



Impact of thiamine metabolites and spent medium from *Chlorella sorokiniana* on metabolism in the green algae *Auxenochlorella protothecoides*

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ABSTRACT

Auxenochlorella protothecoides is a known thiamine auxotroph but our past work has shown that it can synthesize thiamine if provided with the precursor molecule 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP). Partial thiamine auxotrophy is common in microalgae with important ramifications for global phytoplankton productivity as well as engineering applications of algae. While thiamine deficiency can greatly depress algae growth and lipid content, the detailed metabolic impacts of thiamine deficiency are not well understood. We used metabolomics to study the response to thiamine-limited and replete conditions in mixotrophic *A. protothecoides*. We also investigated the impacts of exogenous HMP addition and the use of spent medium from another green algae, *C. sorokiniana*, as a source of thiamine metabolites. This is the first study, to our knowledge, that addresses metabolic impacts of thiamine deficiency and alleviation in green microalgae. Thiamine deficient cultures exhibited accumulation of pyruvate and α -ketoglutarate, indicating bottlenecks at the pyruvate dehydrogenase (PDH) and oxoglutarate dehydrogenase (OGDH) complexes. Both PDH and OGDH require thiamine pyrophosphate (TPP) as a cofactor. Transketolase also requires TPP but we only observed build-up of ribose-5-phosphate when glucose was supplied as a substrate. As expected, thiamine and HMP addition could alleviate these metabolic bottlenecks while greatly increasing algal growth, neutral lipid and starch content. Spent medium from *C. sorokiniana* only appeared to partially alleviate thiamine deficiency and resulted in build-up of isocitrate and glycolate, metabolites that appeared relatively unaffected by the presence or absence of thiamine. Interestingly, longer culture time of *C. sorokiniana* when preparing the spent medium led to much higher availability of thiamine metabolites. Thus, under the right conditions, it may be possible to co-culture mutually beneficial algae species and/or recycle spent cultivation medium to overcome auxotrophy in algae.

1. Introduction

The green algae, *Auxenochlorella protothecoides*, holds great potential for lipid production and wastewater treatment, even in the presence of wastewater microbes [1,2]. Under optimized heterotrophic conditions, it can accumulate 55% of biomass as lipids [3]. This strain also grows very rapidly under mixotrophic conditions in which both photosynthesis and an organic carbon source provide energy [4,5]. *A. protothecoides* is understood to be a thiamine auxotroph, but our past work has shown that it can synthesize thiamine if provided with the precursor molecule 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP) [6]. Specifically, *A. protothecoides* can synthesize the thiazole

precursor of thiamine but lacks the HMP synthase gene. A recent study has shown that loss of HMP synthase is common among a range of marine algae species [7].

Green algae use thiamine to synthesize thiamine pyrophosphate (TPP). TPP is a known cofactor in carbohydrate and amino acid metabolism [8]. It is required by pyruvate dehydrogenase (PDH), which converts pyruvate into acetyl-CoA. The latter can enter the tricarboxylic acid (TCA) cycle or can be used for fatty acid synthesis. TPP is also a cofactor for oxoglutarate dehydrogenase (OGDH), which converts α -ketoglutarate into succinyl-CoA in the TCA cycle and for transketolase (TK), which catalyzes reversible reactions in the pentose phosphate pathway as well as the Calvin-Benson cycle for CO₂ fixation. In addition

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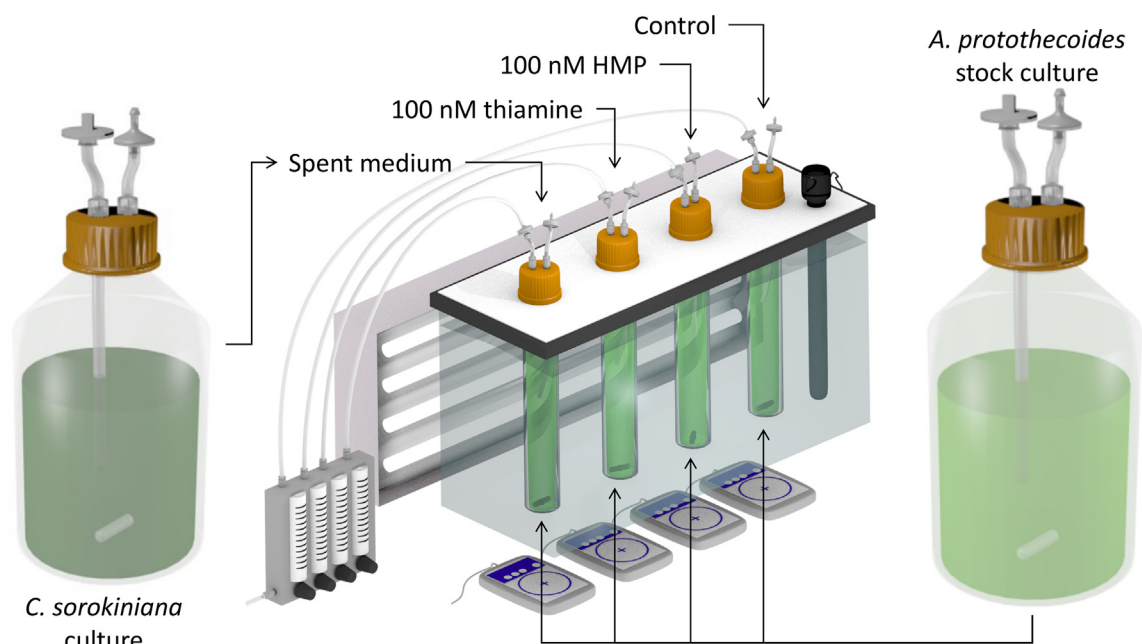


Fig. 1. Scheme of experimental set-up showing 1 L bottles used for algae stock cultures and 200 mL airlift reactors for culture experiments. The experimental scheme shown was carried out in triplicate. HMP is 4-amino-5-hydroxymethyl-2-methylpyrimidine.

to providing ribose-5-phosphate for nucleic acid synthesis, the pentose phosphate pathway also produces NADPH, the principle supplier of electrons for lipid biosynthesis. Finally, TPP is a known cofactor for enzyme complexes that catalyze reactions of keto acids generated during catabolism of the branched amino acids, leucine, isoleucine, and valine. Much of our understanding of thiamine nutrition stems from extensive research in humans and animals [9,10]. In a recent review, Helliwell raised the critical question of how transferable this knowledge is to the microbial realm, particularly that of phytoplankton [11]. This is a highly relevant question given that half of algae are likely B-vitamin auxotrophs with roughly 22% exhibiting thiamine auxotrophy [12]. In nature, these auxotrophs are understood to scavenge B-vitamin metabolites from bacteria [12–14] whose populations can vary with environmental conditions. The consequences of B-vitamin deficiency can lead to vast changes in phytoplankton community abundance and consequent marine primary production [15]. Likewise, B-vitamin deficiency can lead to significant impact on algal growth for engineering applications including biofuels, bioproducts, and wastewater treatment [6].

In past research, we showed that supplementation of *A. protothecoides* cultures with exogenous thiamine and HMP resulted in rapid growth compared to un-supplemented control cultures [6]. Under mixotrophic conditions, thiamine supplementation increased neutral lipid content significantly and eliminated pyruvate secretion by *A. protothecoides*, a common side-effect of thiamine deficiency in this organism. Similar effects were observed when co-culturing *Escherichia coli* with *A. protothecoides* [5,16]. In these conditions, *E. coli* provided thiamine metabolites to *A. protothecoides*, particularly the thiamine precursor, HMP. Thus co-culturing algae with bacteria is a promising engineering strategy to overcome B-vitamin deficiency.

The use of mixed cultures to promote algae growth through mutualistic interaction is an emerging research frontier in algal biotechnology [17]. Emulating natural symbioses, engineers can design processes that utilize mutually beneficial organisms with biotechnological applications. As an example of this, we have cultured *A. protothecoides* on spent medium from another industrially-relevant green algae, *Chlorella sorokiniana* [6]. *C. sorokiniana* is an autotrophic strain and has the ability to synthesize thiamine de novo, similar to *E. coli*. Co-culturing multiple green algae species, as a means of overcoming

thiamine deficiency, is highly attractive for engineering applications of algae, especially when both strains can be used for biofuel and bio-product synthesis. Our results showed that *C. sorokiniana* spent medium supported faster growth than control cultures but that the growth rate appeared to fall short of thiamine-replete cultures [6]. This raised questions about whether *C. sorokiniana* could fully alleviate thiamine deficiency in *A. protothecoides*.

Thiamine deficiency has been shown to decrease growth rates, nutrient uptake, and lower cellular lipid and starch contents. However, detailed impacts of exogenous thiamine and cellular secretions on *A. protothecoides* metabolism have not been investigated. Moreover, extensive database searches revealed no studies that specifically address metabolic impacts of thiamine deficiency and alleviation in microalgae. The objective of the work presented here was to determine how thiamine deficiency and alleviation in *A. protothecoides* impacts its metabolome and conversion of organic substrates into biomass components. We used a combination of culture studies and metabolomics to reveal the impact of exogenous thiamine and HMP on mixotrophic metabolism in *A. protothecoides*. We also tested the metabolic impacts of culturing *A. protothecoides* on spent *C. sorokiniana* medium as a potential source of algae-derived thiamine metabolites. This is the first study, to our knowledge, to examine the effects of exudates from one green algae species on the metabolome of another.

2. Methods

2.1. Culture experiments

A. protothecoides (UTEX 2341 [18]) was cultivated in lab-scale bioreactors to understand the impacts of exogenous thiamine, HMP, or spent *C. sorokiniana* medium on algae growth, composition, and metabolism (Fig. 1). All reactors contained N8-NH₄ medium supplemented with either 8 g/L glucose or 8 g/L acetate as described in previous work [5]. Control reactors were filled with fresh medium only. For treatment reactors, fresh N8-NH₄ medium was supplemented with either 100 nM thiamine or 100 nM HMP, levels that were previously determined to fully alleviate thiamine deficiency in *A. protothecoides* [6]. To prepare spent medium, *C. sorokiniana* was cultured in bottles filled with N8-NH₄ medium without any organic carbon source. Culture time was five days

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