**Addition of Sericin, cell-activating factor, together with Carbon Sources into Mammalian Cell Culture for Improving its Biologics Productivity.**

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**Highlights**

* Sericin induces the proliferation of the cells under glucose-limitation condition.
* Sericin improves cell survival under glucose-limitation condition.
* Glucose-limitation increases monoclonal antibody productivity.
* Sericin further increases antibody productivity of the cells under glucose-limitation.

**1. Introduction**

In recent years, biologics production is increasing. Among the biologics, protein pharmaceuticals such as erythropoietin and antibodies are produced by mammalian cell culture. For the productions, expensive equipment and media are required and so the cost is too expensive.

In this study, we focused on cellular glucose metabolism for reducing the cost of culture medium. Although carbon sources including glucose are pivotal in the cell culture, large part of carbon sources in the medium is not efficiently consumed by the cells. Most of culture media contain abundant amount of glucose and so the cells tend to consume glucose and to produce lactate. But soon glucose concentration decreases and the cells cannot get glucose adequately. Thus, at the first stage of the culture, medium contains excessive amount of glucose that induces cells consume glucose uselessly, and it results in wasteful consumption of glucose and inefficient cell culture.

In this study, to avoid consuming nutrition wastfully, medium was diluted with PBS and at day 2 or day3, condensed medium was added. Or the culture was started with lowered glucose concentration, and then fed with glucose. Further, in order to prevent the starved cells from death and to activate the cells, sericin hydrolysate obtained from silk was added into the diluted or glucose-limiting culture. Sericin hydrolysate is potent culture supplement.

**2. Methods**

Murine hybridoma cells producing anti-TNP monoclonal antibody were used. Commercially available serum-free medium ASF 104 (Ajinomoto, Japan) was used for dilution culture using by phosphate buffered saline (PBS). For glucose limitation culture, we used RPMI1640 medium (Wako, Japan). RPMI medium can be purchased with and without glucose, and so we easily prepared medium containing glucose at different concentration.

Silk protein sericin hydrolysate (Wako) and pyruvate was also added to the culture. Proliferation and antibody production of the cells were measured and the culture conditions were evaluated to find optimal condition.

**3. Results and discussion**

Dilution of ASF104 medium by half largely decreased the maximum cell density, but antibody production was slightly decreased. Antibody concentrations were similar, indicating that the yield of antibody production per medium powder weight was doubled (Figure 1). In addition, sericin improved the viability of the starved cells.

Limitation of glucose in RPMI1640 medium affected both cell proliferation and antibody production. On the other hand, addition of sericin hydrolysate improved glucose-limitation culture. In the presence of sericin hydrolysate, glucose concentration did not affect the cell proliferation (Figure 2).

 **Figure 1.** Antibody productivity.

**Figure 2.** IVC between day 0 - 4.

Gluc. conc. (g/L)

Sericin － ＋

− － ＋

+ － ＋

**4. Conclusions**

Glucose- or nutrients- limitation culture are useful in cell culture. But the limitation often induce cell death and so decrease the productivity. Addition of sericin hydrolysate activates the starved cells. In this way, culture supplements such as sericin are potent tools for mammalian cell culture.