Expression and purification of human DNA topoisomerase I in pichia yeast expression system

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Human DNA Topoisomerase I (Topo I) plays an important role in the transcription and replication processes. The enzyme can relax negative and positive supercoil DNA by introducing a transient nick on the single stranded DNA, rotation of the free stranded DNA and religation of the broken strand. As the enzyme has been used as the targets of anticancer drugs for chemotherapy, the enzyme can be developed as a molecular target for screening of potential anticancer compounds. Therefore, producing an in house Topo I through recombinant technology will facilitate our project in screening of anticancer compounds. In this study, the coding region of Top I from cDNA of MDA-MB 231 cell was amplified by PCR with Phusion enzyme. The cDNA which is 2298bp in length was ligated into a yeast expression vector, pPICZA-alpha and transformed into Top'10 electrocompetent cells. The positive clones with the desired insert were isolated and plasmid was purified for sequencing. The recombinant plasmid was linearized with Sac I restriction enzyme and transformed into pichia strain, GS 115 by electroporation. The positive integrated transformants was grown in BMGY(Buffered Glycerol-complex medium) and expression of the protein was induced in BMMY (Buffered Methanol-complex medium), pH 6.0 at 28°C, 250 rpm with addition of 0.5% (V/V) methanol every 24 hour. Activity of the recombinant enzyme was determined with DNA relaxation assay using 1 µl of the culture supernatant. The enzyme was purified with HiTrap CM FF weak cation exchanger column (GE healthcare, Sweden) using ÄKTAprime plus protein purification system. SDS-PAGE and Western blot analyses showed the recombinant human Topo I with a molecular weight apparently of 85 kDa which is approximately 10 kDa less than the actual molecular weight of human Topo I. This might due to the proteolysis of the protein during the extracellular expression.

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Asteromonas gracilis (Prasionphyceae) as a model for production of β-carotene and total lipids

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The effect of nutrients deficiency (phosphorus, nitrogen and sulphur) on growth, ultrastructure, β-carotene and total lipid contents of the green alga Asteromonas gracilis has been examined. Algal cells were cultured under conditions of nutrients deficiency. Completely absent of phosphorus, nitrogen and sulphur caused significantly decreased of chl. a in A. gracilis compared to control. The maximum growth rate and minimum generation time calculated on basis of chl. a were 0.032 and 21.9, respectively for control culture. The total carbohydrates were decreased with deficiency of phosphorus and sulphur, but increased in case of nitrogen deficiency. On the other hand, the production of β-carotene and total lipids was enhanced with phosphorus, nitrogen and sulphur deficiency. The most changes in ultrastructure of A. gracilis observed when cells were cultivated without nitrogen source, the changes are in the structure of chloroplast, where the degradation of thylakoids was observed and the grana structure of thylakoids had almost disappeared as well as accumulation of starch grains were easily detected. Asteromonas gracilis has been identified as promising producers of useful lipids for biofuels production with cells containing about 37% oils. The aim of this work was to study and evaluate the effect of nutrient deficiency such as phosphorus, nitrogen and sulphur on the β-carotene and lipids accumulation of Asteromonas (local isolate) in addition, the scanning and transmission electron microscopy were made under these conditions.

Biography
Mustafa Ahmed Fawzy has completed his PhD at the age of 31 years from Assiut University. He is lecturer in Botany & Microbiology Department. He has published 5 papers in reputed journals.

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