Magnetotactic bacteria and bacterial magnetite

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1. Introduction

Magnetotactic bacteria which orient and swim along a geomagnetic field have been found in both fresh water and marine sediments [1,2]. These bacteria precipitate magnetite (Fe_3O_4) crystals intracellularly with a similar size. The magnetic particles are generally small in size (500 - 1000 Å) and aligned in groups of 10 to 20 particles, called the magnetosome, because they are covered with stable lipid membrane [3]. Since it has been difficult to culture magnetotactic bacteria on a large scale, these bacterial magnetites have thus far not been used for practical purposes.

The author's group has investigated various applications of the magnetic particles isolated from magnetotactic bacteria, including enzyme immobilization [4]. The activity of an enzyme (glucose oxidase) immobilized on bacterial magnetites was 40 times higher than that immobilized on artificial magnetites, and it retained its activity when it was reused 5 times. Bacterial magnetites also can be introduced into red blood cells by cell fusion technique [5], and granulocytes and monocytes by phagocytosis [6]. The movement of red blood cells containing bacterial magnetites was controlled magnetically. Magnetotactic bacteria have also been used to identify the magnetic south pole on selected iron-nickel grains from a meteorite [7]. Recently, a

new strain of magnetotactic bacteria as collected by use of a magnetic harvesting apparatus, and cultured in a chemically defined medium.

This paper presents the isolation and cultivation of a magnetotactic bacterium capable of growing in an air atmosphere and new applications of bacterial magnetites.

2. Isolation and Cultivation of Magnetotactic Bacterium Capable of Growing aerobically.

Many fresh water sludges and sediments were obtained from ponds at Koganei in Tokyo, and magnetotactic bacteria were isolated from them by using an isolation apparatus modified from Wolfe's work [8]. This apparatus allowed the selective separation of magnetotactic bacteria from an initial sample which contained contaminants. A sterile solution was placed on the inner side of a wetted cotton plug and the impure sample which contained magnetotactic bacteria was placed on the other side. Magnetotactic bacteria migrated through the cotton plug toward the south pole of a samarium-cobalt (Sm-Co) magnet (produced by TDK, Tokyo, Japan) placed on the side of the sterile solution. The magnetotactic bacterial sample which was purified in this way was inoculated into fresh medium. This isolation medium contained (per 1.0 l distilled water) 2 ml of Wolfe's mineral solution [9], 0.2g of potassium dihydrogenphosphate, 0.12 g of sodium nitrate, 0.02g of yeast extract, 0.02g of malt extract and 0.05 g of L-cysteine hydrochloride$\cdot H_2O$, 10 μM ferric gallate (The ferric gallate solution was prepared in 100 ml distilled water containing 0.27 g $FeCl_3$ and 0.19 g of gallic acid.), and 0.5 mg

of biotin. The medium was adjusted to pH 6.75 with NaOH solution before sterilization (120 °C, 2 atm, 10 min).

Helical shaped magnetotactic bacteria were isolated from fresh water sediments collected from a natural spring at Koganei in Tokyo. The pH of water samples from this site was 6.5-7.0. These sediments contained 7-10 species magnetotactic bacteria with many types of morphology, and about 90 % of these cells were distributed in the surface of the sediment (0-2 cm from its surface). Although many types of magnetotactic bacteria were inoculated into the isolation medium, only helical shaped magnetotactic bacteria were able to grow, and a single strain was isolated and designated AMB-1. Cells of strain AMB-1 have intracellular magnetic particles with an average diameter of 50 nm, and contained in a chain of over 15 particles. The cell diameter was 0.4 to 0.6 μm, and cell length was over 3 μm. A single flagellum was present at each pole (Figure 1).

Strain AMB-1 is oxygen tolerant, and can grow in an air atmosphere. The cells had oxidase activity. The ratio of non-magnetic AMB-1 cells increased, when magnetic cells were grown aerobically. The color cell pellet changed from black-brown to white (colorless).

The pH range of the medium which can grow was 5.0 to 8.2. AMB-1 was able to grow on 12 kinds of carboxylic acids such as α-ketoglutarate, succinate, fumarate, malate, oxaloacetate, pyruvate, lactate, acetate, propionate, n-butyrate, and β-hydroxybutyrate). After the cultivation for 7 days in the presence of these compounds, the cell number was 10 times larger

than that of the control. The cells grew and showed magneto-sensitivity in the presence of the following iron sources such as ferric chloride, ferric citrate, ferric malate, ferric quinate, ferric catechol, ferric protocatechuate, ferric gallate, ferric L-DOPA, ferric EDTA and ferric ferrioxamine B. In particular, when ferric malate, ferric catechol, ferric protocatechuate, ferric gallate, ferric L-DOPA and ferric EDTA were used, the cell concentration reached 1.4 to 2.9×10^8 cells/ml after 4 days microaerobic growth, and over of the 90 % cells were magneto-sensitive.

3. Separation and Preparation of Bacterial Magnetites

Magnetotactic bacteria suspended in HEPES buffer (pH 7.4) were disrupted using an ultrasonic disrupter (Tomy Seiko Co. LTD., UR-200P) operated 10 times for 5 min at 0 °C. Using a Sm-Co magnet which produces an inhomogeneous magnetic field (0.4 T on the surface of the magnet and an average gradient of 0.2 T/cm), bacterial magnetites were separated from the sonicated cell fraction. Magnetite particles isolated in this fashion were washed with HEPES buffer at least 5 times and were then treated with chloroform-methanol (2:1, v/v) for 1 h at room temperature in order to remove the covering organic thin film. Extracted magnetites were then placed on carbon-coated collodion-covered copper mesh grids and air-dried for electron microscopy. Electron microscopy was carried out using a JEOL JEM 200CX high-resolution transmission electron microscope and a Hitachi H-700H transmission electron microscope.

Separated bacterial magnetites were only slightly aggregated

and well dispersed in buffer. This result was confirmed by electron microscopic observation (Figure 2 (a)). As described above, each magnetites have the average diameter of 50 nm. However, the median diameter of bacterial magnetites was 120 nm when particle size was measured using a particle size analyzer (Shimadzu, Kyoto, Japan; SA-CP3: Multi mode, Accel 240 rpm/min). Because bacterial magnetites aggregated, each aggregates consisting of 2-4 bacterial magnetites. In contrast, bacterial magnetites were aggregated (12.5 μm in median diameter) in the case of chloroform-methanol treatment (Figure 2 (b)) and they were not covered with organic thin film (magnetosome membrane). Magnetosome membrane consisted mainly of phospholipids and glycolipids [3]. Palmitoleic acid and oleic acid account for 90 % of the total fatty acids. Therefore, the bioactive substances such as enzymes and antibodies were directly immobilized on bacterial magnetites separated by ultrasonication.

4. Application of Bacterial Magnetites

4.1. Immobilization of Antibody on Bacterial Magnetites

Fluorescein isothiocyanate (FITC) conjugated anti immunoglobulin G (IgG) antibody was directly immobilized onto the bacterial magnetites by modifying the lipid membrane with glutaraldehyde. Bacterial magnetites (5 mg) were dispersed by sonication, and incubated with 2.5 % glutaraldehyde solution in 1 ml of phosphate buffered saline (PBS, pH 7.4) for 1 h at room temperature. The modified bacterial magnetites were washed with PBS, dispersed, and incubated with FITC conjugated anti IgG antibody for 12 h at 4 ℃. Antibody-ccupled bacterial magnetites

were washed with PBS several times to remove excess antibody and kept at 4 °C in PBS. On the other hand, bacterial magnetites of which lipid membrane was removed b chloroform-methanol extraction was employed as a control. They were washed with PBS and dispersed by sonication, and 5 mg of particles were incubated with 1 ml of γ-aminopropyltriethoxysilane (γ-APTES) for 10 min at room temperature. Magnetite particles coated with γ-APTES were washed with PBS, dispersed, and incubated with 2.5 % glutaraldehyde solution for 1 h at room temperature. The modified magnetite particles were incubated with FITC conjugated anti IgG antibody. FITC-anti IgG antibody-magnetite particles were kept at 4 °C in PBS. The concentration of antibody in the solution was determined by the Lowry method [10] before and after immobilization and the quantities of antibody immobilized on magnetites were calculated. FITC conjugated anti IgG antibody was immobilized on bacterial magnetites with and without lipid membrane. Equal amounts of particles were used. The extent of antibody coupling with bacterial magnetites covered with lipid membrane was 263 μg/mg particles. Whereas 112 μg/mg particles of antibody was immobilized on bacterial magnetites without lipid membrane.

4.2. Fluoroimmunoassay of Mouse IgG by Using FITC Conjugated Anti IgG Antibody Immobilized on Bacterial Magnetites

IgG standard samples were prepared by appropriately diluting the IgG whole molecule in phosphate buffered saline (PBS; pH 7.4). They (10 μl) were diluted into 100 μl gelatin veronal buffer (GVB; pH 8.3) containing Tween 20 (0.16 vol%). Bacterial

magnetite suspension (1 ml) containing 100 ug of FITC-anti IgG antibody conjugates and 100 ul of each diluted standard samples were mixed and incubated in a test tube (16.5 mmϕ) for min at 37 °C. The agglutination reaction was enhanced by applying an inhomogeneous magnetic field (the test tube was placed on a Sm-Co magnet) which increased aggregation of FITC-anti IgG antibody-bacterial magnetites when they reacted with IgG. The strength of the applied magnetic field was adjusted by changing the position of the magnet and measured using a flux meter (Shimadzu, GK-3). Then the mixtures were added to 2 ml of GVB containing 0.16% Tween 20, and mixed for a few seconds. The fluorescence intensity of FITC-conjugated anti IgG antibody-bacterial magnetites decreased because of agglutination. Fluorescence intensity of FITC-labeled magnetic particles was determined using a fluorescence spectrophotometer (Hitachi, F-1200) with the excitation wavelength set at 490 nm and the emission wavelength at 520 nm using a 10 x 10 mm glass cuvette at 25 °C. The value was estimated after 15 min, when the fluorescence intensity stabilized.

IgG concentration was measured using FITC-conjugated anti-mouse IgG-bacterial magnetites. Figure 3 shows the relationship between the relative fluorescence intensity and IgG concentration. The relative fluorescence intensity decreased with increasing mouse IgG concentration. A linear relationship was obtained between the relative fluorescence intensity and IgG concentration in the range of 0.5 - 100 ng/ml. The minimum detectable concentration of mouse IgG was 0.5 ng/ml. Fluorescence intensity was reproducible with an average relative

error of 5% when a sample containing 10 ng/ml of IgG was measured 5 times.

The assay described here is based on the agglutination of bacterial magnetites covalently coated with FITC conjugated anti IgG antibody and IgG present in the sample. The decrease of fluorescence inten-sity of immunomagnetic particles was caused by the agglutination. The aggregation of FITC-anti IgG antibody-bacterial magnetite conjugates was enhanced during the agglutination reaction by applying a magnetic field. This resulted in a shortening of the required incubation time. Furthermore, the high sensitivity of this assay for IgG determination is achieved by combining an agglutination reaction with fluoroimmunoassay also non-radioactive.

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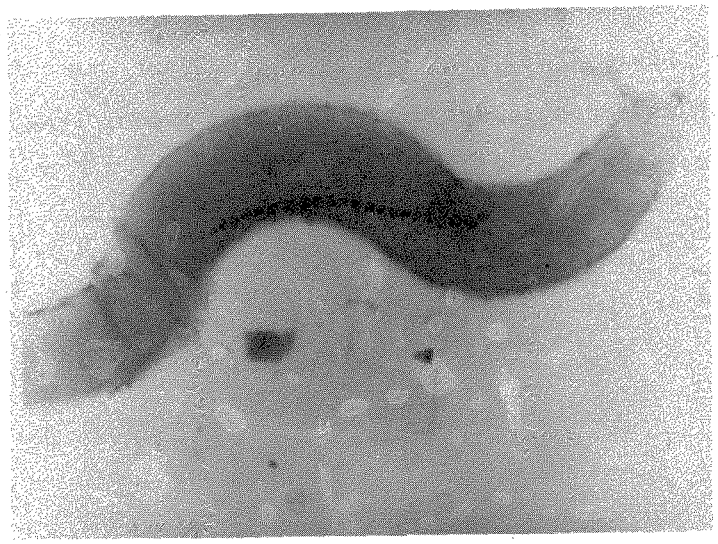
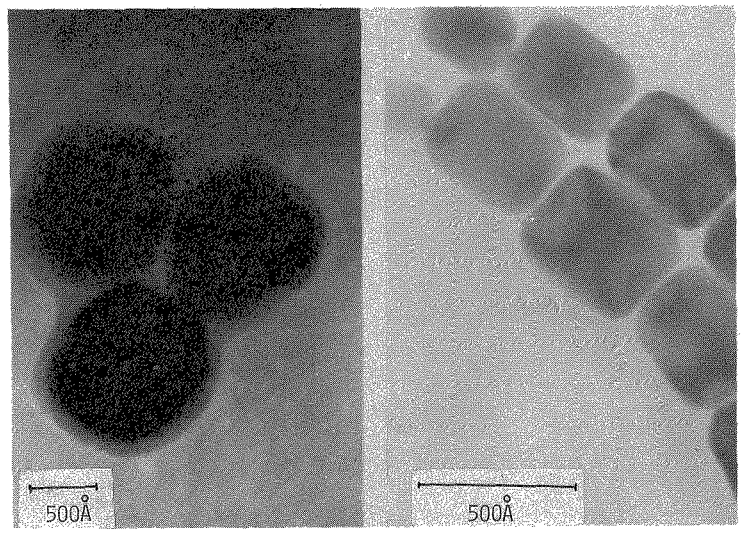


Figure 1. Transmission electron micrograph of magnetotactic bacteria, strain AMB-1.



(a) (b)

Figure 2. Transmission electron micrographs of bacterial magnetites (a) separated by ultrasonication and (b) treated with chloroform-methanol (2:1 v/v).

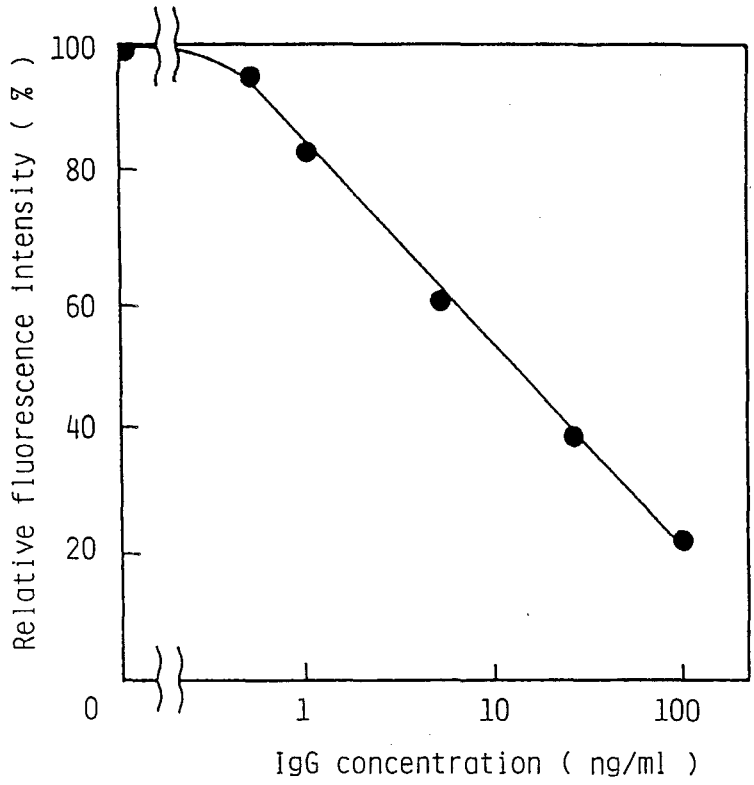


Figure 3. Relationship between mouse IgG concentration and relative fluorescence intensity.

Temp. : 37 ℃
pH : 8.3