PC-11

Functional expression of flounder growth hormone gene in transformed microalga, *Chlorella ellipsoidea*

Dae-Hyun Kim[1], Jung Jong Cho[1], Sung-Bum Hur[2],
Tae-Jin Choi[1], Young Tae Kim[1]
[1]Department of Microbiology and [2]Department of Aquaculture, Pukyoung national University, Namgu, Busan, 608-737, Korea

Introduction

Escherichia coli is the most widely used heterologous overexpression system. But, it has several limitations, such as poor or no expression of some proteins, proteins that lack biological activity, proteins that are toxic to E. coli, and formation of insoluble inclusion bodies.

Chlorella is unicellular eukaryotic green microalgae and therefore may produce complex protein that requires post-translational modification in biologically active form. These characteristics provide a rationale for the usage of *Chlorella* as a bioreactor for foreign protein overexpression.

Despite of these advantages, trial of *Chlorella* transformation is very limited. Jarvis and Brown (1991) detected transient expression of firefly luciferase in protoplasts of *Chlorella ellipsoidea*. Stable transformants were recovered from nitrate reductase deficient *Chlorella sorokiniana* mutants transformed with nitrate reductase gene from *Chlorella vulgaris* (Dowson *et al.*, 1997). However the expression was transient or the introduced gene was from same genus.

Here we report stable transformation and expression of two foreign protein, green fluorescent protein and flounder growth hormone gene in *Chlorella ellipsoidea*. Also, it is reported the usage of Sh ble gene, a phleomycin resistance gene, as a selection marker in *Chlorella* transformation.

Matrials and Methods

Strains and culture *Chlorella epillsoidea* was provided from Korea Marine Microalgae Culture Center of Pukyong National University (Strain No. KMCC C-20). Cells were cultured without bubbling in f/2 medium under *3000 lux fluorescent lamp at* 25℃ with 18:6 h light:dark cycle.

Protoplast formation of *Chlorella* The cells were suspended in 5㎖ of 25mM phosphate buffer (pH 6.0) containing 0.6M sorbitol, 0.6M mannitol, 4%(w/v) cellulase (CALBIOCHEM, USA), 2%(w/v) Macerase (CALBIOCHEM), and 1%(w/v) pectinase

(Sigma). The cell suspension was incubated at 25℃ for 16 h in a dark shaker with gentle shaking.

Transformation of protoplasts with foreign gene Protoplasts were gently suspended with 5㎖ f/2 medium containing 0.6M sorbitol/mannitol and washed by centrifugation at 400×g for 5 min. The pellet was suspended in 1㎖ 0.6M sorbitol/mannitol with 0.05M $CaCl_2$. Then, $10^7 \sim 10^8$ protoplasts in 0.4㎖ were placed into a fresh microcentrifuge tube and 5㎍ vector DNA was added with 25㎍ calf thymus DNA as carrier (Sigma Chemicals, St. Louis, Mo.). After a 15 min incubation at room temperature, 200㎕ of PNC [0.8M NaCl, 0.05M $CaCl_2$, 40% PEG 4000 (Sigma)] was added with gentle mixing. After 30 min incubation at room temperature, 0.6㎖ f/2 medium containing 0.6M sorbitol/mannitol, 1% yeast extract and 1% glucose was added, and the cells were incubated at 25℃ for 12 h in dark. The transformed cells were transferred to fresh f/2 medium and cultured.

Results

Protoplasts were produced by treating *C. ellipsoidea* with enzyme mixture of cellulase, macerase and pectinase for 16 hrs in the presence of osmotic stabilizer. Protoplasts of *C. ellipsoidea* were transformed with a modified plant transformation vector containing the flounder growth hormone gene (fGH) under control of the cauliflower mosaic virus 35S promoter and the phleomycin resistant Sh ble gene as a selection marker under control of *Chlamydomonas* RBCS2 gene promoter. Transformants were selected in f/2 medium containing 1.0㎍/㎖ phleomycin. Chromosomal DNA extracted from the transformants was used for PCR with a growth hormone gene and a Sh ble gene specific primer sets. PCR products of expected size were detected only from the transformed *Chlorella*. The PCR products were confirmed by Southern blot analysis. Western blot analysis showed the expression of fGH protein in transformed *Chlorella*. The introduced DNA and expressed protein were detected throughout seven transfers in the medium not containing phleomycin. There was no difference of the growth of transformed and non-transformed *Chlorella*. The fGH expressed in transformed *Chlorella* was measured by immmunoassay, and values for expressed fGH of about 0.4~0.5㎍/㎖ were obtained. *In vivo* test, fGH expressed in transformed *Chlorella* was biologically active.

References

Dawson, H. N., Burlingame, R., and Cannons, A. C. (1997). *Current Microbiology* 35, 356-362.
Jarvis, E. E., and Brown, L. M. (1991). *Current Genetics* 19, 317-321.