BIO_14 - Recombinant monoclonal antibody (mAb) accumulation in chloroplast of *Chlamydomonas reinhardtii*

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Introduction: The introduction of monoclonal antibodies (mAbs) in the pharmaceutical market occurred in 1986 with the monoclonal antibody Muromonab CD3 commercialization. Since then many mAbs were developed and applied for the treatment of a range of diseases thanks to the success of its targets specificity. These mAbs are industrially produced in non-human mammalian cells and the Chinese hamster ovary (CHO) cell platforms, however, the production is too expensive, which makes attractive the search for alternative platforms. Among these alternatives the green microalga *Chlamydomonas reinhardtii* appears as a great alternative because of its low cost, easy genetic and biochemical machinery manipulation, and it is considered Generally Recognaized as safe. In this paper we aimed to study the antibody Adalimumab, a recombinant fully human monoclonal antibody that has been commercialized since 2002 for the treatment of rheumatoid arthritis, an immune-mediated inflammatory disease.

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

Methodology: The expression vector containing the gene of interest sequence (Adalimumab) was transformed into the CC503 C. reinhardtii strain by the glass-bead transformation technique. Selective medium containing kanamycin was used to select the transformed colonies, followed by PCR (polymerase chain reaction) gene positive confirmation and Western blot (WB) assays to confirm the protein accumulation.

Results: Transformed colonies were detected on agar plates containing the antibiotic kanamycin. The presence of the gene of interest were confirmed by the PCR technique. After successive colonies stricking, ten colonies showed homoplasmy also by PCR technique however four colonies showed an accumulation of protein after the Western blot assay.

Conclusion: So far we have succeeded in detecting and accumulating the protein encoding for the antibody Adalimumab in the chloroplast of *Chlamydomonas reinhardtii*. This finding is of great relevance because Adalimumab is a large and complex protein with a molecular weight of approximately 148 kilodaltons [kDa], which makes it difficult to manipulate in other organism genetic machinery.

Keywords: Monoclonal antibodies, Platforms, Microalgae