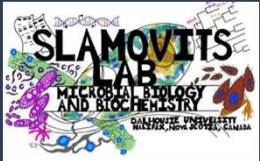


Genomic Analysis of the Bacterium *Roseibium aggregatum* and its Role as a Symbiont of *Oxyrrhis marina*



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Background

In the ocean, **microorganisms engage in intricate interactions** vital for ecosystem functionality and life sustenance. Their influence is limitless, as they work with other organisms to build ecosystems and essentially make life possible to sustain.

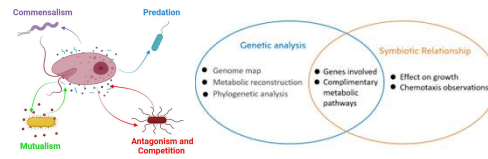
Symbiotic relationships are very common in the ocean, usually among organisms that are not related phylogenetically (1). Previous research has established that **bacteria and dinoflagellates interact** since they have been found to coexist together, with various possible symbiotic relationships, however the exact relationship is often unknown (2).

The impact of Bacteria on biogeochemical cycles is evident as photosynthetic bacteria in the ocean have been found to be important in iron cycling and nitrogen fixation (3). Further, the metabolites produced by marine bacteria have also been found to have applications in pharmacological research and other commercial fields (4). *Roseibium aggregatum*, the focus of this study, is a **gram-negative bacterium belonging to the alphaproteobacterial group**. This relatively understudied bacterium, with limited research, shares some commonalities with similar strains. *Labrenzia aggregata* has been studied in relation to its metabolic & biogeochemical functions, functions of its genes and other features (5). The genome of this bacteria, its application in bioremediation, and overall **ecological role is currently unknown**, necessitating further investigation.

Oxyrrhis marina, also researched here, is a **heterotrophic, unicellular microeukaryote**. It is considered a model organism, especially in ecology, evolution, genomics, and biogeography (6).

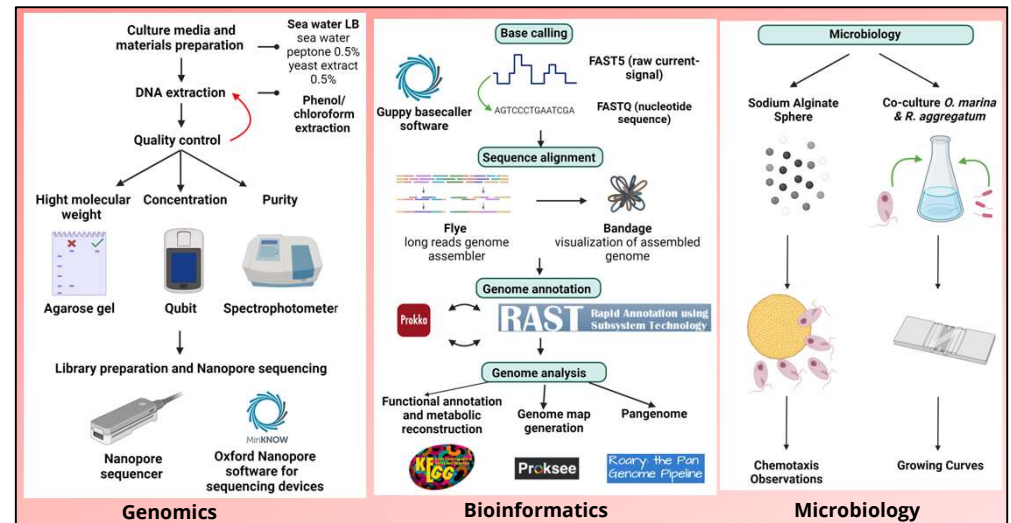
Objectives

This study aimed to analyze the genome of *R. aggregatum*, followed by the evaluation of its symbiotic relationship with *O. marina*. It also explored other interesting features found in the genome of *R. aggregatum* using various biological assays. It was hypothesized that the bacterium *R. aggregatum*, found within the microbiome of *O. marina*, plays a role as a symbiont of *O. marina*.



- Understand *R. aggregatum*'s genome by performing nanopore DNA sequencing, followed by functional genome annotation, metabolic reconstruction, and comparative genomic analysis.
- Determine the effect of *R. aggregatum* on *O. marina*'s growth.
- Identify if *O. marina* shows positive or negative chemotaxis toward *R. aggregatum* and characterize their symbiotic relationship.
- Identify the presence of genes and complementary metabolic pathways in the *R. aggregatum* genome involved in symbiosis.
- Perform tests to experimentally confirm features of *R. aggregatum*.

Methodology



Results

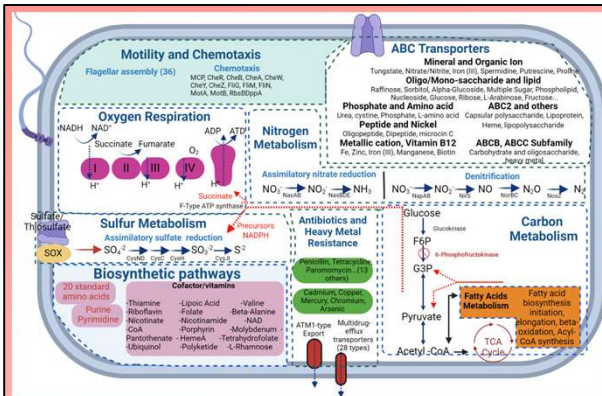


Figure 1 (up): Metabolic reconstruction of *R. aggregatum* revealing interesting features like contribution to biogeochemical cycles, antibiotic and heavy metal resistance, inability to metabolize sugars, and high production of cofactors and vitamins (i.e., B-12).

Figure 2 (left): Sugar biochemical test. Positive control, Glucose, Fructose, Galactose. (+) = Yellow; (-) = Red
Figure 3 (Right): Antibiotic resistance test.

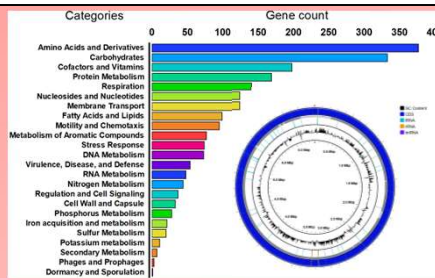


Figure 4 (up): Functional annotation of *R. aggregatum*'s genome (Size = 6417359 bp; GC% = 59.07; CDS = 6565; rRNA = 9; tRNA = 52; 2 mega-plasmids; 30X depth).
Figure 5 (down): Pangenome analysis of *R. aggregatum*. (Core genome = 4704 genes, accessory = 3907 genes).

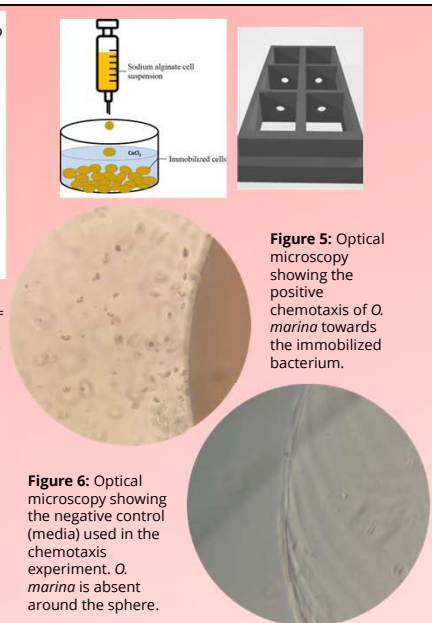
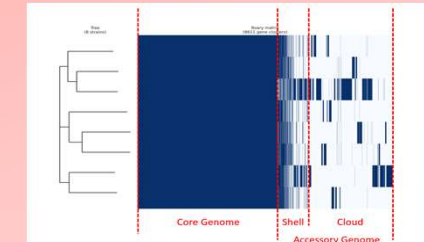


Figure 5: Optical microscopy showing the positive chemotaxis of *O. marina* towards the immobilized bacterium.
Figure 6: Optical microscopy showing the negative control (media) used in the chemotaxis experiment. *O. marina* is absent around the sphere.

Conclusion & Future Research

Although the project is ongoing, the following was concluded about *R. aggregatum*:

- Large genome with regions of potential **lateral gene transfer**.
- Genes for antibiotic resistance (not Chloramphenicol), and heavy metal resistance (MerA and SVPC), useful in **bioremediation**.
- Role in **biogeochemical cycles**- nitrogen and sulphur metabolism.
- Possesses **gramicidin**, known to be harmful to other gram-positive bacteria, and **surfactant** (rhamnolipids) which are compounds with emulsifying properties, with applications in the oil industry.
- Lacks the Entner-Duodoroff pathway and 6-phosphofructokinase, making it **incapable of metabolizing sugars**.

The following is concluded about their symbiotic relationship:

- O. marina* showed **positive chemotaxis** towards *R. aggregatum*
- R. aggregatum* has a **positive effect on the growth** of *O. marina*.
- Mutualistic** or commensalism relationship- Preliminary results.

This work has various implications:

- Deeper understanding of the symbiotic relationships of a **model organism**, i.e., *O. marina*, helping expand our insight.
- Clearer understanding of the **genomic properties** of *R. aggregatum*, and its relationships with other microorganisms, providing the most in-depth analysis of the bacterium to date.
- Potential role of *R. aggregatum* in **bioremediation** has been revealed, as it seems to be capable of reducing heavy metals and possess surfactants.

Since this project is ongoing, future work will explore our results in-depth, and search for genes and complementary metabolic pathways involved in symbiosis. Growth curve experiments, and tests for heavy metal resistance will be conducted. Lastly, a scanning electron microscope will be used to explore their symbiotic relationship.