Unveiling magnetosome biomineralization in magnetotactic bacteria

Alfred Fernández-Castané and Charlotte Clarke-Bland (Aston University, UK)

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Here, Alfred Fernández-Castané and Charlotte Clarke-Bland from Aston University, used their grant to purchase the necessary chemicals to run confocal microscopy experiments and more importantly, it has allowed them to start a collaboration.

Magnetotactic bacteria (MTB) are a group of microorganisms that naturally synthesize single-domain ferrimagnetic nanoparticles of the magnetic iron minerals magnetite (Fe_3O_4) or greigite (Fe_3S_4) contained within subcellular membrane bound organelles called 'magnetosomes'. They are formed through a biomineralization process in which soluble iron is crystallised into a magnetic mineral. Magnetosomes have tremendous potential for biotechnological applications and their transmembrane proteins can be used as anchors for diverse functionalization, that can be introduced either genetically or chemically. For instance, immunoassays, ligand-receptor binding assays, cancer therapy, antimicrobials and target cell separation techniques or DNA extraction applications have been developed.

Production of magnetosomes at large scale is generally performed in bioreactors by growing cells at relatively high densities. It is recognized that future widespread applications of magnetosomes will, to a large extent, depend on the development of intensified high yielding biomanufacturing platforms for magnetosome production. Fundamental to this, are the appropriate means for analysing MTB growth and physiology and, relevant to this project, unveiling the biomineralization of magnetic iron minerals which is still poorly understood.

In this study, we have developed a methodology to image and quantify intracellular iron in PG-SK stained *Magnetospirillum gryphiswaldense* MSR-1 cells using time-lapse confocal microscopy. PG-SK is a chelatable fluorophore whose fluorescence is quenched by metal ions including Fe^{2+} and Fe^{3+}. By using confocal microscopy, we have been able to obtain direct imaging of the dynamics of the intracellular pool of iron. As seen in Figure 1, fluorescence intensity of PG-SK stained cells decreased over a period of 15 minutes when analysing at a single cell level. This might be due to an increase of iron ion availability in the cytoplasm, for example, iron released by metalloproteins such as ferritins being present in MTB or by magnetosomes. This could also be due to a photo bleaching effect caused by the cells being exposed to the microscope laser for relatively long periods of time.

Interestingly, upon observation of PG-SK stained cells at a population level, we found that a significant fraction of cells did not fluoresce over the incubation period, as shown in Figure 2. As can be seen in the fluoresce (Fig. 2A) compared to bright filed (Fig. 2B) images, some cells showed higher fluorescence intensity than others with cellular regions showing peaks of intensity. However, not all cells were fluorescent, thus indicating that the intracellular distribution of soluble iron among the population was heterogeneous. This

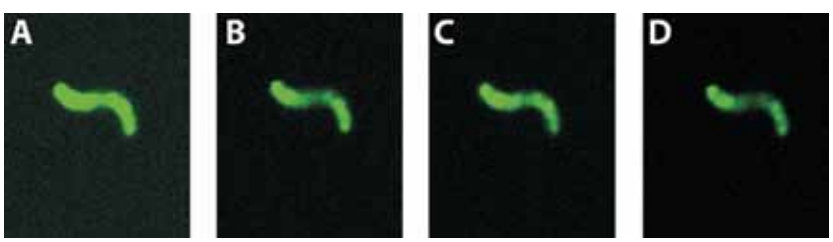

Figure 1. Confocal microscopy images over a 15 min period of a MSR-1 cell stained with PG-SK at (**A**), 0 min, (**B**) 5 min, (**C**) 10 min and (**D**) 15 min incubation.

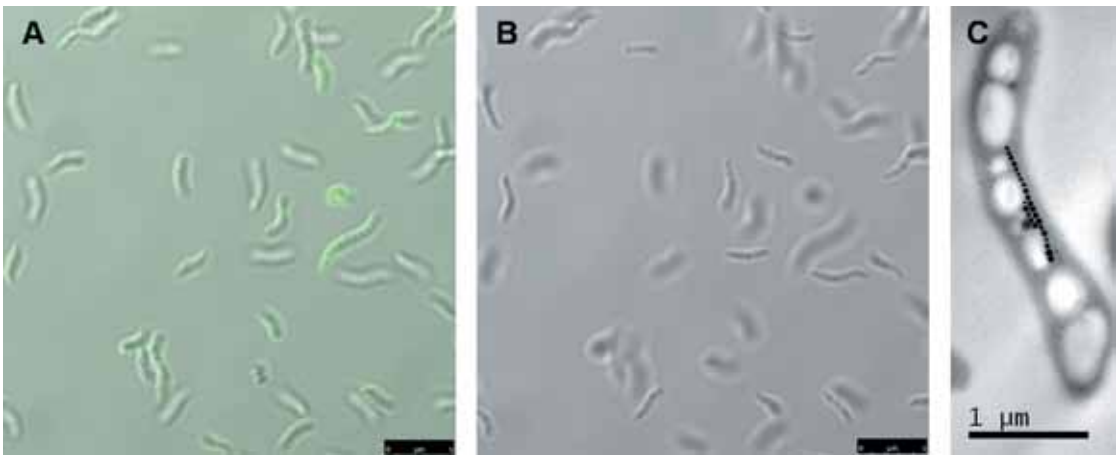

Figure 2. Microscopy images of MSR-1 cells. (**A**) Confocal microscopy and (**B**) bright filed microscopy of cells stained with PG-SK. (**C**) Transmission Electron Microscopy (TEM) micrograph.

might be correlated to the number of magnetosome units per cell as it is known that there is a variation in the length in the magnetosome chain when observing MSR-1 in TEM (Fig 3C). However, further evidence of this correlation will need to be provided.

Our preliminary results have improved our understanding on iron uptake by the cell and assessed intracellular iron content. Further experiments will aim to identify current limitations of magnetosome production related to the availability of iron in the cell as well to fully understand the dynamics of how iron ions enter the cell and undergo biomineralization to make magnetite crystals within magnetosomes. ■

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Further reading

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