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Respiring cellular nano-magnets

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ABSTRACT

Magnetotactic bacteria provide an interesting example for the biosynthesis of magnetic (Fe_3O_4 or Fe_3S_4) nanoparticles, synthesized through a process known as biologically controlled mineralization, resulting in complex monodispersed, and nanostructures with unique magnetic properties. In this work, we report a novel aerobic bacterial strain isolated from sludge of an oil refinery. Microscopic and staining analysis revealed that it was a gram positive rod with the capability to thrive in a medium (9K) supplemented, with Fe^{2+} ions at an acidic pH (~3.2). The magnetic behaviour of these cells was tested by their alignment towards a permanent magnet, and later on confirmed by magnetometry analysis. The X-ray diffraction studies proved the cellular biosynthesis of magnetite nanoparticles inside the bacteria. This novel, bio-nano-magnet, could pave the way for green synthesis of magnetic nanoparticles to be used in industrial and medical applications such as MRI, magnetic hyperthermia and ferrofluids.

1. Introduction

Microorganisms have inhabited the earth for billions of years, and their activities are vital strengths forming our planetary surroundings through biogeochemical cycles [1,2]. Microbial biomineralization specifically takes up natural components, and stores minerals either intracellularly or extracellularly. This is of prodigious interest, as they do not only serve as vital biosignature for searching hints of past life, but also have numerous commercial applications [3,4]. Despite the fact, that microbes make an extensive assortment of biominerals, very little is known about these microorganisms' minerals and their mechanisms of biomineralization [5–7]. The best considered among them are magnetotactic bacteria (MTB), which biomineralize iron oxide (magnetite, Fe_3O_4) or the iron sulfide (greigite, Fe_3S_4), inside membrane-bound organelles called magnetosomes (Fig. 1) [8,9].

MTB are widespread, motile, morphologically, phylogenetically, and physiologically diverse group of ubiquitous gram-negative bacteria, which have the ability to orientate, and migrate along magnetic field lines of the earth [10–12]. This capacity of these bacteria relies on the magnetic structures, which are present intracellularly, the magnetosomes. They consist of membrane bound, nano-sized crystals of iron oxide, synthesized by a tightly regulated cellular mechanism, known as Biologically Controlled Mineralization (BCM) [13,14]. The magnetic crystals of iron are organized into chains by means of a devoted cytoskeleton, and are in charge of the cell magnetotaxis behaviour [10,15]. The magnetosome synthesis by all accounts is a complicated procedure that comprises a few discrete strides including the formation of a vesicle for magnetosome, uptake of iron by the cell, transport of this iron into the magnetosome vesicle, and biomineralization of Fe_3O_4 or Fe_3S_4 in a controlled manner in the cellular nanoreactors [16–18].

Magnetosomes synthesized through BCM by MTB have many interesting attributes, compared with chemically synthesized magnetic nanoparticles. They have high substance purity, limited size extent, specie-specific crystal morphologies, and display specific cellular arrangements of these crystals [19,20]. These obvious advantages make them convenient bearers for the coupling of relatively higher quantity of bioactive materials, which can later on be alienated by the application of a magnetic field [21]. They can find applications in the recognition of nucleotide polymorphism [3], immunoassays [10], DNA extraction, and pathogens detection in food, in magnetic hyperthermia and (targeted) drug delivery [22].

MTB are a group of heterogeneous aquatic prokaryotes and due to their high abundance (in marine and freshwater), they play a vital role in numerous sediments, and biogeochemical cycling of iron, as well as other elements [2,23]. They are mostly microaerophiles or anaerobes, and are attracted towards an environment that contains practically no oxygen [24,25]. The MTB presence depends on opposing gradient of reducing and oxidizing sulfur species and oxygen. Availability of soluble iron is one of the reasons for their abundance in the particular environment. MTBs are considered as a typical

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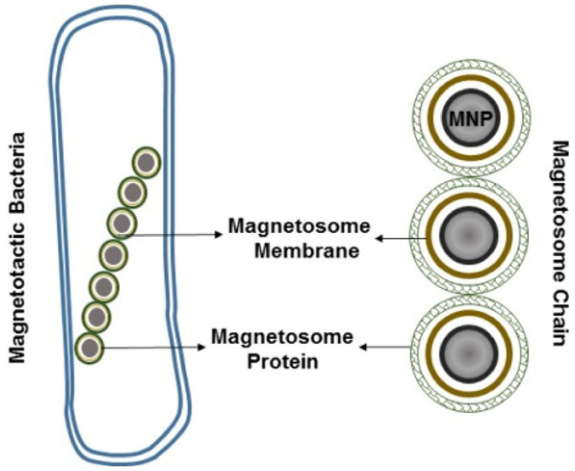

Fig. 1. A cartoon showing the arrangement of magnetosomes inside magnetotactic bacteria. Magnetosomes consist of a double membrane bound magnetic nanoparticle (MNP) core.

example of gradient organisms [26]. They reside in the first top centimetres of marine sediment, within the oxic/anoxic interface (OAI), where oxygen is present at low concentrations. MTB isolation and culture in laboratory is difficult as they have diverse nutritional requirements, and are redox-sensitive. These conditions are not easy to mimic in laboratory set-ups [27,28]. MTB that have the capacity to biomineralize under aerobic conditions do exist in our environment, but there is limited published literature available about their isolation, optimization, and culture in lab controlled conditions [29–31]. For instance, Matsunaga et al. isolated helical shaped MTB from fresh water sediments in a carbon medium with ferric gallate [30]. Similarly, Elcey et al. and Jun et al. reported the isolation of MTB capable of thriving under aerobic conditions. They both described the isolation of rod shaped MTB; the former in 9K while the latter in liquid 2219 (ferric quinate) medium [29,31]. The current study reports an aerobic magnetotactic bacterial strain characterized (bio/chemical and physical) and optimized for the biosynthesis of magnetic nanoparticles.

2. Materials & methods

2.1. Sampling & culturing

Samples were collected from oil refinery sludge in pre-sterilized 50 mL conical tubes, wrapped in aluminium foil to prevent it from direct sun light, and stored in cool/dark place. Each time 2 g of sample was dissolved in 50 mL distilled water/filtered. The samples were cultured initially (12 mL) LB broth, and inoculated with 2 μL of sample filtrate and incubated overnight at 30 °C. The cultured bacterial samples were grown in 9K medium [32], i.e. 15 mL conical tubes were filled with 9K and were inoculated with 100 μL inoculum. The tubes were incubated overnight at 30 °C on continuous shaking (120 rpm) (LSI-100C, KWF China) under aerobic conditions. Bacterial growth was determined at regular intervals of every 2 h, by recording the optical density (OD) at 600 nm using spectrophotometer (Genesys 10-S, Thermo Spectronic).

2.2. Magnetic movement & Gram staining

Preliminary tests were performed to check if the cultured bacteria are magnetic (magnetotactic) or not. The magnetic movement of bacteria was checked by using a permanent magnet. 5 mL of actively growing bacteria were collected, and centrifuged at 4000 ×*g*. The pellet was washed in 0.9 M NaCl solution three times. The purified cells were resuspended in 0.9 M normal saline solution. A permanent magnet was applied at one end of a glass tube to align the cells towards the magnetic field gradient. To estimate their relative magnetic behaviour, commercially purchased magnetic nanoparticles were also tested the same way. The Gram staining was performed as per standard procedure.

2.3. Magnetosomes extraction

The bacterial culture (45 mL) was taken in 50 mL tubes and spun at 12,000 ×*g* (Eppendorf 5810 R, Germany) for 2 min. The pellet was washed thrice with 0.9 M normal saline, and suspended in 0.2% SDS for 45 min at room temperature. The cell-SDS mixture was then sonicated (Cole Parmer CPX 130) at 20 kHz for 5 min at 40% amplitude (pulse on 2 s, pulse off 1 s). The sonicated mixture was purified by placing a strong magnet to one side of the tube, followed by removal of fluid

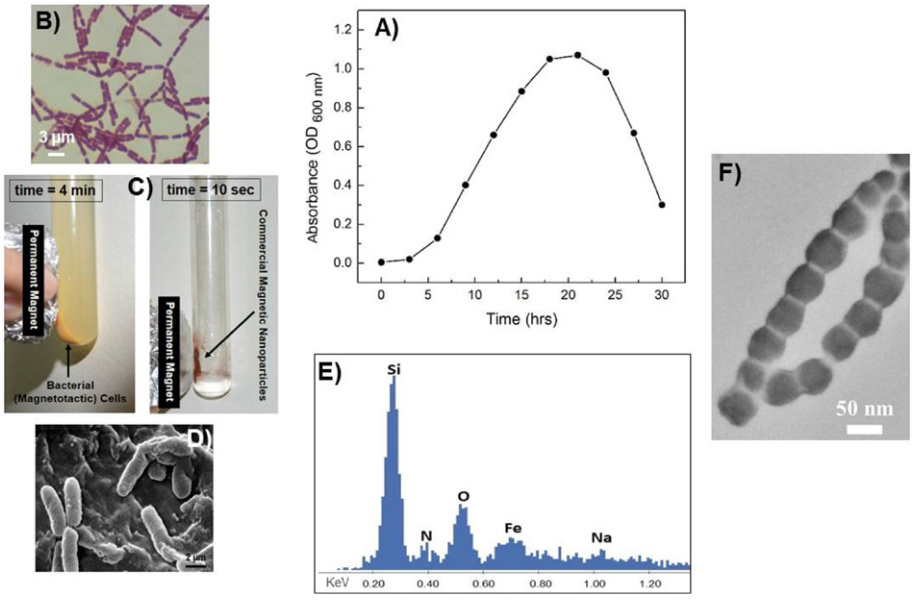

Fig. 2. A) Growth plot of isolated MTB strain in 9 K at 30 °C (B) Isolated MTB strain: a gram positive *Bacillus* (C) Magnetic response towards a permanent magnetic; isolated MTB cells slowly aligned towards the magnetic field gradient as compared to commercially synthesized magnetic nanoparticles (D) SEM image of isolated MTB rods (E) EDX spectrum of extracted magnetosomes showing clear peak of Fe (F) Scanning Transmission Electron Microscopy image of isolated magnetosomes.