

Isolation And Characterization of Magnetic Bacteria from different water environment sources in Saudi Arabia



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P18

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Introduction

Magnetotactic bacteria (MTB) are aquatic fastidious prokaryotes whose direction of motility is directed by the Earth's geomagnetic and externally applied magnetic fields. This response is due Nano-sized magnetic crystals inside the cells. These crystals called magnetosomes and known to consist two types of minerals i.e., iron oxides magnetite (Fe₃O₄) and iron sulfides greigite (Fe₃S₄). They vary in morphology, size, composition as well as arrangements; and a single cell may synthesize one-multiple chains. MTB vary in Shapes including bacillus, cocci and multicellular forms adding diverse ecological habitats ranging from aquatic (marine and freshwaters), to deep sea sediments. The highest numbers of MTB are reported close to the oxic-anoxic transition zone. In this work, collection method was designed to isolate MTB from different water environment sources in Saudi Arabia.

Objective

Isolation and characterization of Magnetic Bacteria from different water environment sources in Saudi Arabia.

Materials and methods

Sites of collection:



(Fig. 1) Schematic diagram showing the samples were collected from different water environment sources in Saudi Arabia.

Incubation at room temperature

Enrichment and observation

Nutrient agar and Luria-Bertani and Charcoal agar solid media were used for bacterial cultivation.

Isolation and Cultivation of MTB

The magnet is an effective tool that was used as a MTB trap (several days to months).

Microscopic examination

We used both Light and digital microscope, MTB were found to be highly motile and refractile and exhibited clumping under the application of magnetic field.

Transmission electron microscopy

Transmission electron microscope (TEM) is the technique of choice for observing individual magnetosomes and to absolutely classify a bacterium as capable of producing magnetosomes.

Energy dispersive x-ray spectroscopy

EDXS analyses have been used to evaluate single-element incorporations into magnetite crystals produced by MTB.

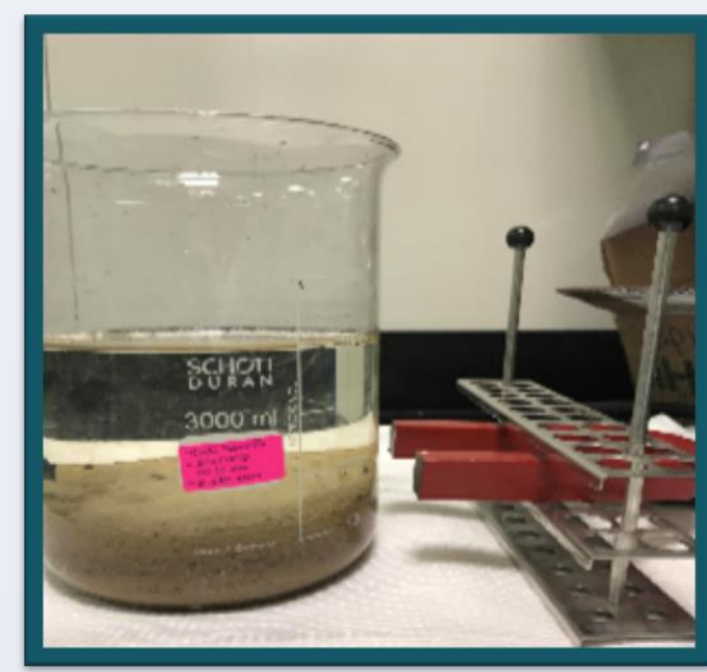
Isolation and Enrichment of the samples from natural environment



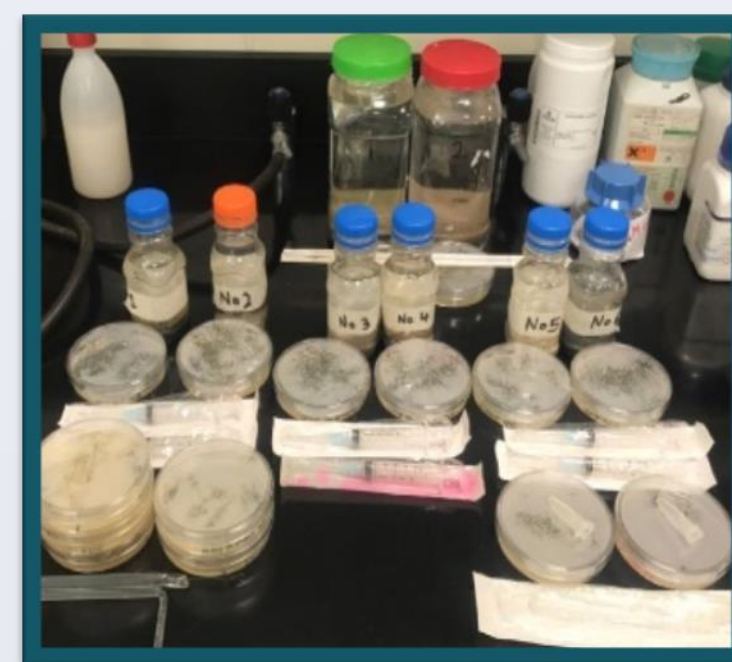
(Fig 2)



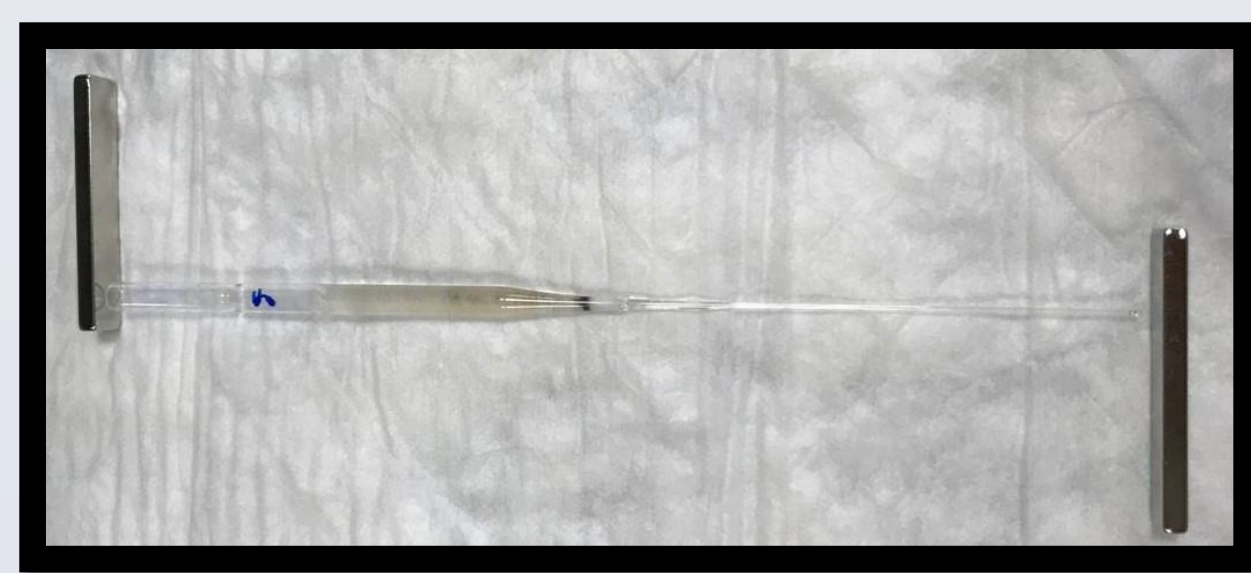
(Fig 3)



(Fig 4)



(Fig 5)



(Fig 6)

Result

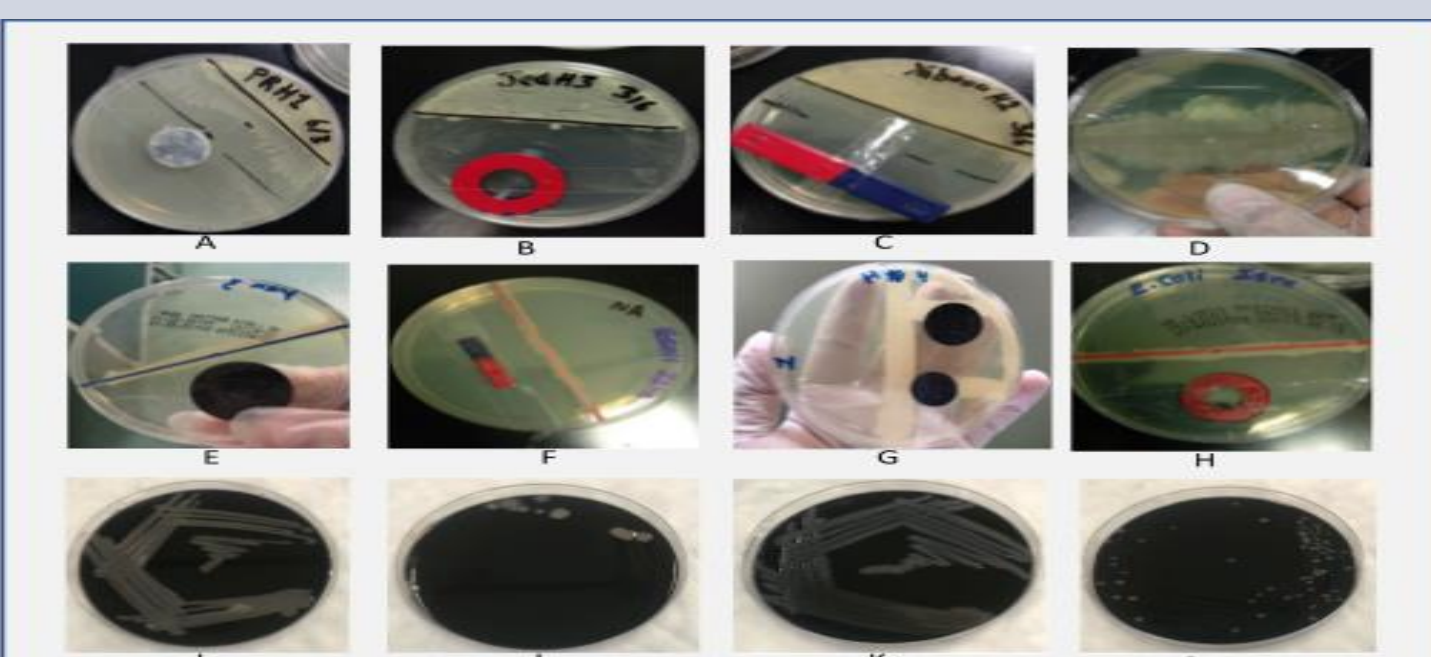
A- Results of the different cultures collection:

The following table lists site, type and date of collection, incubation period, enrichment and first hanging drop result for each sample

Sample code	WH	JB	YB	KG	Control
Locations	Hanifa Valley, Riyadh	Red sea, Jeddah beach	Red sea, Yanbu beach	Arabian gulf, Al-khafi beach	---
Type of sample	Fresh water	Seawater and sediment	Seawater and sediment	Seawater and sediment	<i>Escherichia coli</i> bacteria
Date of collection	The 6th of Feb 2018	The 9-10th of Feb 2018	The 15th of Feb 2018	The 1st of Mar 2018	---
First incubation period	22 days	18 days	13 days	---	---
Second incubation period	238 days	234 days	229 days	215 days	---
Hanging drop method result	Moderate number of bacteria attracted to the magnetic pole	Small number of bacteria attracted to the magnetic pole	Moderate number of bacteria attracted to the magnetic pole	Small number of bacteria attracted to the magnetic pole	---
Cultivation on NA (by spread plate procedure)	Dense growth of bacterial Colonies	Weak growth	Dense growth of bacterial Colonies	---	Dense growth of <i>Escherichia coli</i> Colonies
Cultivation on NA (by streaking) from Previous culture + incubated with a magnetic pole	Showed attraction to the magnet after 3 days (Fig. 7A)	No attraction observed after 3 days (Fig. 7B)	Weak attraction to the magnet after 3 days (Fig. 7C)	---	---
Cultivation on LB (as a straight line) from Previous culture + incubated with a magnetic pole	Attraction to the magnet after 3 days (Fig. 7D)	---	No attraction observed after 3 days (Fig. 7E)	---	No attraction observed after 3 days (Fig. 7F)
Cultivation on NA (as a straight line) from Previous culture + incubated with a magnetic pole	Attraction to the magnet after 3 days (Fig. 9C)	---	---	---	No attraction observed after 3 days (Fig. 7H)
Cultivation on Charcoal (by streaking) from Capillary trace-track	Dense growth of bacterial colonies (Fig. 7I)	Very weak growth of bacterial colonies (Fig. 7J)	Dense growth of bacterial colonies (Fig. 7K)	Weak growth of bacterial colonies (Fig. 7L)	---

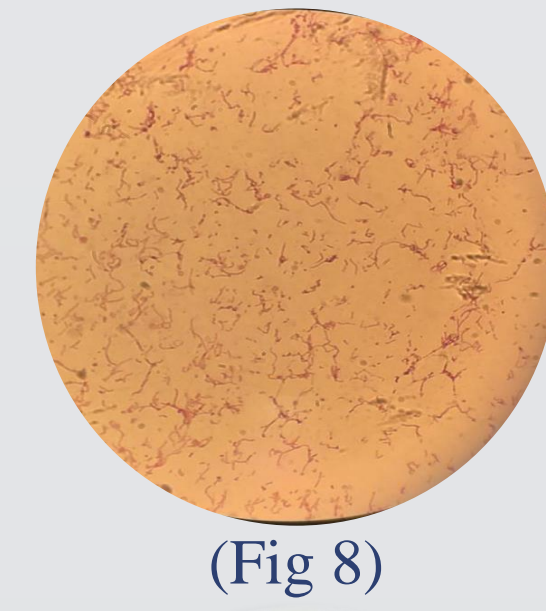
1.*: The sample was excluded in the mentioned stage.
**.: The test was not performed

2. (LB) IS Luria-Bertani, And(NA) is nutrient agar..

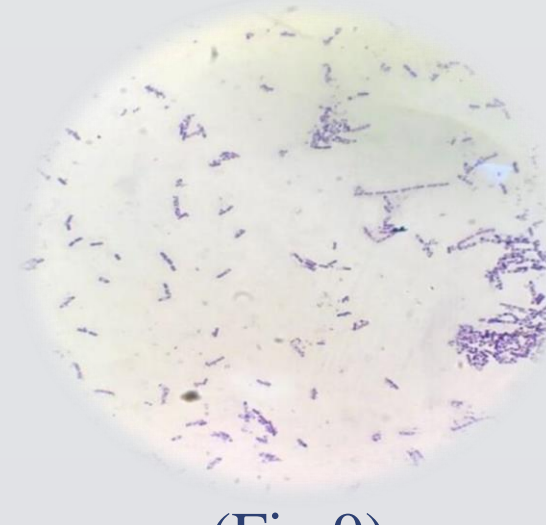


(Fig 6)

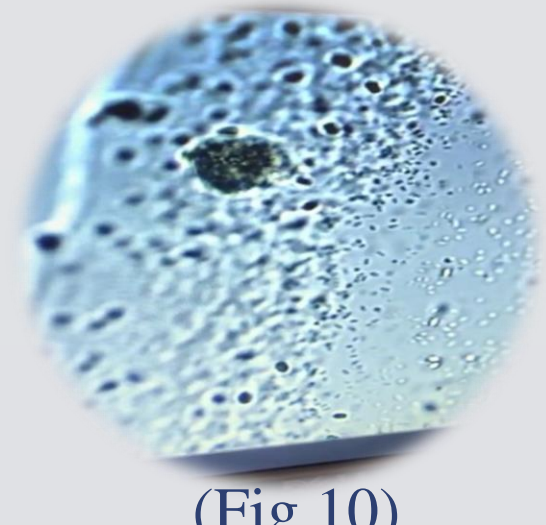
B-Light and digital microscopes :



(Fig 8)



(Fig 9)

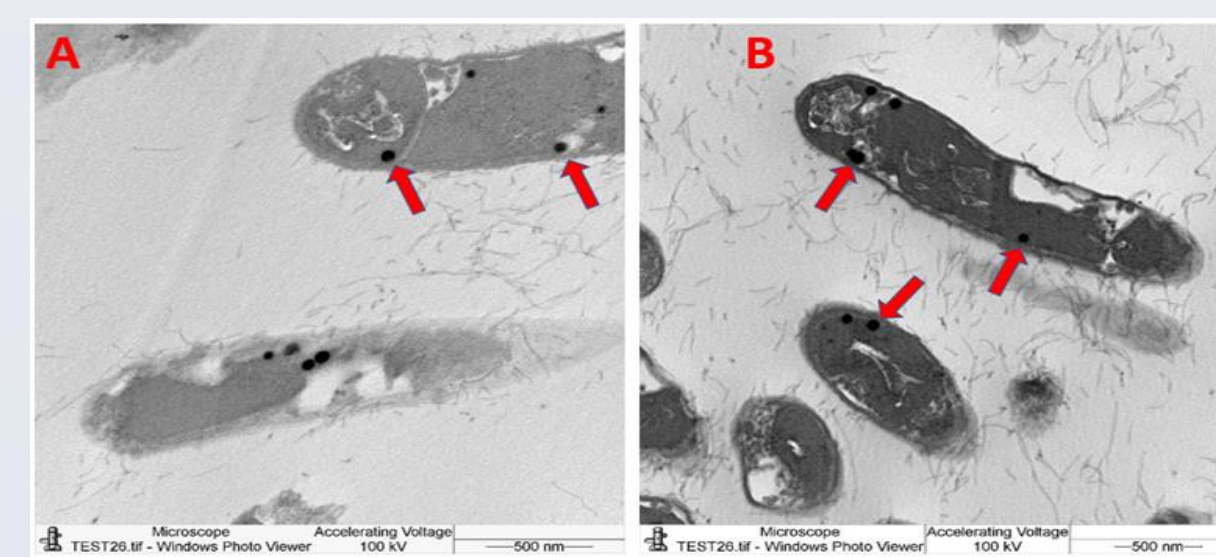


(Fig 10)

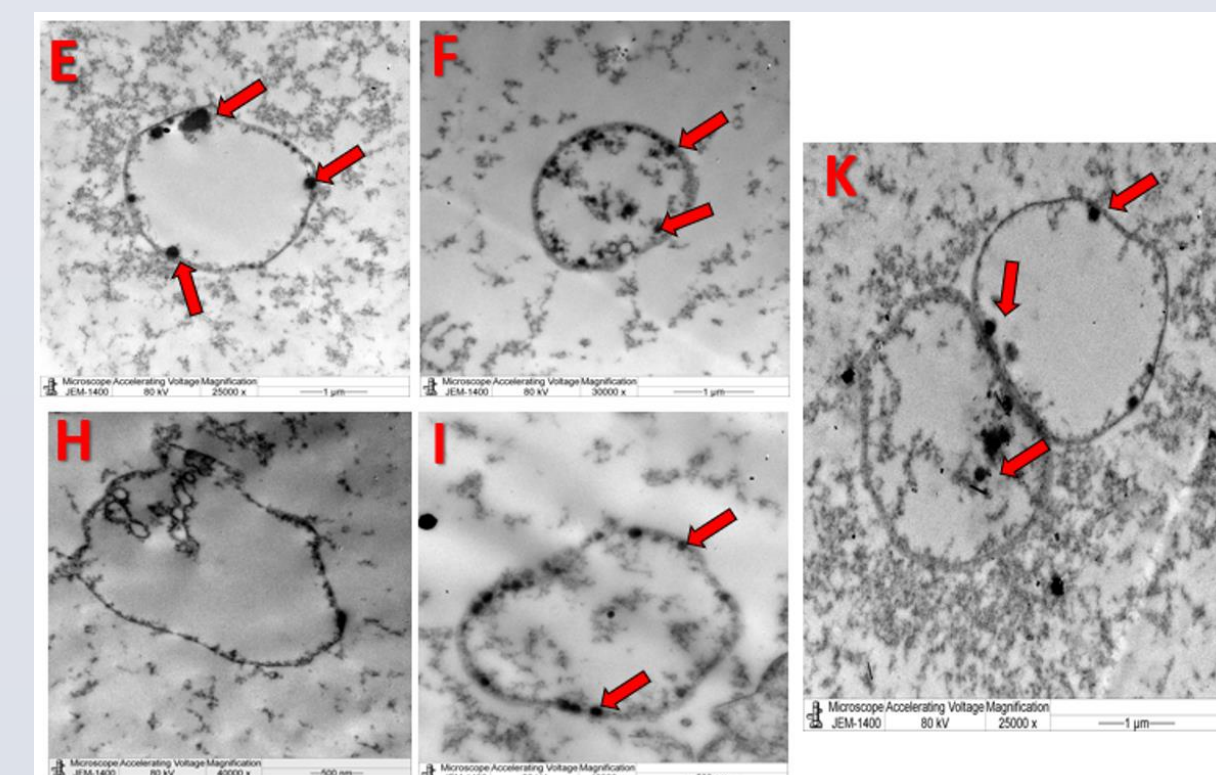
Gram stained bacteria appeared in different shapes such as cocci and bacilli, even though all of them were gram positive and negative .

Bacteria were assembled and attracted to the edge of the hanging drop during magnet placement, under both of light and digital microscope.

C- Transmission electron microscope :

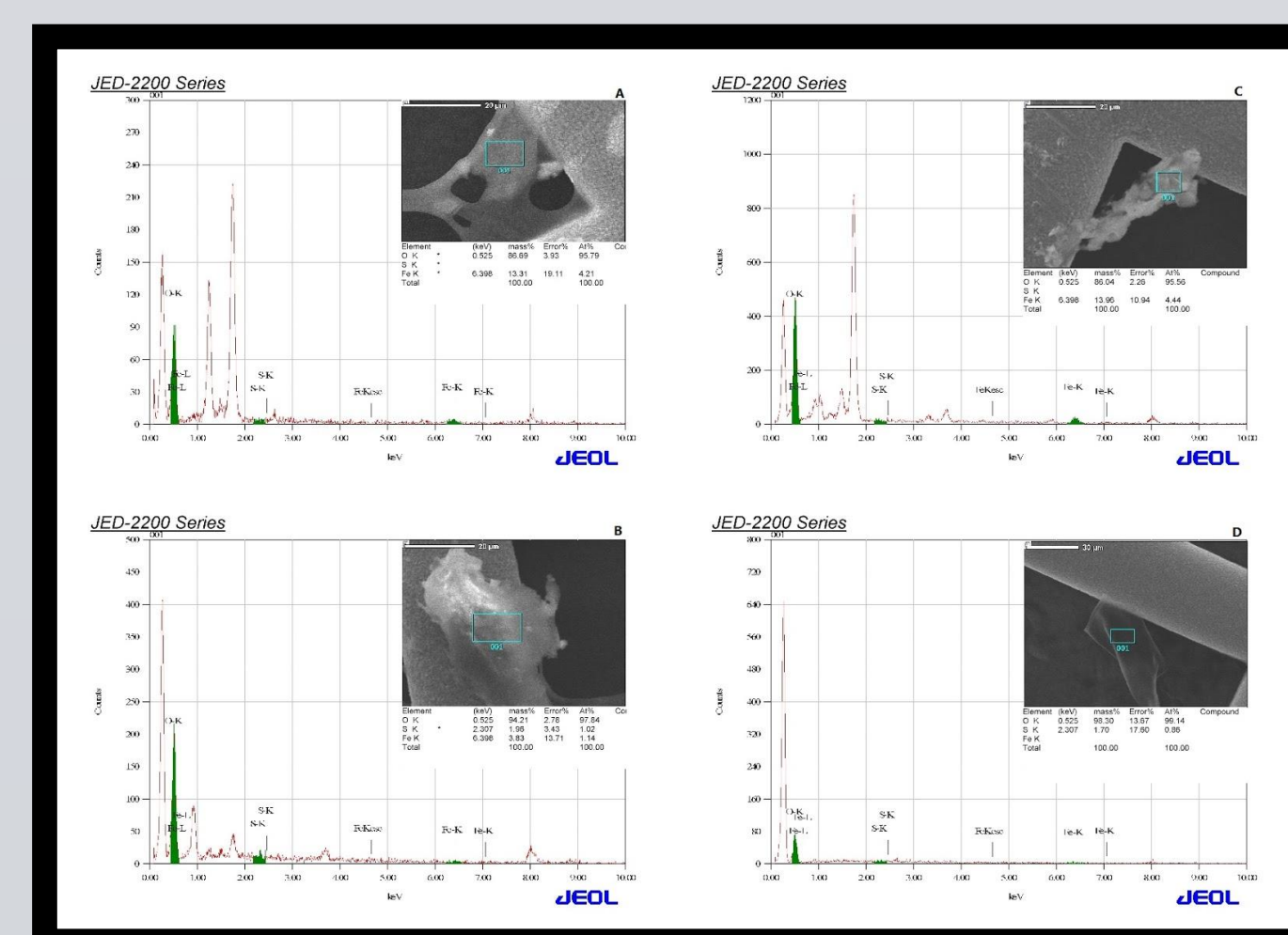


(Fig. 11) Transmission electron micrograph of a thin-sectioned cell of MTB showing growing magnetite crystals within the vesicles of the magnetosome membrane (MM).



(Fig. 12) Transmission electron micrograph of another thin-sectioned cell of MTB revealed the magneto particles to range between 10 to 20 nm in size presented in Hanifa Valley (freshwater) (E, K), Jeddah (F) and Yanbu (I) (Red Sea) All of them were coccoid. Negative result appeared in Al khafji city (H) (The Arabian Gulf sample).

D- Energy dispersive x-ray spectroscopy:



(Fig. 13) EDXS microanalysis of the grid (A) analysis revealed that Fe and O are present in the specimen of Hanifa Valley. (B) S, Fe and O are present in the specimen of Jeddah. (C) Fe and O are present in the specimen of Yanbu. (D) Fe does not exist in the specimen of AL-Khafji.

Conclusion

The present study, the effort mainly focused on isolation and characterization of magnetotactic bacteria from different water environment sources in Saudi Arabia based on a combination of different techniques that used by researchers around the world and our personal experiments.

MTB were isolated from Hanifa Valley sample in a mixed culture and their circular magnetite magnetosomes arrangement were observed under TEM as singles, chains and clusters. Species was not determined due to the time limitation, difficulties in separation methods as well as unavailability of selective media in the place of this study.

Moreover, there are several great applications for this group of bacteria in nanotechnology such as use the magnetosomes as a potential drug-carrier for tumor treatment and as contrast material for Magnetic resonance imaging (MRI), which attract our attention to complete research on this area in the near future.

References

- Jogler C, et al. 2009. Towards cloning the magnetotactic metagenome: identification of magnetosome island gene clusters in uncultivated magnetotactic bacteria from different aquatic sediments. *Appl. Environ. Microbiol.* 75:3972–3979.
- Lefèvre, C.T., and Abreu, F., Lins, U., Bazylnskim, D.A. 2011. A bacterial backbone: magnetosomes in magnetotactic bacteria. *Springer* 15, 75-102.
- Yan L, Zhang S, Chen P, Liu H, Yin H, et al. (2012) Magnetotactic bacteria, magnetosomes and their application. *Microbiol Res* 167: 507-519.

Acknowledgement

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through the Undergraduate Research Support Program, Project no. (URSP -3-18-142).

We are thankful to the Botany and Microbiology department, College of Science at King Saud University for giving us the opportunity to complete this research.

We also would like to express our very great appreciation to Dr. Abeer Hashem for her valuable and constructive suggestions during the planning and development of this research work. With a special thanks to Jeddah divers league for collecting and shipping JED samples and Dr. Manal Al khulifi for discussions and our supervisor Roua Alkufeidy.