Intracellular Inclusions and Nanocrystals of Magnetotactic Cocci Isolated from the Cispata Beach Estuary, Colombia

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ABSTRACT

Magnetotactic bacteria are aquatic microorganisms which share the ability to orient themselves along magnetic field lines. Magnetic orientation is due to the presence of magnetosomes, which are intracellular membrane-bound nanocrystals of magnetite or greigite [4,5]. The bacteria may also have intracellular inclusions [7]. Magnetosome formation in magnetotactic bacteria provides a novel magnetic nanomaterial that is generated by a mineralization process that controls the chemical composition, morphology and size of the magnetic mineral [8]. The aim of this work was to study magnetotactic cocci present in the estuary environment in Colombia, through transmission electron microscopy. Samples of sediment and water were collected at Cispata Beach, and these were incubated in the laboratory under low-light conditions at room temperature for several weeks in order to isolate magnetotactic bacteria using a magnetic field. Cells were studied using Morgagini and TECNAI G2 20 D345 (FEI) transmission electron microscopes operating at 80 kV and 200 kV respectively. Results indicate that these magnetotactic cocci range from 0.5 to 1 μm in diameter. Each bacterium has two pairs of magnetosome chains with elongated prismatic crystals from 60 to 190 nm in length. Energy dispersive X-ray spectrometry (EDX) microanalysis of a magnetosome showed mainly iron and oxygen peaks, probably of magnetite crystals. EDX microanalysis in other globular inclusions in the bacteria shows mainly sulfur. This work contributes to the knowledge of estuary magnetotactic bacteria and their nanocrystals in Colombia, which will facilitate the study of their future biotechnological and biomedical applications.

Keywords: magnetotactic bacteria, magnetosomes, intracellular inclusions, transmission electron microscopy

1 INTRODUCTION

Magnetotactic bacteria are aquatic microorganisms which biomineralize intracellular single-domain crystals of iron oxides (magnetite) and/or iron sulfides (greigite). Each cell contains one or more chains of magnetosomes, which produces a net dipole moment sufficient to align the cell with local geomagnetic fields. The populations of magnetotactic bacteria are typically found at or below the interface between oxygenated and reduced sediments or waters [3,5,6,13]. Magnetite-producing magnetotactic bacteria are found in both freshwater and marine environments [14]. Magnetite nanocrystals of magnetotactic bacteria provides a novel magnetic nanomaterial that is generated by a mineralization process that controls the chemical composition, morphology and size of the magnetic mineral [8]. These crystals are individually covered with a thin organic membrane, which confers high and even dispersion in aqueous solutions compared with artificial magnetite, making them ideal biotechnological materials [2].

In addition to magnetosomes, other intracellular inclusions are found in the cytoplasm of magnetotactic bacteria, including phosphorus-containing granules, polyhydroxyalkanoate inclusions, and sulfur globules [7]. Analyses of the composition of these intracellular inclusions provide some clues about the physiology of the bacteria and the characteristics of the microenvironments where they live. The aim of this work was to study nanocrystals and intracellular inclusions of magnetotactic cocci isolated from an estuary environment in Colombia, through transmission electron microscopy and EDX Microanalysis. This work contributes to knowledge of estuary magnetotactic bacteria and their nanocrystals in Colombia, which will facilitate the study of their future biotechnological applications.

2 MATERIALS AND METHODS

2.1 Collection and Isolation of Magnetotactic Bacteria

Samples of water and sediment in a 1:2 ratio were collected from the oxic/anoxic interface of the Cispata Beach (9°20'–9°23'N and 75°40'–75°50'W), an estuary near the city of San Antero, Cordoba, Colombia. Samples were analyzed for redox potential, pH, dissolved oxygen concentration and temperature. Redox potential values were measured using an Ag/AgCl reference electrode (HANNA HI 3619), pH values were measured with a pH electrode (Handylab 1), oxygen concentration was measured using an oxygen meter (HANNA HI 9143) and temperature values were measured with a conventional mercury thermometer (B & C Germany).

Approximately 50 ml of sample water was analyzed for ferrous iron, total iron and sulfate concentration using a spectrometric technique. The spectrophotometer used was a GENESYS™ with 10 UV scan. The ferric iron concentration was estimated by subtracting the ferrous iron concentration from the total iron concentration. The samples were stored in plastic bottles and left undisturbed in the laboratory at

ambient temperature (25-27° C) and under dim light for four weeks. During this period, the sediment stratified and opposite gradients of sulfides and oxygen formed, and populations of magnetotactic bacteria increased more than other populations of non-magnetotactic microorganisms.

Magnetotactic bacteria were magnetically separated as described by [9]. Briefly, water and sediment were transferred to a specially designed glass flask containing one large opening at the top through which the samples were inserted, and a lateral capillary at the end through which water containing large amounts of magnetotactic bacteria was collected after exposure for 30 minutes to a magnetic field generated by a coil with the appropriate polarity. Drops of water containing magnetically separated microorganisms were collected in plastic Eppendorf tubes and used in subsequent experiments.

2.2 Transmission Electron Microscopy and EDX Microanalysis

A drop of water containing harvested magnetotactic bacteria was placed onto a Formvar-coated grid with its edge covering half of the grid area. A common magnet was then used to drive the bacteria to the center of the grid. The magnet was positioned on the opposite side of the drop with the alignment of the magnetic field in such a way that the bacteria would swim to the side closest to the middle of the grid. After several minutes, bacteria had accumulated at the drop edge. After 2 minutes, the water from the estuary was replaced by distilled water, and the grid was air-dried [9]. For observation of whole bacteria and elemental analysis of magnetosome and intracellular inclusions, imaging and energy-dispersive X-ray (EDX) analyses were carried out using Morgagini and TECNAI G2 20 D345 (FEI) transmission electron microscopes operating at 80 kV and 200 kV respectively, equipped with an EDX analysis system. To quantify the crystal-size distributions of nanocrystals, statistical analyses of particle size was performed by measuring crystal length and width in images obtained by transmission electron microscopy using OriginPro 7.5 software.

3 RESULTS AND DISCUSSION

Samples collected in Cispata Beach estuary had pH values close to neutral and slightly alkaline typical of estuarine environments, ranging from 7.5 to 7.9. The redox potential values ranged from 75 to 132 mV, values typical of reductive environments. The assimilation of iron for magnetite synthesis in magnetotactic bacteria occurs very efficiently from relatively low environmental concentrations. Magnetotactic bacteria are able to accumulate iron and form magnetite crystals in environments with a relatively low abundance of iron (15–20 μM) [11]. The concentration of total dissolved iron from natural samples in the Cispata estuary ranged between 0.28 and 0.51 ppm, ferrous dissolved iron concentration between 0.15 to 0.32 ppm and ferric dissolved iron concentration between 0.15 and 0.21 ppm, indicating a low concentration of iron forms in this estuary. Besides the availability of micromolar amounts of iron, microaerobic conditions are required for magnetite formation [10]. The dissolved oxygen concentration values from the oxic-anoxic interface in the Cispata estuary ranged from 0 to 3.4 ppm, which showed that magnetotactic cocci live under microaerobic conditions. Tipically, the amount of dissolved oxygen that allowed growth of magnetotactic bacteria in aquatic environments ranged from 0 to 3% [5]. In *M. gryphiswaldense,* maximum magnetite synthesis occurred at 0.25 mbar oxygen [11]. Sulfate concentration ranged from 650 to 2160 ppm.

Environmental samples contained magnetotactic cocci that ranged from 0.5 to 1 μm in diameter. These magnetotactic bacteria in a water drop when placed under light microscopy after application of a magnetic field exhibited active swimming as well as their characteristic "ping-pong" movement at the north edge of the drop. Transmission electron microscopy of magnetotactic cocci showed cells with two spherical dark regions corresponding to intracellular inclusions that were very similar in size and elemental composition. The bacteria contained two pairs of chains of magnetosomes, which seem to be organized at opposite sides of the cell or in a crossed arrangement (see Fig 1A and 1B). Each chain contained eight elongated prismatic crystals with a highly regular structure with faceted edges at the corners (see Fig. 1C and 1D).

Crystals in magnetosomes generally range from 30 to 200 nm [10]. Mature magnetite crystals produced by magnetotactic bacteria typically fall within the stable single magnetic domain range of between 30 and 120nm, and thus are of the optimum size for magnetotaxis. Particles within this size range are perfect bar magnets. While crystals of this size range are permanently magnetic, smaller sizes would not efficiently contribute to the cellular magnetic moment, as such crystals are superparamagnetic at ambient temperatures, which means they do not show persistent magnetization. In crystals larger than 120 nm, multiple magnetic domains of opposite magnetic orientation can be formed, which reduces the total magnetic remanence of the crystal [1,12,15]. The existence of unusually large magnetosomes from 120 to 200 nm in size (these correspond to the theoretical size of two-domain magnetite crystals) can be explained by the fact that the theoretical size of a magnetic domain calculated for the zero strength of a magnetic field may not correspond to the actual size of such domains under the conditions typical of the cell interior [1].

Another characteristic in biogenic crystals of magnetotactic bacteria is that they display restricted width/length ratios and volumes within the single-domain size range in order to obtain a competitive advantage in the natural environment, with crystals that are perfect bar magnets [15]. The magnetite crystals in magnetotactic cocci from Cispata estuary had lengths from 60 to 190 nm, with 80% of crystals having lengths of up 120 nm, falling within the single-domain size, 15% having lengths from 60 to 110 nm falling within the superparamagnetic range, and 5% having lengths from 140 to

190 nm and falling within the multi-domain range. These crystals were elongated (width/length ratio of 0.8) along [111] their axes. At the ends of some chains smaller superparamagnetic crystals could be seen. (see Fig. 1C and 1D).

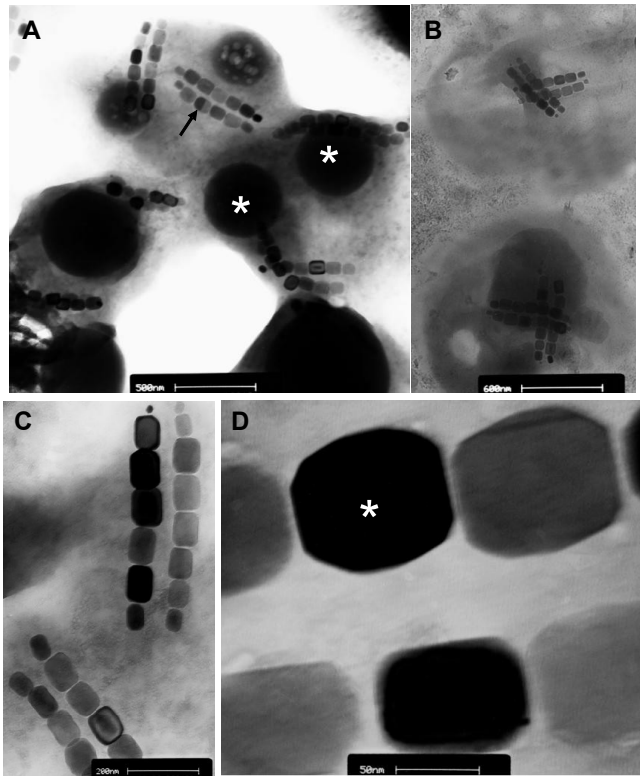

Figure 1: Transmission electron micrograph of magnetotactic cocci from Cispata Beach estuary. (A) Image of whole-mount magnetotactic cocci showing two pairs of magnetosome chains and two sulfur globules (asterisks). A crystal with twin (arrow). Bar = 500 nm (B) Two pairs of magnetosome chains arranged in two lines cross. Bar = 600 nm. (C) Section of two pairs of magnetosome chains with elongated prismatic crystals. Bar = 200 nm. (D) High-magnification image of the elongated prismatic crystals from the chains. Crystals had faceted edges on corners. Bar = 50 nm.

Magnetite produced by magnetotactic bacteria is pure stoichiometric Fe_3O_4, lacking minor or significant trace elements such as Ti, Cr, Mn, and Al. Magnetotactic bacteria exclude these other elements from the growing magnetite crystals, even though the elements may be readily available in the environment [4,15]. EDX analysis of magnetosomes showing mainly iron and oxygen peaks demonstrated that these crystals are of magnetite with a high chemical purity (see Fig. 2). EDX analyses of spherical dark inclusions in the same cell showed that these were very similar in elemental composition and size, mainly consisting of sulfur (see Fig. 3).

The presence of intracellular S-globules shows that these bacteria are sulfide- and/or thiosulfate-oxidizers, which are found mainly at chemical gradients of brackish, marine, or hypersaline environments. S-globules are S°-containing structures; they are enveloped by a protein coating or a membrane. Sulfur results from hydrogen sulfide or thiosulfate oxidation. When sulfide and thiosulfate are no longer present in the culture medium, the stored sulfur is oxidized to sulfate [7]. These results suggested that these marine cocci are chemolithoautotrophic and probably sulfide oxidizers. They live under microaerobic conditions, with their lifestyle adapted to sediments and chemically stratified aquatic habitats as well as estuaries.

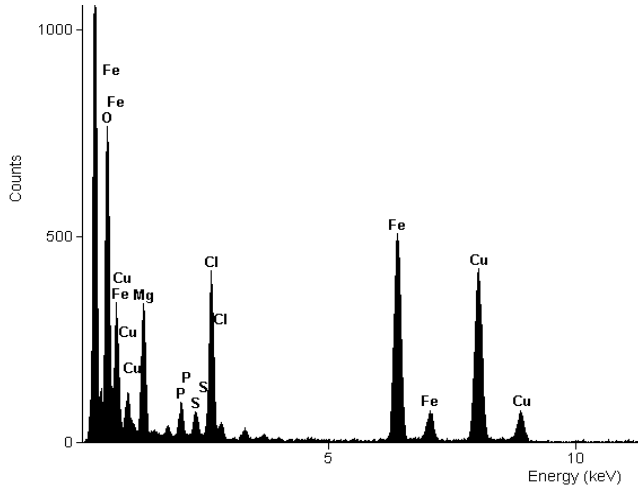

Figure 2: EDX espectrum of a nanocrystal (asterisks in Figure 1D) showing mainly iron and oxygen peaks. Small Mg, P, S, and Cl peaks are derived from the cytoplasm and membranes surrounding the magnetosome. Cu peaks were derived from the supporting grid.

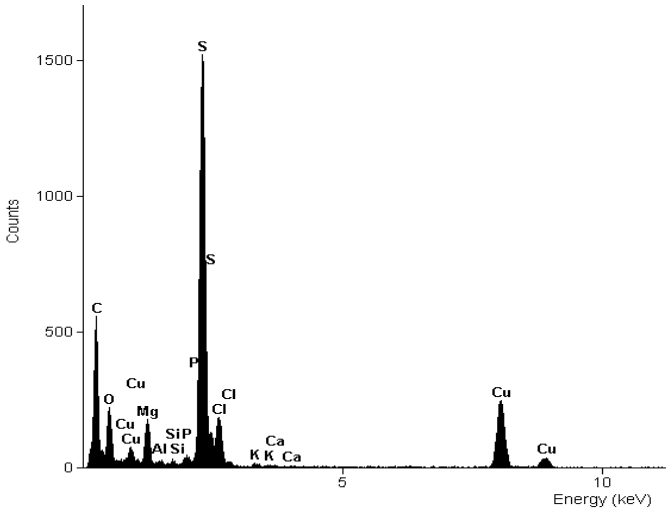

Figure 3: EDX spectrum of a sulfur globule of a magnetotactic coccus (asterisks in Figure 1A) showing that the globules contain mainly sulfur. Small peaks readings for C, O, Mg, P, Cl, K and Ca are derived from the cytoplasm surrounding the globules. Cu peaks were derived from the supporting grid.

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