SCREENING OF MAGNETOTACTIC BACTERIA FROM LAKES IN UNIVERSITI SAINS MALAYSIA, PENANG

Nurulatika binti Minhad[1*]

[1]*Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA Pahang, Bandar Tun Abdul Razak Jengka, 26400 Pahang, Malaysia*

**Corresponding author: nurulatika@uitm.edu.my*

Abstract

Magnetotactic bacteria (MTB) is a Gram-negative prokaryote which depends on the magnetic influence called magnetotaxis imposed upon them. MTB produces intracellular encased magnetic grains, magnetosomes. The bacteria are hard to isolate in axenic culture due to its fastidious lifestyle. The objective of the study is to screen water samples from two lakes in Universiti Sains Malaysia (USM), Penang for magnetotactic bacteria. Approximately 500 microliter (ml) of samples were collected in triplicates and incubated in the dark for 2 days. For isolation of MTB, a modified method of racetrack purification, hanging drop and centrifugation technique of MTB were used. The samples were enriched in a modified medium and incubated in a shaker incubator for one week. Pour plate and streak plate methods were done. For characterization, microscopic analysis using optical and scanning electron microscope (SEM) was carried out to observe swimming behaviour of MTB and its magnetosomes if any. The screening of MTB showed that all the samples were Gram-negative but there was no movement of bacteria towards magnetic force and no magnetosomes found. Therefore, it can be assumed that water samples from the two lakes in USM do not have magnetotactic bacteria due to its fastidious growth, hard to isolate in axenic culture and exists in a place with abundant of soluble ferrous form.

Keyword: Magnetotactic, Magnetosome, Magnetotaxis

Introduction

Magnetotactic bacteria (MTB) were discovered in the early 1970s (Blakemore, 1975; Moench & Konetzka, 1978) by Richard P. Blakemore when these bacteria responded magnetically to the attraction of magnet when observed under a microscope. MTB is a heterogeneous group of bacteria that are capable of aligning themselves along the magnetic field (Zhu et al., 2010). MTB is several micrometres long, aerobic and with flagellum are pervasive in nature. The bacteria are generally found in sediments of aquatic habitats and also in layered water columns (Bazylinski et al., 1995). Usually, they can be found at the anoxic area or oxic-anoxic transition zone (OATZ) or both (Bazylinski & Williams, 2007; Spring & Bazylinski, 2007; Lefèvre et al., 2007). OATZ is a condition of microaerophilic environments.

The manoeuvrability of magnetotactic bacteria is facilitated by flagella, the nano engine that leads to magnetotaxis. Magnetotaxis indicates the movement, orientation and swimming direction of the cells that is conducted by a magnetic field (Blakemore, 1982). Their motility is affected by geomagnetic of Earth, and magnetic force applied (Lefèvre et al., 2011).

MTB produce an important intracellularly encased magnetic grains known as magnetosomes. The magnetosomes are the membrane-bound crystal responded to magnetic influence (**Figure 1**). This intracellular magnetic grains consist of two types of nano sizes crystals, either magnetite (Fe_3O_4) or greigite (Fe_3S_4) (Bazylinski & Frankel, 2004; Faivre & Schüler, 2008).

The magnetosomes will allow MTB to travel along the magnetic field, line up and locate themselves to produce an intracellular magnetic dipole moment (Balkwill *et al*., 1980, Lefèvre *et al*., 2007, Scheffel & Schüler, 2006).

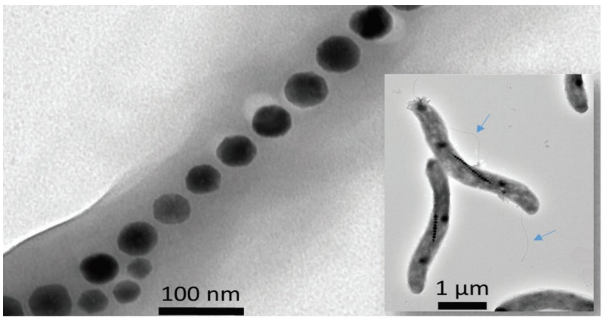


Figure 1 TEM image of MTB with its magnetosomes. In the inset, the whole bacteria are shown, and their flagella are indicated with blue arrows (Adapted from: Gandia et al., 2019)

MTB have potentiality in biotechnology as well as geobiology for bionics and biomagnetism field (Wenbing *et al*., 2007; Bazylinski & Frankel, 2004; Lang & Schüler, 2006). Magnetotactic bacteria provide a significant use in geochemistry as MTB taking in the iron source from the environment. Magnetosomes are used widely in many fields including pharmacy, electronics, optics, and electrochemistry (Bazylinski & Frankel, 2004). The application also includes magnetic separation, immobilisation of biomolecule, drug delivery, hyperthermia, and detection of analytes (Jacob & Suthindhiran, 2016; Gandia et al., 2019).

Due to their rapid growth and powerful metabolic diversity, only a few strains are ready to be used in pure culture (Flies *et al*., 2005). MTB also cannot easily grow on solid agar. Most of the isolated MTB are phylogenetically associated with *Alphaproteobacteria Magnetospirillum spp*. that are abundant in isolated cultures that come from freshwater habitats (Blakemore *et al*., 1979; Matsunaga *et al*., 1991; Schleifer *et al*., 1991; Flies *et al*., 2005; Wenbing *et al*., 2007). *Desulfovibrio magneticus* is included in genus *Desulfovibrio* within *Deltaproteobacteria* that can be found in axenic culture from freshwater is sulfate-reducing bacteria (Sakaguchi *et al*., 2002; Lefèvre *et al*., 2007). Pure cultures that are able to isolate are *Magnetospirillum magnetotacticum, M. gryphiswaldense, M. magnetotacticum* AMB-1 and MGT-1, coccus MC-1, *Vibrios* MV-1 and MV-2, RS-1, and *Marine spirillum* MV-4 (Li *et al*., 2007).

Due to their fastidious growth, MTB are very difficult to culture and sub-culture. Only little groups of these bacteria have been isolated in axenic culture. There were many efforts and trials to isolate MTB in axenic culture. However, the attempts failed (Blakemore, 1975). MTB were usually found at the interface of the water sample, and it is difficult to obtain a sample that is precisely at the interface zone. These conditions are challenging to prepare in laboratory cultures thus making it difficult to isolate MTB axenically (Faivre & Schüler, 2008). Therefore, the study was carried out to screen water samples from two lakes in Universiti Sains Malaysia (USM), Penang for magnetotactic bacteria. Different isolation methods were carried out to screen the water samples followed by the characterization of the bacteria.

Materials and Methods

Culture medium

Modified culture medium was used. The isolation of magnetotactic bacteria was performed by using the simplest medium. Nutrient agar and $FeCl_3$ were used to replace quinic acid. The preparation of media started with the addition of 1.6221g of $FeCl_3$ with 100ml distilled water

which was autoclaved at 121°C for 20 minutes. Then, the solution was mixed with nutrient agar and autoclaved at 121°C for 20 minutes. The mixture of both substances was poured on a petri dish and left in laminar flow to avoid contamination. The absence of water vapour in the mixture was confirmed during the process. The other media used was nutrient broth (NB). NB was used to enrich the bacteria in the shaker. 13g of NB was diluted in 1 Litre (L) of distilled water, and the pH was adjusted to 7.6-7.8. The broth was poured approximately 50ml for each flask. Ten-fold dilution up to 10^{-5} was used for culturing of the bacteria, and Gram-staining procedure was carried out later.

Sample collection

Samples of sediments and water were collected from Fajar lake and Aman Lake, Universiti Sains Malaysia (USM), Penang. The samples were collected at the first 15cm depth sediments (He et al., 2018). The samples were taken by dropping a 500ml plastic bottle into the lake at the interface of water containing mud. The samples were kept in a dim place for 2 days.

Isolation method

a) Racetrack Purification

Modified racetrack purification method was carried out. Firstly, magnetotactic bacteria were enriched by attaching the south pole of permanent magnets (0.37 mT) outside the bottles for 20 to 30 minutes (**Figure 2**). The cells would accumulate at the area where the magnet was placed. It was assumed that the magnetotactic bacteria would accumulate at the wall of the bottle due to the presence of magnetic influence. Cells were accumulating as dark spots underneath the magnets were removed with a Pasteur pipette and saved as magnetically collected samples. These samples were further magnetically purified in Pasteur pipettes according to the racetrack purification method (Wolfe *et al*., 1987). Secondly, the sample was poured into a 100ml beaker with a magnet tied and hung on top of the water (**Figure 3**). The magnetotactic bacteria supposedly was accumulated under the magnet and collected by using Pasteur pipette. Thirdly, a drop of the water sample was put on the center of a glass slide, and the magnet was put at the edge of the water drop. It was assumed that the magnetotactic bacteria would swim to the edge of the drop. Then, the bacteria were collected by using a micropipette.

Figure 2 The magnet was attached outside of the bottle containing samples.

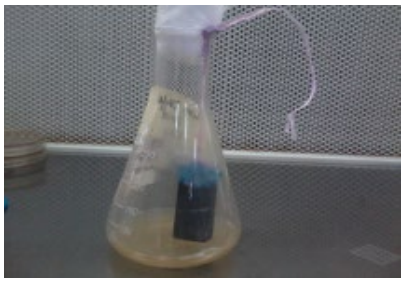


Figure 3 The magnet was put hanging on top of the sample.

b) Hanging drop method

The hanging drop method was performed and modified (Schüler, 2002). A plasticine was used to hold the glass slide, and approximately 100µl of the sample was pipetted and placed onto the glass slides. The magnet then was attached to the glass slide, and the bacteria movement towards the magnet was observed under a microscope (**Figure 4**).

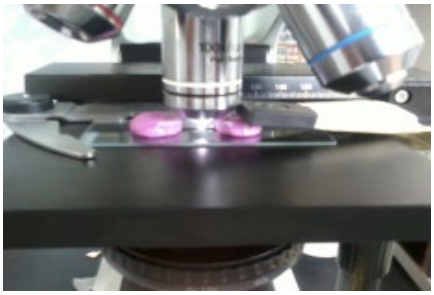


Figure 4 The sample was viewed under microscope with the magnet attached at the end of the water drop.

c) Centrifugation Technique of MTB

Approximately 1ml of the samples was pipetted out and centrifuged with 10,000 rpm for 10 minutes. The supernatant was removed, and 1ml of sterile distilled water was added. The steps were repeated twice. The magnet was brought near to the wall of centrifuge tube. The magnet was moved slowly up and down to see any movement of the sample.

Characterization of MTB Using Optical and electron microscopy observations

The swimming behaviour of MTB was observed using an OLYMPUS CX21 microscope. Transmission electron microscope (TEM) was used to observe the magnetosome if any. The sample was centrifuged, and the supernatant was discarded. Phosphate buffer (0.1M, 100ml) was added into a pellet and centrifuged for 8 minutes. The step was repeated, and 1% of osmium tetroxide was prepared in phosphate buffer and centrifuged. The pellet was resuspended in distilled water and the step was repeated. The tube with the only pellet was placed in a water bath at 45 °C for 15-30 minutes. Two percent of the agar solution was prepared and added into the pellet. The solidified agar was cut into small cubes and placed in vial containing 50% alcohol. Cutting, grinding, smoothening, and staining process was carried out, and the sample was observed.

Results and Discussion

The isolation methods show that there were brown-blackish spots formed when a magnet was attached near the sample. The sample was assumed to be magnetotactic bacteria. However, the methods did not show any movement of bacteria towards the magnetic force when observed under a microscope. Through all isolation methods, no organisms responded to the magnetic field imposed to them. There was no movement of accumulated bacteria in all isolation methods. The bacteria were plated on agar medium using the spread plate method and streak plate method to obtain a single colony (**Figure 5**). The bacteria colony was purified several times in an enriched nutrient broth.

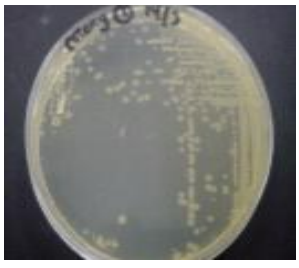
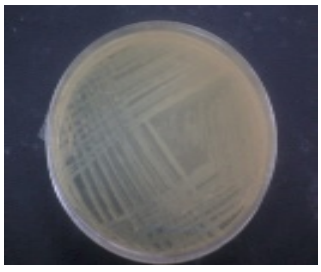
(a)  (b) 

Figure 5 The plates that were purified to obtain single colony.

The Gram staining procedure was carried out. The results show that the bacteria were rod-shape and pink in colour (**Figure 6**). The bacteria were Gram-negative. The bacteria formed

based on Gram staining show that the characteristics were a bit similar to magnetotactic bacteria. However, if based on the movement of bacteria towards the magnet, the result was negative. Through electron microscopy, the cells were coccoid and not in a chain manner (**Figure 7**). There are only a few species of MTB successfully isolated in axenic culture (Lefèvre et al., 2007, Schüler, 2008, Lefevre et al., 2009, Moench & Konetzka, 1978). There was no formation of magnetosome inclusion in the bacteria membrane using TEM. Therefore, the bacteria were not magnetotactic bacteria. The cultivation of MTB is very hard because of the needs of microaerophilic or anaerobic condition (Moench & Konetzka, 1978, Jun et al., 2006). These bacteria are hard to isolate due to their fastidious style which are highly selective in their growth medium and makes the culture difficult to grow.

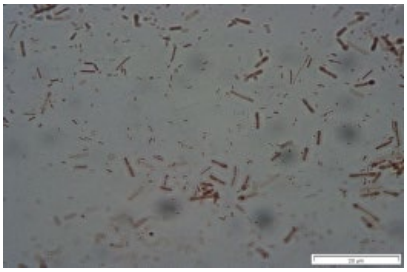


Figure 6 The image of bacteria with total magnifying power of 1000x using optical.microscope.

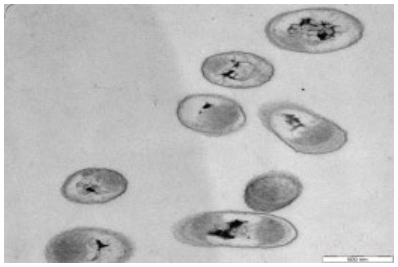


Figure 7 The cells captured using TEM with magnification power of 10K.

MTB are generally found at or below the oxic-anoxic transition zone (Lefèvre et al., 2007) because the bacteria prefered condition where there is a little concentration of oxygen. They can be found at sediment that has soft and slimy feel. The mud or the sand was black and produced foul smell. If there is plants or photosynthetic algae, that is assign of no MTB because this floral environment produce oxygen. Magnetotactic bacteria was chemolithoautotrophic compound (Postec et al., 2012, Farhad et al., 2010) which obtains energy by oxidizing inorganic chemicals and carbon from CO_2. The bacteria were assumed to gather at the particular area that has magnetic influence because MTB have intracellular organelle that was specific to the bacteria called magnetosome. The magnetosome consists of greigite and magnetite that was enclosed by a membrane. The function of magnetosome was to direct the bacteria to line up and align according to force by geomagnetic when they swim (Lefevre et al., 2009).

There were a few methods to isolate magnetotactic bacteria. However, based on previous researches, it was proved that these bacteria were hard to isolate due to their characteristic, which is a fastidious style. Fastidious organisms have a complex nutritional requirement. In other words, a fastidious organism will only grow when specific nutrients are included in its culture medium. Based on Gram staining result, the bacteria were rod shaped and pink colour. Magnetotactic bacteria have various cell shapes, including cocci, rods, vibrios and spirilla. However, coccoid morphotype is the dominant one (Postec et al., 2012; Xiao et al., 2007; Jun et al., 2006). Based on the conducted study, it shows that the bacteria were Gram negative. Through previous researches, it was also stated that magnetotactic bacteria were Gram negative (Farhad et al., 2010; Wenbing et al., 2007).

The cultivation of MTB is very hard because of the needs of microaerophilic or anaerobic condition (Moench & Konetzka, 1978, Jun et al., 2006). There was no movement of bacteria when the magnet was brought near the sample under a microscope. Therefore, it was assumed that both samples from lakes in Universiti Sains Malaysia did not have magnetotactic bacteria. There were a few reasons why the experiment showed a negative result. Detection of iron should be performed before isolation of the MTB. Both sample sites were for

recreational purpose. Therefore, there was no sign of iron production at that sites. Magnetotactic bacteria exist in a place where there is a reducing anoxic zone that showed abundant of the soluble ferrous form (Lefèvre et al., 2011). Due to its lifestyle that was highly selective in its growth medium makes the culture difficult to grow. From all readings based on previous research, most of them used complex culture media to fulfil the requirement of the bacteria. However, the study was carried out using simple media to fulfil the bacteria needs. In the future, if the enriched medium is hard to prepare, the aqueous solution from the collecting site can be filtered, and combine in the complex medium prior autoclaving.

Conclusion

The screening of water samples from Fajar and Aman lakes in Universiti Sains Malaysia, Penang showed Gram negative bacteria through characterization using optical microscope and TEM. However, there were no movement of bacteria towards magnetic field imposed to them. There were no magnetosome chains found in the bacteria. There were no magnetotactic bacteria in both lakes in USM, Penang.

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Conflict of interests

The authors declare that there are no conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influences its outcome.

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