Glycolate Utilization in Two Species of Marine Bacteria

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Glycolate Metabolism in Marine Bacteria

Interactions between bacteria and phytoplankton are crucial for the cycling of organic matter in marine environments. Around 50% of organic carbon taken up by marine bacteria is converted into inorganic carbon. The uptake of organic carbon by marine bacteria exuded from phytoplankton is a key factor in regulating the marine carbon cycle. One such molecule that is exuded by phytoplankton and then uptaken by marine bacteria is called glycolate - the anion of glycolic acid, a two carbon molecule. Glycolate is exuded by phytoplankton during photorespiration and 10-50% of dissolved organic carbon in marine environments is comprised of glycolate. Additionally, production of glycolate is thought to be a way to regulate excess energy from sunlight. Concentrations of glycolate in marine environments fluctuate from night to day. Concentrations are up during the day, when photorespiration is taking place, while concentrations are down during the night while only bacterial uptake is taking place. Glycolate is a major source of energy that drives uptake of other nutrients. Knowledge of its utilization can help to understand the dynamics between marine bacteria and phytoplankton.

Genes and Bacteria Used

Two species of bacteria were used. *Thalassospira sp.* 3-2 and *Marinobacter sp.* 13M1. Specific primers were used to target genes of interest during RT-qPCR. A housekeeping gene, gyrA was used to normalize expression rates between samples. glcC is a master regulator of the gly operon and encodes a regulatory protein. glcB encodes malate synthase G, which converts glyoxylate into malate. It signals the use of glycolate by central metabolism and its incorporation into aerobic respiration. Glc D encodes a subunit of glycolate oxidase, which converts glycolate molecules into glyoxylate. A final gene monitored was lldP, a L-lactose transporter that is though to also transport glycolate into the cell.

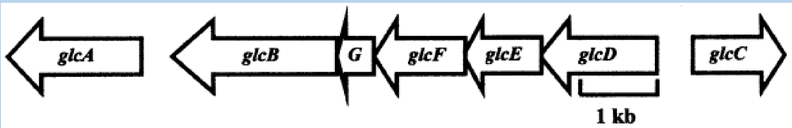


Fig. # - Diagram of the glc operon. glcC, the master regulator controls the functions of glcD, glcE, and glcF.

Energy and Biosynthesis Utilization of Glycolate

Glycolate is primarily used as an energy source by marine bacteria. Other molecules such as proteins and other carbohydrates are more commonly used for biosynthesis. This can explain the low rates of growth for many bacteria. During the experiment glycolate was the sole carbon source in half of the treatments and so only small amounts of growth could occur due to low availability of molecules to perform biosynthesis. However, a notable case was observed in isolate 3-2, which showed steady, logarithmic growth during the experiment. Perhaps there is a gene that is located in 3-2 that allows for inclusion of glycolate into biosynthesis pathways.

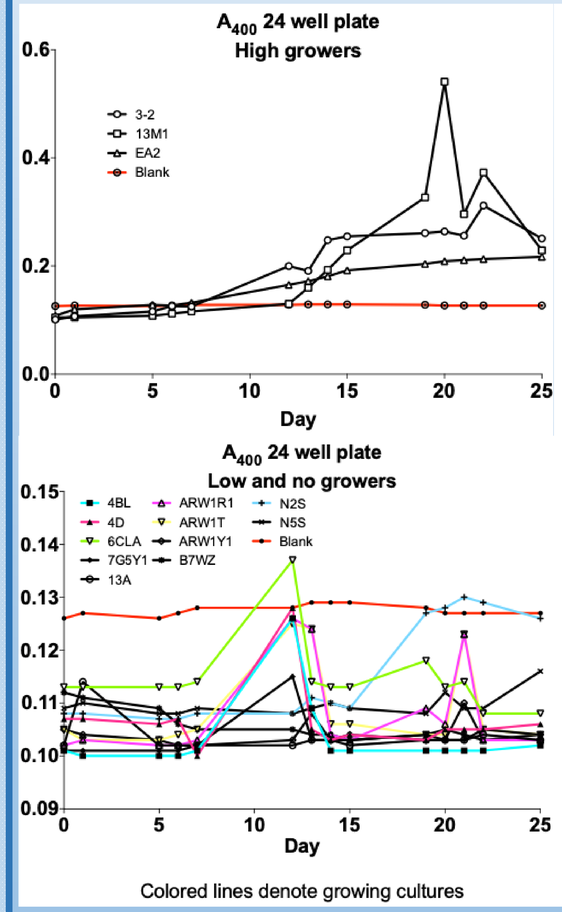
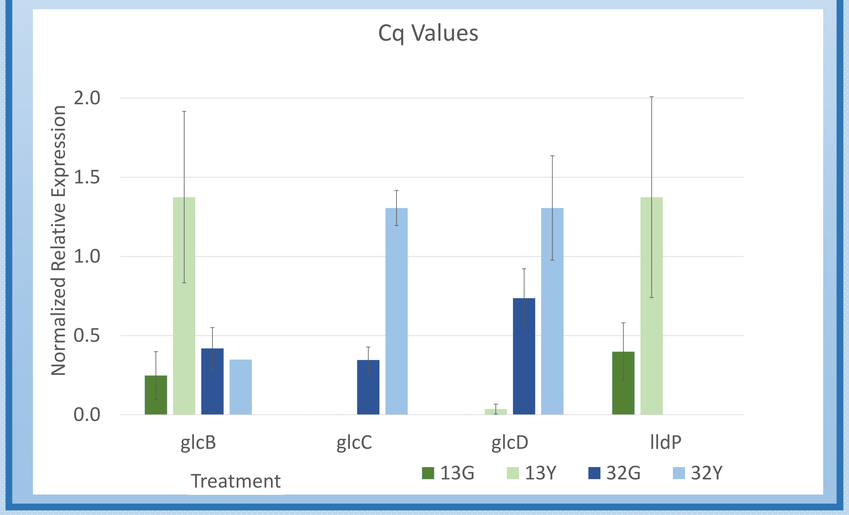


Fig. # - Growth curves from marine bacteria grown in ESAW with 50mM glycolate as the sole carbon source. 3-2 appears to be the only isolate with consistent growth.

Fig. # - Growth curves from marine bacteria grown in ESAW with 50mM glycolate as the sole carbon source. These isolates did not grow well and thus were excluded from RT-qPCR analysis.

Explaining Genetic Differences with C_q Values

RT-qPCR analysis yielded results in line with the hypothesis. There were mostly significant differences in expression of genes involved in the glycolate pathway between the glucose and glycolate treatments. glcB shows increased utilization by 13M1 in the glycolate treatment (13Y) versus the glucose treatment (13G). glcC, the master regulator found only in 3-2, shows increased expression in the glycolate treatment (32Y) versus the glucose treatment (32G). No difference in expression of glcD was found in 13M1 but a difference was found between glucose and glycolate treatments in 3-2. The last gene studied was lldP, a lactase importer gene known to also import glycolate. It too showed increased expression in glycolate treatment indicating that lldP gene product does in fact import glycolate.



Future Directions

- Further analysis of intracellular and extracellular metabolites in treatments where glycolate is the sole carbon source could yield interesting results about how glycolate is utilized in the cells of marine bacteria. This could be done using radiolabeled carbon to track the pathways in which the glycolate is utilized
- Investigation of more genes related to glycolate utilization could provide information on the differences of marine bacteria and how they effect marine environments and carbon cycles. This could provide information on carbon sequestration patterns and their effect on the Earth's atmosphere and oceans
- Further investigation into marine bacteria strains that utilize glycolate as an energy source versus a source of carbon for biosynthesis. Investigation into bacterial utilization of glycolate could provide insight into how marine bacterial communities are organized.

Methods

Cultures of 3-2 and 13M1 were grown up in Zobell media and then in ESAW media where glycolate or glucose was the sole carbon source. At sufficient density, cells were harvested from the triplicates of the two carbon source treatments and RNA was extracted and converted to cDNA for use in a RT-qPCR assay to measure the relative expressions of four genes: glcB, glcC, glcD, and lldP. Statistical analysis was performed to measure Cq values of the RT-qPCR.

References

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