Physiological and Molecular Analysis of Carbon Source Supplementation and pH Stress Induced Lipid Accumulation in the Marine Diatom *Phaeodactylum tricornutum*

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Abstract

A detailed physiological and molecular analysis of lipid accumulation under a suite of conditions including nitrogen limitation, alkaline pH stress, bicarbonate supplementation and organic acid supplementation was performed on the marine diatom *Phaeodactylum tricornutum*. For all tested conditions, nitrogen limitation was a prerequisite for lipid accumulation and the other culturing strategies only enhanced accumulation highlighting the importance of compounded stresses on lipid metabolism. Volumetric lipid levels varied depending on condition; the observed rankings from highest to lowest were for inorganic carbon addition (15 mM bicarbonate), organic acid addition (15 carbon mM acetate) and alkaline pH stress (pH 9.0). For all lipid accumulating cultures except acetate supplementation, a common series of physiological steps were observed. Upon extracellular nitrogen exhaustion, culture growth continued for approximately 1.5 cell doublings with decreases in specific protein and photosynthetic pigment content. As nitrogen limitation arrested cell growth, carbohydrate content decreased with a corresponding increase in lipid content. Addition of the organic carbon source acetate appeared to activate alternative metabolic pathways for lipid accumulation. Molecular-level data on more than 50 central metabolism transcripts were measured using real time PCR. Analysis of transcripts suggested the central metabolism pathways associated with bicarbonate transport, carbonic anhydrases and C4 carbon fixation were important for lipid accumulation. Transcriptomic data also suggested that repurposing of phospholipids may play a role in lipid accumulation. This study provides a detailed physiological and molecular-level foundation for improved understanding of diatom nutrient cycling and contributes to a metabolic blueprint for controlling lipid accumulation in diatoms.