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mamAB Operon: Major Role in Biomineralisation within Magnetotactic Bacteria

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Abstract: ***In this report, we described and identified the gene involved in biomineralisation process with in magnetotactic bacteria MTB. The genomic Magnetosome Island were isolated by using PCR technology, and studied for identification of conserved sequences with in mamAB operon of MTB. This is the evidence that the unknown MTB exhibit similarity with other known MTB and contributes diversity for geomagnetic property and magnetosome crystal formation in MTB.***

Keywords: Magnetotactic Bacteria, Genomic Magnetosome Island, mamAB operon, mamO, mamE

1. Introduction

The ability of MTB is to grow in geomagnetic field, that is done because of presence of magnetosomes; are intercellular organelles of magnetic nanocrystal of iron oxide magnetite (fe_3O_4)[1]. *Magnetospirillum gryhiswaldense* has recently known to us as the modal system to study the biomineralisation process within the microorganism having extreme properties of magnet.

2. Literature survey and Research demonstration

The genetic analysis of MTB is still complicated, as it devoid of genetic tools and technology .Using proteomic approach distinct set of 20 proteins , that is associated with Magnetosome Membrane(MM), have found on genomic magnetosome island(MAI)[2]. These proteins have identified in MM and conserved in other marine MTB, along with MV-1 magnetotactic vibrio strain and MC-1 magnetic coccus strain[3].Most of the magnetosome membrane proteins (MMP) clusters encoded within major operon ; mamAB operon ,mam GFDC operon, mms6 operon and mamXY operon[2][3][4][5]. The genomic region with homology display similar content for protein synthesis, thus can predict to identify the magnetic properties in other bacteria. The mamM and mamB (MMP) belongs to cation diffusion facilitator family and show s magnetosome-directed iron transfer [6]. mamE and mamO ,PDZ domain of mamE and tetra tricopeptide protein (mamA containing domains) express similarities to serine protease. Serine protease involved in processing of MMP and protein with PDZ and TPR motif interacts with other proteins involve in biomineralisation in MTB [1]. mamE and mamO are conserved one which reported as, involve in protein sorting and biomineralisation in MTB. mamE is essential for localisation of several soluble and membrane bounded proteins. mamO reported to involve in biomineralisation. It has been suggested that, mamE and mamO was essential and most important protein reported in mamAB operon [7].

It has been demonstrated that, the mamAB operon can be isolated from the unknown magnetotactic bacteria and can compared with the known MTB '*Magnetospirillum gryhiswaldense*'. The potential of magnetotactic bacteria reverent for biotechnological applications, had already been demonstrated [8][9]]. In this study, we identified the conserve mamAB operon from the unknown magnetotactic bacteria isolate from fresh water lake. This identification provides evidences for most similarities between unknown isolated MTB and known modal system of MTB.

3. Material and Methods

1. Sampling.
We sampled unknown MTB strain from the fresh water lake "ketham" located near city Agra,U.P, INDIA. This sample had showed the geomagnetic property, and stored for experimental work, that was for comparative analysis and analysis of similarity among gene content between the unknown sample of MTB and *Magnetospirillum gryhiswaldense*.

2. Collection of MTB
The pure strain of unknown sample has been isolated by CRT Method (Capillary Rack Track method).And further it cultured under optimum conditions.MTB is a anaerobic bacteria ,thus required contentious subculturing with minimum requirement of oxygen .The hungate technique appropriately utilized for culturing of MTB[9].

3. Isolation and purification of Genomic Magnetosome Island from Bacteria
The cultured MTB was digested and the proteins got removed. The cell wall debris, polysaccharides and remaining proteins were removed by selective precipitation and high molecular weight DNA was removed from the resulting supernatant. The genome ® kit was used here to get proper DNA extract, as the bacteria showed gram –negative nature.

4. Separation of gene of interest
The gene of interest, mamAB operon was isolated by using PCR to identifying the target gene by particular digestion of 15ug bacterial genomic DNA with MbOI to produce DNA fragments. The DNA sequence of about 3Kb was generated by PCR performed by oligonucleotide primers. This sequence contains entire mamAB operon which has been coded for mamO and mamE protein. Amplification was carried out by using 50ul reaction mixture (J.Biotech) 20 ng of purified genomic DNA from isolated strain , 0.7ul of

eIONGase enzyme mixture(cinagene-Inc),10pmol of each primer (Table 1)[10] ,200uml of each dGTP,dATP,dTTP and dCTP.3.0% dimethyl sulfoxide,2mM $MgSo_4$ and 1xelONGase buffer(life tech). The amplification were done with PTC-200 thermal cycler under variant Condition .Initially denaturation were done at 94^0C for 2min followed by 37 cycles of 94 ^{o} C for 1 min ,64^oC for 1min and finally 72^oC for 10 min to finish the program of reaction [11]. The amplified DNA were examined through horizontal gel electrophoreses. The gel electrophoresis thus performed to carry out bands of recombinants, by using 1.5%Agrose Gel, TBE Buffer, 90mM tris borate, 2mM EDTA at pH 8.3 with 8ul of PCRproduct. The gel was stained with ethidium bromide and observed under U.V light of Wavelength 312nm. The comparisons were made between mamAB operon (conserved sequence in MTB) of known MTB; *Magnetospirillum gryhiswaldense* and the unknown MTB collected from lake.

Table 1: Primers for the amplification of genes from Genomic Magnetosome Island

Primer Sequence	Primer Name
CTCGAGATGGCGCAAAGTGTGACGTC	mamAB forward
CATATGTCCCGTCACAATTCACCTCC	mamAB Reverse

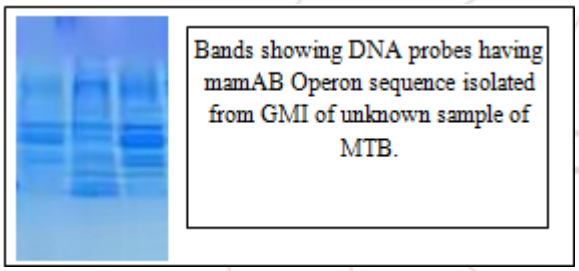

Figure 1: Gel Electrophoresis of PCR Product

4. Result

The Genomic DNA isolated from collected MTB was attempted to amplification by PCR using primer to isolate the mamAB operon from the genomic DNA. The isolated mamAB operon had been screened by gel electrophoresis. The parallel bands (FIGURE-1) of DNA fragments were noted on the gel which emphasise the isolation of similar type of gene from the DNA content of unknown sample of MTB.

5. Discussion

In this work, we reported the conserved sequence from the collected unknown MTB which is similar to the geneof known MTB strain. The primers sequences using in this research were already reported to get used for isolate mamAB operon from *M. gryhiswaldense*. The bands patterns of recombinant PCR product by agarose gel, which result out the similarity between collected magnetotactic bacteria and known MTB's. The analysis of these sequences supports the evidences, as Magnetosome genes are clustered in the mamAB operon of collected MTB.

6. Conclusion

In this study, we identified a Magnetotactic Bacteria which has similarity in Genomic Magnetosome Island of *M.gryhiswaldense*, our study provide the phylogenetic relation of the collected sample with the known MTB that has been already been identified and studied. Additionally, our further studies provide a platform to the unknown MTB by cloning and expressing the genome sequence into a suitable host to analyse the mechanism of magnetosome synthesis in unknown sample of MTB collected from lake. Thus we realized that possibility to increase the matter and improving the further study on collected Magnetotactic Bacteria.

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