GENETIC MODIFICATION of *Chlamydomonas reinhardtii* CHLOROPLAST WITH Ag85b FROM *Mycobacterium bovis.*

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Introduction. Tuberculosis (TB) is one of the infection diseases with the highest incidence worldwide; approximately one third of the world population is infected with *Mycobacterium tuberculosis*, the causative agent. An estimated nine million new cases of active TB and two million deaths are reported annually (1). Currently the vaccine prepared with the bacillus Calmette-Guérin (BCG), obtained by inactivation of *Mycobacterium bovis,* prevents disease but it has been shown sometimes the protection is low (2). To develop the next generation of vaccines against *Mycobacterium*, various strategies are being analyzed, including vaccination with pathogen subunits, where it has been shown that protein secreted Ag85B from *Mycobacterium bovis* has high immunogenicity. It has been found that immunization with this protein is able to protect mice against infection with *Mycobacterium*, inducing a strong T cell proliferation and IFN-g (3). A novel strategy for the production of therapeutic proteins, as is the case of antigen Ag85B, is the genetic modification of green algae chloroplast. *Chlamydomonas reinhardtii* is a non-toxic microalgae with a short doubling time and it has been demonstrated that its chloroplast can accumulate 1-5% of heterologous proteins (4).
In this study, we have demonstrated that *Chlamydomonas* reinhardtii is capable of accumulating the protein Ag85B from *Mycobacterium bovis.*

Methods. Codon usage in gene Ag85b was optimized for *C. reinhardti* chloroplast and synthesized by DNA 2.0 (USA). Chloroplast transformation vector p322 (Chlamydomonas center), which directs the insertion of the genes of interest into the region between the psbA gene-5S-23S, was used to generate p322-Ag85b. Vector p-228 (Chlamydomonas center), which confers resistance to spectinomycin, was used to select transformed lines when co-bombarded with p322. Spectinomycin-resistant lines were screened by PCR with primers specific for the gene of interest. The presence of the protein Ag85b was determined by western blot using anti-Ag85b polyclonal antibody (Abcam ab43019).

Results. Gene Ag85b was first inserted in plasmid (pJ248:GFP) to generate pJ248:Ag85b and then transferred as an expression cassette to vector p322 to generate p322-Ag85b (Fig 1). Using vector p322-Ag85b, 6 bombardment events were carried out, recovering 28 spectinomycin-resistant lines. Of these lines, 7 were confirmed to be carriers of gene Ag85b following PCR with specific primers (Fig 2a). Seven out of these lines were analyzed by western blot. The antibody bound to a protein of apparent molecular weight of 30 kDa, presumably Ag85b. The antibody did not bind to a protein of the same weight in the wild-type extract (Fig 2b).

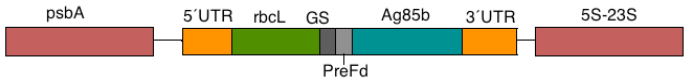

Fig. 1. Schematic representation of transformation vector p322-Ag85b. Expressión of the rbcL:Ag85b fusion protein is under the control of the rbcL 5´UTR and 3´UTR. Ag85b and rbcL are linked by a postranslational cleavable bridge made of glycine and serine (GS) and the ferredoxin signal peptide (preFd).

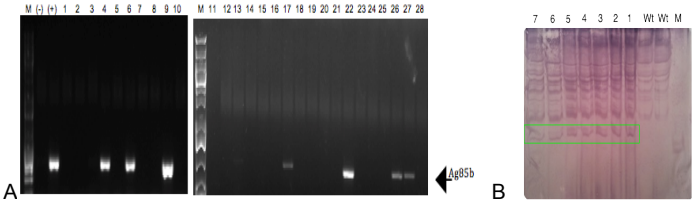

Fig.2. Agarose and polyacrylamide gel electrophoresis of lines obtained with vector p322-Ag85b. a) Confirmation of the presence of gene Ag85b in the chloroplast genome of *C. reinhardtii*. b) Confirmation of the presence of protein Ag85B in *C. reinhardtii* transformed lines.

Conclusions. Following transformation with vector p322-Ag85b, 7 lines of *C. reinhardtii* were recovered and demonstrated to carry the gene Ag85b and to accumulate a protein of apparent molecular weight of 30 kDa.

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References.

1. Maartens G, Wilkinson RJ. 2007. Tuberculosis. *Lancet* 370(9604):2030–43.
2. Feng CG, Palendira U, Demangel C, Spratt JM, Malin AS, Britton WJ. 2001. Priming by DNA immunization augments protective efficacy of Mycobacterium bovis Bacillus Calmette–Guérin against tuberculosis. *Infect Immun* 69(6):4174–6.
3. Gupta UD, Katoch VM, McMurray DN. 2007. Current status of TB vaccines. *Vaccine.* 25(19):3742–51.
4. Soria RE, Paz LM Rosales S. 2012. Chlamydomonas reinhardtii as a viable platform for the production of recombinant proteins: current status and perspectives. *Plant Cell.* 31:479-494.