BIO_13 - The algal chloroplast as a platform expression of full-length monoclonal antibody (Infliximab)

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Introduction: Biopharmaceuticals are mostly therapeutic recombinant proteins obtained by biotechnological processes. The ideal protein expression system should provide recombinant proteins in high quality and quantity involving low production costs. The recombinant monoclonal antibody is a relatively large protein that is heterogeneous due to post translational modifications and carbohydrate attachment. The infliximab protein is a chimeric monoclonal antibody which targets the tumor necrosis factor (TNF-a) pathway. It is used for the treatment of autoimmune diseases, rheumatoid arthritis, Crohn’s disease, and psoriasis. The use of microalgae as cell factories is particularly attractive as a low-cost, low-tech and sustainable approach. These microorganisms absorbs sunlight as their energy source and extract CO2 from the air as their carbon source. Furthermore, the chloroplast contains the proper machinery to form disulfide bonds and assemble large, complex proteins such as full-length antibodies. *Chlamydomonas reinhardtii* is the most widely used microalga for recombinant protein expression. This green microalga has the chloroplast genome sequenced and well-known transformation methods for, making them attractive for the therapeutic proteins production.

Objectives: The aim of this work was to accumulate Infliximab in the chloroplast of *Chlamydomonas reinhardtii*.

Methodology: In this work, an expression vector was inserted in the chloroplast genome as a direct replacement of the endogenous chloroplast psbA gene. The cell wall deficient *Chlamydomonas reinhardtii* strain (CC-400) was genetically transformed with glass beads method. The culture medium with antibiotic allowed the selection of transformed cells. Next, the colonies were tested for presence of the infliximab gene by PCR. Kanamycin- resistance selection led to an acquisition of homoplasmic strains of which a stable, and the protein of interest was detected by western blot.

Results: 100 colonies were obtained from the transformation of *Chlamydomonas reinhardtii* cell wall deficient in agar plates containing kanamycin. These colonies were transferred for new plates containing with culture medium containing kanamycin, and only 12% were selected by the antibiotic. After that, the selected colonies were tested for presence of infliximab gene by PCR, and 7% were positive. The presence of infliximab protein was detected in all strain by western blot.

Conclusion: This study highlights the potential of microalgae as a robust and low-cost expression platform for production of a full-length monoclonal antibody, infliximab.

Keywords: Monoclonal antibody production, microalgae, platform expression