P74

Effects of Osmotic Pressure on Production of Recombinant Human Granulocyte-macrophage Colony Stimulating Factor Production in Plant Cell Suspension Culture

Jin-Ok Lee[1*], Dong-Geun Lee[1], Jae-Hwa Lee[1], Moon-Sik Yang[2]

Department of Bioscience and Biotechnology, Gwaebop-dong 617-736, Busan

[1]Division of Biolosical Sciences, Chonbuk National University

[2]Dukjin-dong 664-14, Jeonju, Cheonbuk 561-756, Korea

Objectives

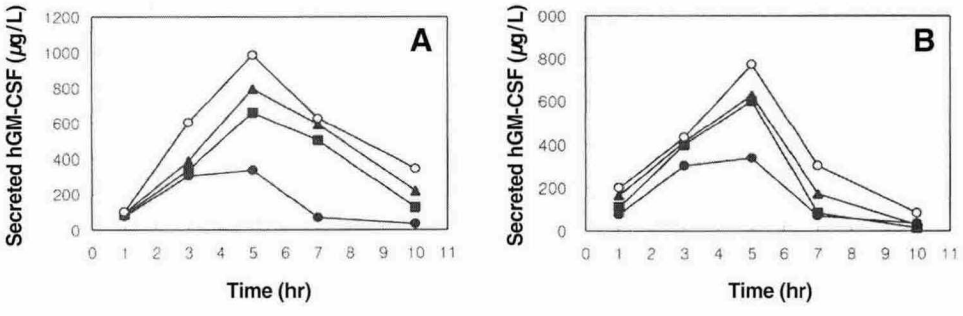
To promote the secretion of hGM-CSF from suspension culture of tobacco cell, osmotic shock was applied.

Materials and Methods

1. Materials: Tobacco (*Nicotiana tabacum* L. cv Havana SR), which was transformed with *Agrobacterium tumefaciens* LBA4404 harboring the hGM-CSF gene, was used.
2. Methods: For osmotic shock condition, cells were incubated in medium containing 30 g/L sucrose and various concentrations of mannitol (30, 60, 90 g/L). Another osmotic agent, sodium chloride (50, 100, 150 mM) was added in the equivalent of the osmolarity of mannitol (30, 60, 90 g/L, respectively).

Results and Discussion

The extracellular hGM-CSF concentration increased dramatically with the addition of mannitol. (A) Increasing the mannitol concentration to 90 g/L resulted in a maximum hGM-CSF concentration of 980.1 g/L at day 5 (2.9-fold higher than that under the normal culture condition. Moreover, the addition of mannitol (90 g/L) resulted in a maximum ratio of hGM-CSF/DCW (306.3 g/g) and hGM-CSF/secreted total protein (2.63%) at day 5 (6.8%, 4-fold, respectively higher than that under the normal culture condition). As mannitol added to culture medium, produced hGM-CSF was enriched by 4-fold. (30(■), 60(▲), 90(○) g/L, control(●)) (B) The addition of NaCl enhanced the secretion of hGM-CSF. Maximum hGM-CSF production of 776.2 g/L was achieved under the culture condition with 150 mM NaCl at day 5. (50(■), 100(▲), 150(○) mM, control (●)) Eventually, we demonstrated that hGM-CSF production was influenced by osmotic pressure generated by mannitol and NaCl during tobacco suspension culture.



*Corresponding author. Tel 051-309-5748 E-mail jhalee@silla.ac.kr