TITLE: PARTIAL STUDY OF TEMPERATURE INFLUENCE IN *Chlamydomonas reinhardtii* GROWTH AND FLUORESCENT PROTEIN ACTIVITY

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ABSTRACT:

Chlamydomonas reinhardtii is a green microalga, considered as a model microorganism in cellular and molecular biology. Several studies were produced using this platform since its genome was fully sequenced, along with the development of transformation techniques for each organelle. Its molecular characteristics enable post-translational modifications that are necessary for proteins' correct function, like glycosylation and disulfide bonds addition, which makes this an interesting platform for biotechnological applications. However, microalgae recombinant protein yield is low in comparison to other platforms (bacteria, for instance), demonstrating the need for more studies focused on increasing microalgal biomass and recombinant protein activity and accumulation. It is well-known that culture conditions can influence microorganism's growth as well as intracellular compounds expression. Temperature is an important variable to assure microalgae's optimal growth. It is known that *C. reinhardtii* usually grows at 25 °C in bench scale. However, previous studies proved that it manages to maintain cell replication between 15 and 37 °C. To demonstrate temperature influence in biomass and fluorescent protein activity, a recombinant *C. reinhardtii* strain expressing the fluorescent protein mCherry was grown at 15, 25, and 35 °C. Experiments were carried out in Erlenmeyer flasks using TAP medium, under constant illumination of 60 µmol photons m^{-2} s^{-1} in a rotary shaker at 110 rpm. Microalgal growth was measured through cell counting, and mCherry fluorescence was detected in wavelengths of 575 nm (excitation) and 608 nm (emission). Results indicate that microalgal growth was higher at 25 °C (11.3 cells x10^6/mL), but mCherry highest fluorescence was achieved in cultivations carried out at 35 °C (40,438 fluorescence unit). Meanwhile, the cultivation carried out at 15 °C resulted in the lowest cellular growth (4.96 cells x10^6/mL) and mCherry fluorescence (28,866 fluorescence unit). Therefore, we can conclude that higher temperatures increase mCherry fluorescence. This indicate that temperature is an important parameter to be studied to increase recombinant protein accumulation.

Keywords: *Chlamydomonas reinhardtii*, microalga growth, fluorescent protein activity

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