Sugar, Spice and a Glycan Slice: Investigating Changes to *Oxyrrhis marina* Surface N-Glycans During Prolonged Starvation.

Abigail Enck[1], Amanda Montalbano[2], Susanne Menden-Deuer[2] & Christopher Reid[3]

[1]Science & Technology, Bryant University, North Smithfield, RI
[2]Graduate School of Oceanography, University of Rhode Island, Narragansett, RI
[3]Chemistry, Bryant University, North Smithfield, RI

Oxyrrhis marina is a globally distributed heterotrophic dinoflagellate commonly found in marine and saltwater coastlines. Their cell surfaces are covered in N-linked polysaccharides (N- and O-linked). These carbohydrates are used by cells for communication, adhesion, and regulation. In a previous study with *O. marina*, changes to their neutral lipid content during starvation was observed, which ultimately led to curiosity of their surface glycan profiles. In this study, *O. marina* was used as a model to investigate the changes to the cell's surface N-glycans under saturation and starvation conditions. The *O. marina* cells in the saturated condition were actively fed with the prey Isochrysis Gambana, a common alga found in saltwater. The prey's surface glycan profiles were also analyzed. In order to label the surface carbohydrates, they first needed to be taken off the cell itself. The N-glycans were liberated via treatment with PNGaseF, purified by cold ethanol precipitation, and labeled with 2-aminobenzamide (2AB) followed by analysis by MALDI-TOF mass spectrometry. PNGaseF effectively liberated N-linked oligosaccharides from glycoproteins from intact cells. The cold ethanol precipitation allowed contaminating protein to be removed from the aqueous solution. Carbohydrates ionize poorly in mass spectrometry, labeling with 2AB provided a fluorescent label and ionizable group on the glycans through reductive amination. During active feeding on the cyanobacteria Isochrysis, the *O. marina* surface should present a specific set of N-linked glycans. No detectable Isochrysis N-glycans were observed in the satiated *O. marina*. Over a 3-week starvation period, a shift in the *O. marina* surface N-glycan profile occurred. Starvation conditions result in a unique array of carbohydrates. Our characterization of the *O. marina* surface provides opportunities for a greater understanding of cell signaling and communication in saturation and starved conditions.

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