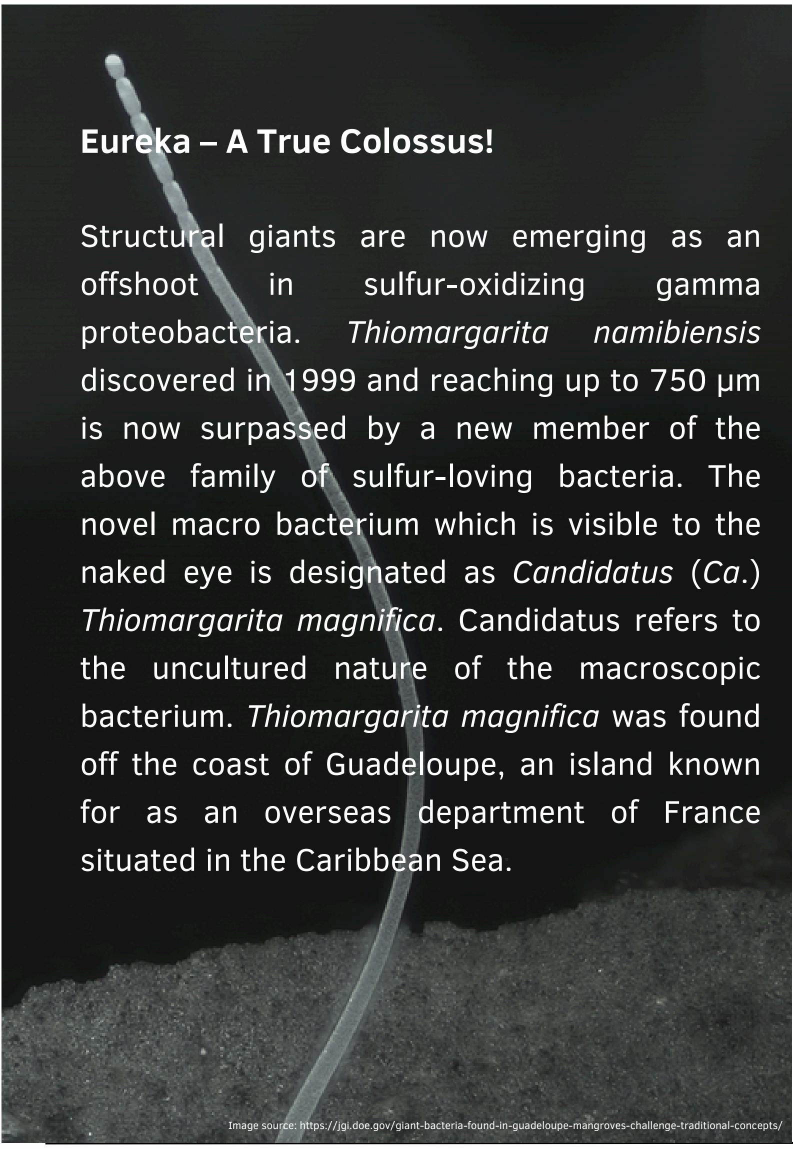
FEATURE ARTICLES

From Serendipity to its Secret Sauce: Connecting the Dots of *Candidatus* (*Ca.*) *Thiomargarita magnifica*, a Giant Macroscopic Bacterium



Eureka – A True Colossus!

Structural giants are now emerging as an offshoot in sulfur-oxidizing gamma proteobacteria. *Thiomargarita namibiensis* discovered in 1999 and reaching up to 750 µm is now surpassed by a new member of the above family of sulfur-loving bacteria. The novel macro bacterium which is visible to the naked eye is designated as *Candidatus* (*Ca.*) *Thiomargarita magnifica*. Candidatus refers to the uncultured nature of the macroscopic bacterium. *Thiomargarita magnifica* was found off the coast of Guadeloupe, an island known for as an overseas department of France situated in the Caribbean Sea.

Image source: https://jgi.doe.gov/giant-bacteria-found-in-guadeloupe-mangroves-challenge-traditional-concepts/

Thiomargarita magnifica has been identified as sessile, being able to move while being fixed to a substratum but not having the capacity of free locomotion away from the bedrock. The environment that *T. magnifica* was identified from can be classified as a sulfur rich environment, where sulfur oxidation plays the central role in energy production. The sulfur oxidizing bacterium is known to outsize the next-in-line giant bacterium - *Thiomargarita namibiensis* - by a whopping 50 fold.

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Connecting the dots: a healthy dose of biology

In order to characterize the structural details of *Ca. T. magnifica*, the membranes of this prokaryotic giant were highlighted by using osmium tetraoxide and a fluorescent dye known as FM 1-43X, to observe the 3-D arrangement of the cell, using X-ray tomography and new tricks in confocal microscopy. Electron microscopy allowed the scientists to measure the size of cells spanning the whole length to the apical extremity. There was some degree of tapering towards the distal end with the cytoplasm narrowing till the apical tip. There were no septa or walled structures separating the filamentous cell into smaller subunits. The “budding” of daughter cells took place at the apical end and was instrumental for the generation of new cells.

A large vacuole sits at the center of the cell, which took up 73.2 ± 7.5% (n = 4) of the total volume of the elongated bacterium. The cytoplasm took a narrow path around the vacuole being only 3.34 ± 1.48 μm thick, therefore allowing for efficient metabolic diffusion to be the cornerstone of its biochemical needs. Lucid vesicles made of deposits of sulfur showed a dotted appearance in the cytoplasm.

The unprecedented took the shape of compartmentalized DNA and ribosomes surrounded by membranous structures, showcasing that there was a jump in bio-complexity for this marine bacterium. These “blebs of cytoplasm” either contained ribosomes with DNA fibrils or exclusively enveloped nuclear material or took the shape and identity of “endobionts”. The scientists of the study could not find relevant answers to these membrane-bound organelle-like structures in their initial dive into the biology of this macroscopic wonder. However, it is suspected that compartmentalized DNA bolsters the polyploidy of the identified species, a common signature in many giant bacteria identified this far.

Biological appartments: floor plans and furniture

The compartmentalization of functions of bacteria has been shown to offer anaerobic ammonium oxidation, photosynthesis, and magnetic orientation as a bacterium’s newly acquired skill sets. Therefore, it was not totally surprising that to accommodate such a giant structure, there was the need to compartmentalize both the DNA and protein making machinery. Deep but incomplete invaginations have been shown to occur in many bacteria (such as *Gemmata obscuriglobus*) prior to the discovery of *T. magnifica*. These incomplete prototypes - stepping stones of future membrane-bound compartments -, show the way in which biology evolves in steps to nestle critical molecules (DNA, etc.) and assembly units (ribosomes) in nurtured easy-to-control environments. FISH (Fluorescence In Situ Hybridization) which entails targeting probes to ribosomal RNA, was the method of choice to demonstrate that ribosomes were indeed compartmentalized. The compartments harboring ribosomes and DNA were named as “pepins”, the etymology derived from pips that are encountered in watermelons and kiwi fruits, a term for “smallness” that is symbolic of the miniature size of the compartment next to eukaryote concentrates bordered by enveloping membranes.

ATP synthase, which is the custodian of energy production (to build chemical blocks called ATP) is known to be located on the inner mitochondrial membrane in eukaryotes, while in bacteria, ATP synthases are localized on the exterior cell membrane. Immunohistochemistry narrowed down the ATP synthases to pepins and to the interconnected network of membrane compartments but not to the exterior cell envelope. This heralds new life into the taxonomic unit we call bacteria where the compartmentalized production of ATP will be beneficial to the distribution of ATP synthesizing units, and consequently the logistics and availability of the chemical ATP for bioenergetics.

Lost in translation

Bioorthogonal noncanonical amino acid tagging (BONCAT) was the method of choice for the detection of protein production. BONCAT revealed that protein synthesis was localized to certain pepins but it was not universally distributed to all pepins. The apical areas of the filamentous bacterium possessed stronger protein production activity than the basal parts of the cell and consequently these "active pepins" were called protein synthesizing hotspots. The growth of an axenic culture will be a requirement of the future, to zoom in on the molecular events that are symbolic of the pepins, as well as the growth and proliferation events of this macro-bacterium. It was observed that the *T. magnifica* did not have to double its cell volume to engage in fission, since apical budding was the method of choice to form bacterial offspring.

Diversity in ploidy: genetic stockpiles

Polyploidy leads to higher degrees of gene duplication, reassortment, and divergence that could be the impetus for a rich diversity of unique and rare biochemical signatures. In all, *T. magnifica* contains the highest estimated number of genome copies of any living organism, and this apical level of genome duplication, containment, compartmentalization, and preservation, requires studies that will hone in on the remarkable biology of this macro bacterium. A largely homogenous genome population sets the stage for harmonized biology – all cells appeared to contain the same signature of DNA assemblies. *Thiomargarita magnifica* appears to contain up to 11788 genes with half of unknown pedigree, which points to a high degree of neofunctionalization (finding new roles in biology). The gene families for sulfur oxidation and carbon fixation appear to be highly enriched, suggesting chemoautotrophy, and specifically thioautotrophy.

The lower number of epibiotic bacteria is indicative of the opulence of secondary metabolites produced by the assortment of metabolic pathways that may discourage the growth of mutually beneficial bacteria on external surfaces. It was also evident that cell division genes were found lacking in the genome of this macroscopic bacterium but cell elongation genes were found in their entirety, which may shape the filamentous and elongated nature of the macroscopic cell. The budding of apical daughter cells brings into perspective that there will only be a subset of the pepin genomes in the bacterial offspring, and this points to a dimorphic cycle where parent and daughter possess a distinct genomic signature.

This sort of budding phenomena draws parallels to dispersion by fruiting bodies of the social myxobacteria or aerial hyphae of *Streptomyces* spp.

Alice in Ponderland

In a synopsis, this is a wondrous discovery that will make us look through the rabbit holes of under sampled niches that may harbor a richness of macro contenders that may defy the standards of biology but will not defy logic or a gradual switch towards complexity. The most enthralling story in this uncharted new base of biology is the compartmentalization of DNA fibrils and protein production units, together and in seclusion. Harboring two types of molecular contenders in fusion, points to the easy logistics of coupled transcription and translation as a possible phenomenon that will benefit the biological pathways. Energy derivation from internal ATP synthases also points to a function comparable to mitochondria that may offer less labor costs for the maintenance of cells.

Long, narrow and oozing with confidence due to its polyploidy genome architecture containing a rich collection of unique genes, make this specimen a true novelty.

Culturing this giant – or the dispersion prone daughters – will be the next logical step. An axenic culture will help in the further detection of biology, and it is time that scientists believe that Alice in Wonderland is not the only place for freaks of biology, the waters of Guadeloupe are right up there.

From holler to ruler

In Alice's adventures, she encounters a 3 cm long hookah smoking caterpillar that can be compared to this centimeter-long bacterium that smokes sulfur. Just like a caterpillar turns into a butterfly, we do not know the catalog of wonderful findings that await the team of scientists of this study. So let's empower science to define the next paradigm of findings of this megalo bacterium, to culture it on a petri dish, and dissect the secret sauce that makes it what it is in the contemporary – *a freak so far, but only until the next serendipitous discovery*. An incremental trend spiraling upwards is associated with the size of mimiviruses and dinosaurs, and now by extension, centimeters-long filamentous bacteria.

Here's a mighty "Eureka" to the next exception to the rule(r) !!!

Bibliography

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