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Cultivation of Transgenic *Nicotiana tabacum* Suspension Cells in Bioreactors for the Production of mGM-CSF

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Abstract Transgenic *Nicotiana tabacum* cells were cultivated for the production of murine granulocyte macrophage–colony stimulating factor (mGM-CSF) in both a stirred tank bioreactor and an airlift bioreactor with draft tube. Cell growth and mGM-CSF production in the airlift bioreactor were found to be better than those achieved in the stirred tank bioreactor. In the airlift bioreactor, 9.0 g/L of cells and 2.2 ng/mL of mGM-CSF were obtained (11.0 g/L and 2.4 ng/mL, respectively in shake flasks). Although the lag period was prolonged and mGM-CSF production was lowered by 33% in the stirred tank bioreactor as compared to the control culture, the maximum cell density was increased up to 12.0 g/L due to better mixing by agitation at the higher cell density.

Keywords: bioreactor, foreign protein, mGM-CSF, plant cell culture, transgenic plant

Recombinant DNA technology has enabled plant cells to become a promising host system for the expression of foreign protein [1]. For the production of foreign protein, plant cells may be more advantageous due to the ease of scale-up, low cost of the medium, feasibility of a high-density culture, more proper glycosylation, and simplified purification in downstream processing [2]. Many examples of foreign protein production by plant cell suspension cultures, such as monoclonal antibody fragment, interleukin-2 (IL-2), interleukin-4 (IL-4), β-glucuronidase (GUS), and murine granulocyte macrophage–colony stimulating factor (mGM-CSF), have recently been published [3-6]. However, the commercialization of foreign protein production by plant cell cultures is not reported yet. For successful commercialization, enhancement of the production level is a crucial factor [7]. Environmental conditions also significantly influence the productivity of foreign protein in plant cell suspension cultures [8]. It is generally known that the productivity of phytochemicals in bioreactors is lower than that achieved in shake flasks [9]. Therefore, scale-up studies and the optimization of the bioreactor operation are indispensable.

In this study, the cultivation of transgenic *Nicotiana tabacum* cells in both a stirred tank bioreactor and an airlift bioreactor was investigated for the efficient production of a foreign protein, mGM-CSF.

The transgenic *Nicotiana tabacum* was kindly donated by the TS Corporation R&D center (Incheon, Korea) and maintained in a modified Murashige and Skoog (MS) medium containing 30 g/L of sucrose, 0.1 g/L of myo-inositol, 2 mg/L of 2,4-dichlorophenoxyacetic acid, and 0.02 mg/L of kinetin. For continuous selection of transgenic cells, 100 mg/L of kanamycin prepared by filter-sterilization was added after autoclaving. The pH of the medium was adjusted to 5.9. Cell suspension was maintained in 500-mL Erlenmeyer flasks with 180 mL of fresh medium on a gyratory shaking incubator operated at 120 rpm under dark conditions and a culture tem-perature of 25°C. Every week, 70 mL of cell suspension was transferred to fresh medium.

Cell cultures were performed in a 5-L stirred tank bioreactor and a 3-L airlift bioreactor (Kobiotech Co., Incheon, Korea) with 2 L of working volume. In the stirred tank bioreactor, the agitation and aeration rates were fixed at 80 rpm and 0.2 vvm, respectively. In the airlift bioreactor, the aeration rate was increased up to 0.4 vvm according to the increase of cell mass. Suspension cells cultured in flasks for 7 days were used as an inoculum.

For dry cell weight measurement, the cell suspension was filtered through Whatman No. 1 filter paper under vacuum and washed three times with distilled water to remove residual sugar on the cell surface. The cells were then transferred to a pre-weighed aluminum tray and after drying at 60°C for 2 days the dry cell weight was measured.

In Fig. 1, the cell growth in the shake flasks and in both types of bioreactors is demonstrated. Cell density in the shake flasks reached 11.0 g/L at the 8th day. The maximum cell densities obtained in the airlift bioreactor and in the stirred tank bioreactor were 9.0 and 12.0

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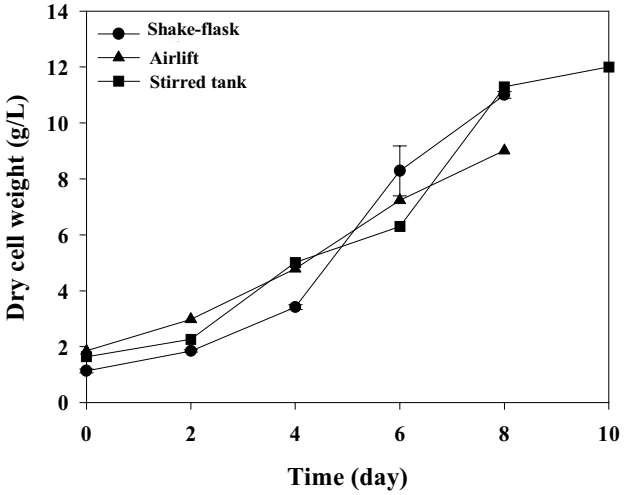

Fig. 1. Time course changes of cell growth in shake-flask, airlift bioreactor, and stirred tank bioreactor.

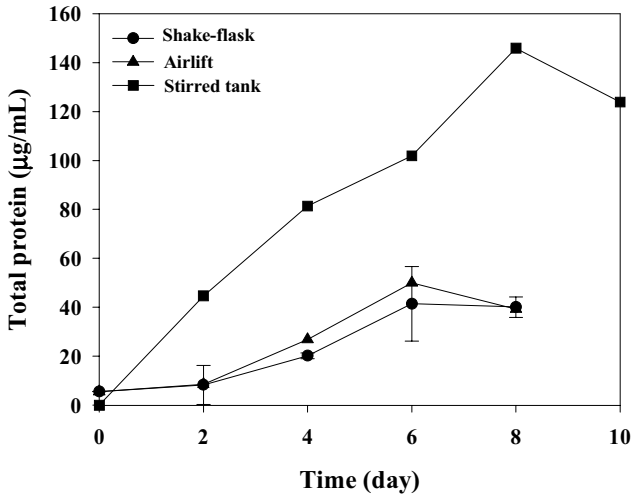

Fig. 2. Profile of extracellular protein concentration.

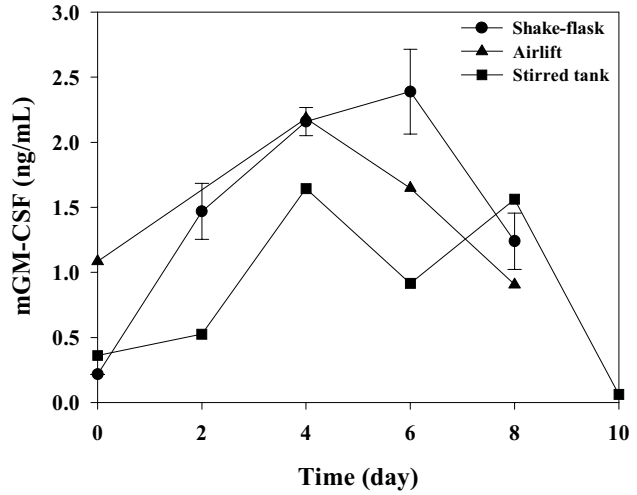

Fig. 3. Production of mGM-CSF in shake-flask, airlift bioreactor, and stirred tank bioreactor.

g/L, respectively. It was observed that lag period was prolonged in the stirred tank bioreactor due to the shear caused by mechanical agitation, although cell growth was quickly recovered following the lag period and maintained for up to 10 days. In addition, the cell density in the airlift bioreactor was observed to be lower than that seen in the stirred tank bioreactor due to the poor mixing at a high cell density.

The total protein concentration in medium is shown in Fig. 2 as a possible indicator of cell lysis. Interestingly, the protein concentration in the stirred tank bioreactor was 145.9 μg/mL at the 8th day, which was much higher than that seen in the shake flasks as well as airlift bioreactor. The high protein concentration may be due to the cell lysis caused by the mechanical agitation, which promotes the excretion of intracellular protein into the medium. In terms of protein concentration in the medium, the pattern of change in the airlift bioreactor was somewhat similar to that in the shake flasks. This result may be due to the mild culture condition in the airlift bioreactor as compared to the stirred tank environment.

The production of mGM-CSF was also affected by the type of culture vessel as shown in Fig. 3. In shake flasks, the mGM-CSF concentration continuously increased to 2.4 ng/mL until the 6th day of culture and then a rapid drop was observed. This decrease may be caused by the degradation of secreted mGM-CSF. As with cell growth and extracellular protein level, the mGM-CSF production in the airlift bioreactor showed a similar pattern to that seen in the flasks. The maximum mGM-CSF concentration was 2.2 ng/mL (at the 4th day). However, the production of mGM-CSF in the stirred tank bioreactor was much lower in spite of a higher cell mass. The maximum concentration obtained in the stirred tank was 1.6 ng/mL, which was a 33% decrease as compared to the value achieved in the shake flasks. After reaching maximum values, a decrease of mGM-CSF concentration was observed in all cases. Since it is thought that the decrease originated from the degradation, stabilization of the secreted protein is necessary for the enhancement of productivity. Gelatin or polyvinylpyrolidone (PVP) could act as a stabilizing agent and it has been reported to increase the productivity of heavy chains of monoclonal antibodies by transgenic plant suspension cells [10,11]. The secretion of product could simplify the recovery process and lower the cost of the purification step [2]. Therefore, culture conditions for facilitating product secretion and stabilizing excreted protein should be further investigated.

In terms of the volumetric productivity of mGM-CSF, the flask culture was superior, the airlift bioreactor second, and the stirred tank bioreactor third (Fig. 4). From this result it was apparent that the airlift bioreactor provided a more moderate condition for cell growth due to the fact that there was no significant shear caused by agitation.

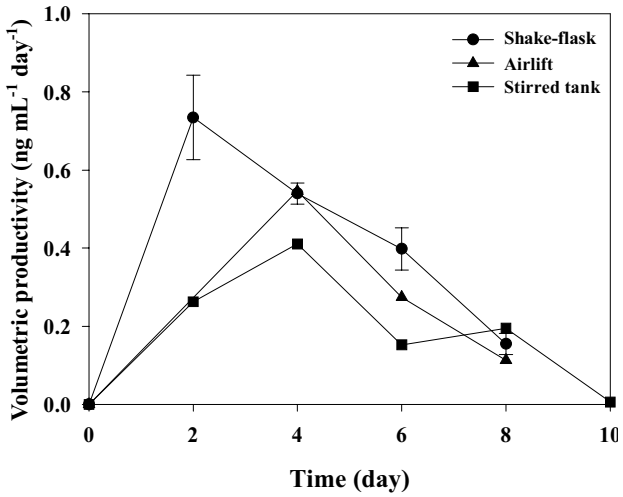

Fig. 4. Effects of bioreactor types on volumetric productivity of mGM-CSF during the culture period.

In conclusion, cell growth and mGM-CSF production using an airlift bioreactor showed patterns similar to those in culture flasks due to the mild culture condition. However, it was difficult to obtain a high cell density because of the mixing problem. To improve the productivity, a high-density culture should be considered [12]. For high-density culture, stirred tank bioreactor would be better. In addition, further study of the stabilization of secreted protein product is necessary.

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