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Temperature effects on growth rates and fatty acid content in freshwater algae and cyanobacteria

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A B S T R A C T

Mass cultivation of algae for biofuel and other bioproduct production in outdoor, open raceway ponds has some considerable economic advantages. However, these systems would be subject to fluctuations in temperature (among other environmental factors), which can have dramatic effects on the growth rates of algal species and impact the overall productivity and quality of targeted algal crops. This study sought to elucidate the effects of temperature on algal growth rates, biomass accumulation, fatty acid production and composition. We surveyed 26 algal species from 5 different functional groups, growing them at 6 different temperatures between 9 and 32 °C. For each surveyed species, we collected eco-physiological trait data including maximum growth rate, thermal optimum (T_{opt}), thermal niche width, and lower and upper temperature limits for growth (CT_{min} and CT_{max}, respectively); these data were also pooled for analysis at the functional group level. Responses to temperature varied among species, but at the functional group level we determined that the cyanobacteria have the highest thermal optimum (30.6 ± 2.3 °C), followed by chlorophytes (25.7 ± 0.1 °C) and diatoms (24.0 ± 0.4 °C). Temperature-specific fatty acid (FA) production was mostly controlled by growth rates, though some change in production was attributable to modification of intracellular FA stores. Temperature affected FA profiles in diverse ways, with no consistent trends across species or functional groups. In sum, temperature significantly impacts the overall productivity of algal biofuel systems by influencing species growth rates and fatty acid production. While algal growth rates varied predictably with temperature, we did not find the generalizable trends in temperature dependence of FA composition, suggesting that some aspects of algal cultivation for bioproducts in outdoor, open-air systems may be less predictable. However, a compilation of algal growth and FA composition responses to temperature, such as ours, may be useful for choosing appropriate species for given temperature regimes.

1. Introduction

Microalgae hold significant potential to help meet the ever-growing societal need for food, feed and fuel [1–8]. To achieve the mass production of these high value bioproducts, industrial-scale cultivation of microalgae will be necessary. Two strategies for mass cultivation have been explored: outdoor, open cultivation in shallow raceway ponds and culture in closed photobioreactors. Closed photobioreactors have more controlled conditions with little risk of undesired invasions by parasites and zooplankton grazers, but they can suffer from extreme internal temperatures and dense cultures leading to self-shading [3,9]. Outdoor ponds may be the most economical option for mass production, but these pond systems are highly susceptible to fungal infection, invasion by undesired local phytoplankton and zooplankton (and crop loss due to herbivory by the zooplankton), and fluctuating environmental conditions [10,11]. Of major concern in both of these systems are the effects of varying temperature on the growth and fatty acid production of algal crops. Although a small number of studies have investigated the influence of temperature on lipid production and growth of various microalgae, the differences among these studies limit cross comparison and general conclusions of how temperature affects algal eco-physiology, specifically in terms of biofuel production [12–15]. In this study, we use the same growth conditions and uniform experiments to test the effects of a range of temperatures on a diverse set of freshwater algae and cyanobacteria.

Algal species vary greatly in the thermal ranges within which they can maintain positive growth (the thermal niche) [16,17]. Falling outside of this range leads to negative growth, whereas within the thermal niche there is an optimal growth temperature at which growth is maximized (T_{opt}). Temperature dependence of most biological rates, including population growth rate, can be described by a left-skewed, unimodal curve, such that positive growth is possible at a wider range of temperatures below T_{opt} than above, where growth rates precipitously decline, crossing the temperature axis at the upper critical temperature (CT_{max}) (Fig. 1). The increasing portion of this thermal performance curve (*TPC*) can be described, in part, by Arrhenius-style (exponential) temperature dependence of photochemical reactions that ultimately govern the growth rate [18,19]. Temperatures above the

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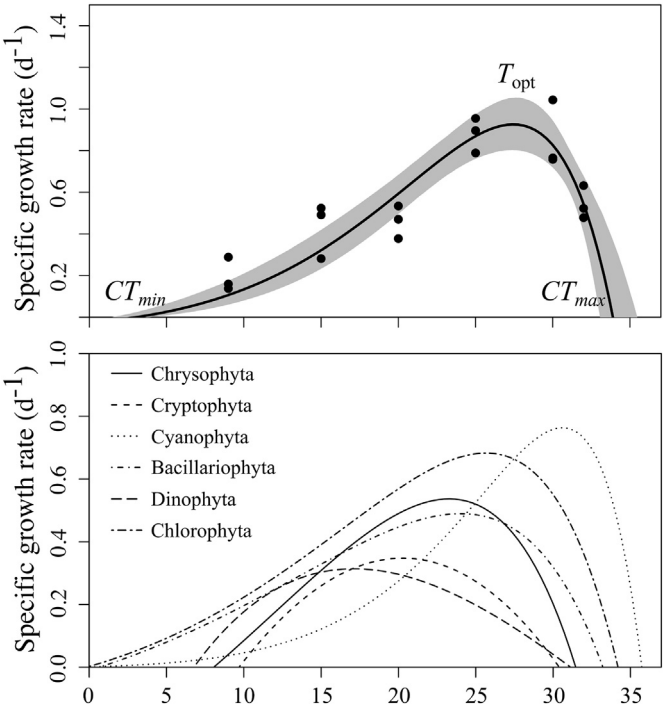

Fig. 1. (A.) Growth curve for *Chlamydomonas reinhardtii*. Growth optimum (T_{opt}), and minimum and maximum critical temperature (CT_{min} and CT_{max}, respectively) are illustrated. (B.) Comparison of temperature curves for different algal functional groups: Chrysophyta (solid), Cryptophyta (dashed), Cyanophyta (dotted), Bacillariophyta (dashed and dotted) and Dinophyta (dashed).

thermal optimum lead to heat stress, resulting in decreased enzyme activity, modifying or denaturing of photosynthesis-related proteins, and even triggering the synthesis of stress proteins [20–22]. These responses to high temperatures limit the downstream production of necessary cellular products and ultimately lead to the observed precipitous decline in growth.

Temperature directly affects the lipid content and fatty acid (FA) composition of organisms but the nature and magnitude of this effect varies across species and is not well characterized [23,24]. At lower temperatures, cells often modify their cellular membrane to incorporate more unsaturated FA, conferring greater membrane fluidity, while at higher temperatures the cellular membrane becomes more saturated in order to increase rigidity [25,26]. The cellular response of modifying FA composition has been widely viewed as a mechanism for maintaining normal cellular function across temperature [27]. For example, by increasing the trienoic fatty acid (16:3 and 18:3) content in tobacco plants through the introduction of an omega-3 fatty acid desaturase gene (*fad7*), the cold tolerance of these plants was improved. Conversely, the suppression of this same gene and subsequent decrease in trienoic fatty acids led to significantly better acclimation of tobacco plants to high temperatures [28]. In non-transgenic plants, low temperatures activate FA desaturases leading to overall FA unsaturation, while at high temperatures the post-translational stability of FA desaturases becomes unstable, resulting in a decrease in trienoic fatty acids [2,27,29,30]. Similar trends (high levels of unsaturation at low temperatures and high levels of saturation at high temperatures) have been documented in various functional groups of microalgae [31–33]. It is important to note that there is also evidence to suggest that this trend is species-specific and not universal in either algae or vascular plants [24,34,35].

The knowledge of these eco-physiological responses to temperature (temperature traits) will not only inform our basic understanding of algae, but can also be used to assemble algal communities that remain stable and productive under variable temperature conditions, e.g., in outdoor ponds. Trait-based approaches can help develop a framework that utilizes known characteristics of target organisms in order to maximize a desired outcome, whether that be prairie restoration, limiting pathogen infection, or optimizing biofuel production. Experimental quantification of the desired traits is at the core of these trait-based approaches [36,37]. Work characterizing microalgae by other key traits has already been conducted, from growth rates to resource requirements, to cell size [16,38–40]. However, research on thermal traits, specifically those concerning overall fatty acid production is quite limited, and studies to date share few methodological commonalities.

This study aims to fill in the knowledge gaps by surveying the temperature traits of 26 freshwater algal species from six different functional/taxonomic groups. We measured growth rates, total FA productivity and composition at six different temperatures over a range of 23 °C (9–32 °C). Assay conditions have been standardized, so that both general, functional group trends and species-specific traits can be compared. Also, the collection of these eco-physiological traits will inform future work on using algal traits to explore the relationship between temperature and algal growth and fatty acid production.

2. Materials and methods

2.1. Microalgal and cyanobacterial cultures

To survey algal thermal responses, we established monocultures of 26 microalgae, including cyanobacterial species from lab grown cultures (Table 1 –species list, UTEX and University of Göttingen). The selected species belong to six different taxonomic groups: Bacillariophyceae, Chlorophyta, Chrysophyceae, Cryptophyta, Cyanobacteria and Dinoflagellata. While more focus in biofuel research has been on eukaryotic microalgae, we included cyanobacteria in our study because they were also shown to have FA suitable for biodiesel production, high growth rates and wide environmental tolerance limits [41].

To assess the impact of temperature on microalgae, we cultured all 26 species at six temperatures (9, 15, 20, 25, 30 and 32 °C); this range was selected so that all 26 strains would likely experience temperatures at the high and low extremes of their thermal niches, as well as temperatures near their T_{opt}. Batch cultures of each species were grown at the intermediate temperature of 20 °C in order to obtain high biomass, and were then divided into 6 smaller batch cultures. These batch cultures were then acclimated for seven days at each of the six experimental temperatures. Triplicate monocultures were then established from these acclimated stocks and standardized to have starting biovolumes (measure of biomass) of $3.33 \times 10^6\ \mu m^3\ mL^{-1}$. Cultures were grown in 125 mL Erlenmeyer flasks with a working volume of 75 mL WC medium [42] and a daytime irradiance of $100\ \mu E\ m^{-2}\ s^{-1}$ (12:12 h light:dark cycle). Monocultures were then grown at the 6 temperatures for a total of 96 h with sampling and culture resuspension taking place every 32 h. The light and nutrient levels were selected to provide hospitable growth conditions for all taxa surveyed, although it is important to note that selecting conditions that would be optimal for all species surveyed would be infeasible.

2.2. Biovolume estimates, growth rates and thermal performance curves

To investigate the impact of temperature on algal growth, we calculated growth rates from the change in biovolume over time. Biovolume (total volume of a given species in culture) is a standard proxy of biomass in algal ecology and can be converted to biomass or carbon content using conversion factors. We measured biovolumes at all three sampling events using the CASY Cell Counter and Analyzer System Model TT (Roche Innovatis AG, Germany). Growth rates (d^{-1}) were calculated as the slope of the linear regression of ln(biovolume) over time [43].