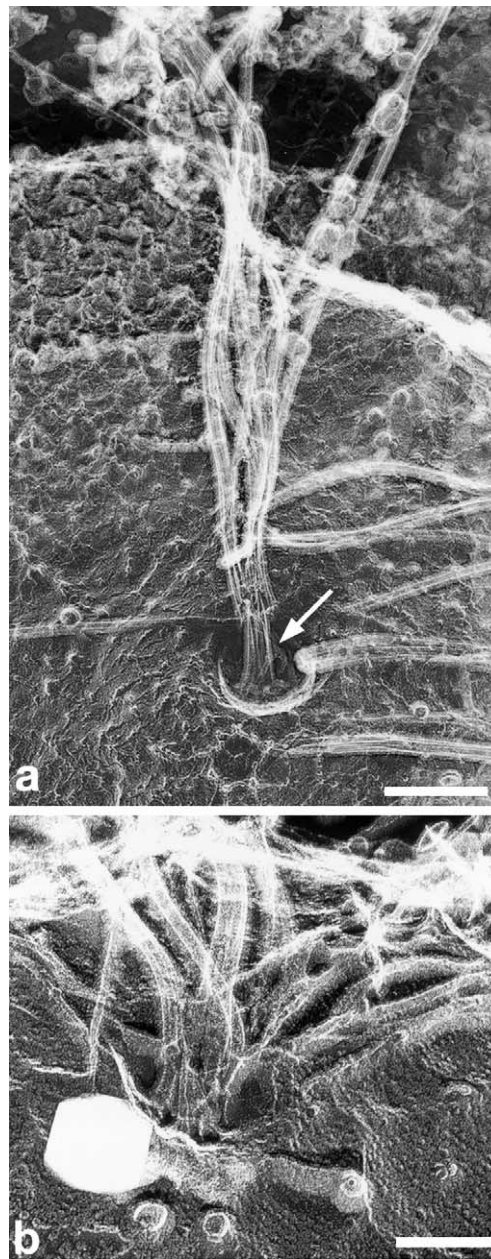


Fig. 3. Structure of flagella in magnetotactic cocci. Flagella are organized in bundles inserted on the bacterial surface in depressions on the envelope (a). Several flagella originated from the same cavity (arrow). Occasionally, an intimate association of the flagellar apparatus with magnetosomes was observed in freeze-etching preparations. Scale bar indicates 300 nm in a and 120 nm in b.

membrane vesicles that project from the surface of all cocci analyzed (Fig. 2b, arrows). No evidence of mineral precipitation on the cell surface was found. The flagella could be observed in the freeze-etching replicas. Numerous flagella were present in bundles (Fig. 3a) that were inserted in the cell surface at depressions. All flagella in a bundle converged to a single point where they attached to the surface (Fig. 3a, arrow). Occasionally, we observed a close association of the magnetosomes that remained attached to the replica and the flagella (Fig. 3b).

4. Discussion

The physicochemical and structural qualities of the cell surface of magnetotactic bacteria provide a basis for the interaction of these microorganisms with the environment and for magnetotaxis. In the present study, several features of the cell surface micro-architecture of magnetotactic cocci have become available with freeze-etching and freeze-fracture. We observed capsules and S-layers on the surface of the uncultured magnetotactic cocci. Capsules of uncultured microorganisms from biofilms collected in diverse environments are known as nucleation sites for a variety of minerals, many containing iron [10,12,26]. On the other hand, studies on S-layer mineralization are sparse. S-layers are a scaffold for gypsum or calcite mineralization in the cyanobacterium *Synechococcus* GL24 [5]. Isolated S-layers found in *B. sphaericus* containing thiol groups have been used as templates for metallic gold mineralization [6]. S-layers are ubiquitous in prokaryotes [3,4] and seem to be a characteristic feature of uncultured magnetotactic cocci. Few reports indicate that magnetotactic bacteria from different environments can express S-layers on their surface [23].

The presence of S-layers and capsules in magnetotactic cocci may have some indirect implications associated with magnetotaxis as previously discussed [23]. Primarily, they may influence the ability of the bacteria to overcome the viscous drag of the environment [23] during the swimming of the cells while they find a suitable place in the environment. The cultured species *Magnetospirillum magnetotacticum* has about 2% of its dry weight as iron [27], which is 100 times higher than the content of *Escherichia coli* cells [28]. No siderophores were identified in spent culture media of *Magnetospirillum gryphiswaldense* [28]. Some studies have shown that magnetotactic bacteria incorporate iron as Fe^{3+} ions [28], which are poorly soluble at neutral pH. This large amount of Fe^{3+} would readily precipitate on any suitable surface. In this way, the absence of mineral particles in both the S-layers and the capsules of magnetotactic bacteria from Itaipu lagoon would indicate that these structures inhibit iron precipitation on them.

Membrane vesicles are a common feature of all Gram-negative bacteria during normal growth. They are commonly filled with components present in the periplasmic space, including cell precursors and enzymes [29]. As far as we know, these vesicles have not been reported in natural communities of magnetotactic bacteria, but have been observed in ultrastructural studies of the cultivated species *M. magnetotacticum* [30]. Our results and previous reports suggest that membrane vesicles are also a structural feature common to all magnetotactic bacteria.

A related aspect of magnetosome organization and magnetotaxis is the question of how magnetosome chains are positioned within the bacterial cell. No evidence for a connection between magnetosomes and the cell surface was observed in cultivated cells. We could not observe

any kind of connection in our samples. The adherence of the magnetosome chains to the replicas in the freeze-etching experiments suggest that the magnetosomes are close to the cell surface. Theoretical estimates of the stability limit of straight magnetosome chains and observation of freeze-dried cells indicate that under normal conditions the chains of magnetosomes are positioned close to the cell envelope [31]. The organization of flagella in magnetotactic cocci seems to be unique in that all flagella in a bundle are attached to the cell inside a depression in the cell surface.

In conclusion, the envelope structure of uncultured magnetotactic cocci is similar to that found in non-magnetotactic microorganisms. The presence of a coat in many magnetotactic cocci observed indicates that this structure could be commonplace in uncultured magnetotactic cocci from marine environments. These structures may be involved in regulation of metal precipitation on the cell surface and may indirectly influence the efficiency of magnetotaxis.

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