PRODUCTION OF MILK-DERIVED BIOACTIVE PEPTIDES AS PRECURSOR CHIMERIC PROTEINS IN CHLOROPLAST OF *Chlamydomonas reinhardtii*

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Introduction. Milk proteins are known to have nutritional, functional and biological properties, these ones are partly attributed to the bioactive peptides, which are considered high value-added ingredients in functional foods, and some sources are milk, egg, plants, among others (1). A major limitation in their commercial use is the cost of production. Although production of bioactive peptides using the tools of genetic engineering recently, eukaryotic algae have been proposed as a new option for the production of biopharmaceuticals, offering the possibility to obtain high yields of recombinant proteins in a faster and cheaper system.

Methods. We design and optimize a synthetic gene (NCQ) encoding a chimeric protein carrying different bioactive peptides with antihypertensive, opioid, antimicrobial, immunomodulatory, antioxidant and hypocholesterolemic activities were elected (2). The NCQ gene was cloned into the p463 and p464 vectors (Fig. 1). A positive clone, selected by restriction analysis and sequencing was used for chloroplast transformations by ballistic. The specific integration of the transgén to chloroplast genome was evaluated by PCR using ch1N and tscA specific primers. Expression and quantity of recombinant peptide in transgenic *C. reinhardtii* was quantified by ELISA and Western Blot.

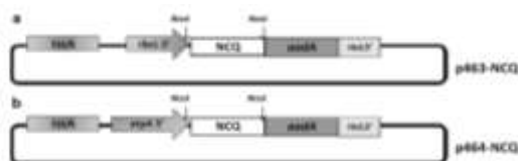

Fig.1 Diagram of transformation vectors p463-NCQ and p464-NCQ. The spectinomycin-resistance gene (aadA) under the control of the rbcL (a) or atpA (b) promoter, and the *tscA* region used for homologous recombination.

Results. To determine if the integration of foreign gene had occurred by homologous recombination in the chloroplast genome at the elected site, PCR was performed using R1 and R2 primers, which align on insertion site flanking regions (tscA and ch1N genes). As shown in the Fig. 2, a 851 bp amplicon was obtained in the wild type strain, but obvious 2.8 and 2.9 kb amplicons were obtained in transformants p463-NCQ and p464-NCQ respectively.

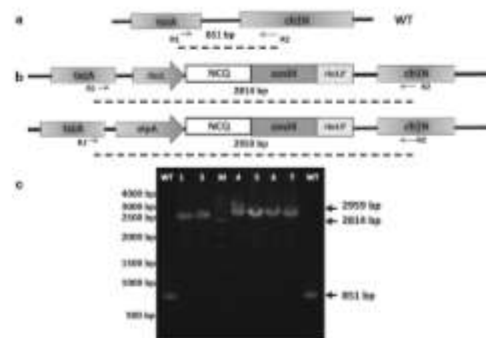

Fig 2. Schematic diagram of C. reinhardtii chloroplast transformation and the PCR assays of C. reinhardtii transformants. a Wild type strain containing the tscA and ch1N genes. b Transformants containing the foreign gene. c Electrophoretic analysis of PCR products from the template DNAs of the wild type and transformed *C. reinhardtii* after five rounds of spectinomycin selection on 1 % agarose gel.

Analysis by ELISA and Western blot revealed that the expressed NCQ protein was accumulated at levels ranging between 0.16 and 2.4 % of total soluble protein (Data not show).

Conclusions: These findings demonstrate that chloroplasts of *C. reinhardtii* could serve as a robust expression platform for production of bioactive peptide.

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References.

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