High-quality protein production in plant cell culture systems

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BASEL – Plant cell cultures have been shown to be an advantageous system for producing high quality human proteins for medical and industrial uses. Newly, scientists in Korea succeded in expressing high-purity human interleukin-12 protein in such a cell culture system.

Recently, Dr. Moon-Sik Yang and his team developed a method to express active human interleukin-12 (IL-12) in plant cell cultures. With their experiments the researchers demonstrated the effectiveness of plant cell cultures for producing small-to-medium quantities of high-quality proteins despit the differences that exist between plant and human cells.

“So far, animal cell culture systems have been used for production of recombinant therapeutic proteins.” sais Dr. Yang. However, with his new patented method the disadvantages of working with animal cell culture systems are overcome.

Dr. Yang explains that: “First, recombinant proteins produced by transformed plant cells are more likely to be safe for human consumption, because plant pathogens, such as fungi and viruses, are easily monitored and usually not pathogenic to humans. Second, sexual crossing can generate multiple transgenic plants. Third, the ease of downstream purification and the low cost of plant culture media, which contain inexpensive major components such sucrose and salt and no macromolecules, make plant cell culture an economically attractive alternative.”

In a first series of experiments the scientists used Interleukin-12 as a model protein. IL-12 is a heterodimeric cytokin naturally produced by macrophages and B-lymphocytes. The two different subunits of the heterodimeric IL-12 are encoded by the genes p35 and p40.

In the human immune system IL-12 plays an important role. It stimulates interferon-gamma production from T- and natural killer cells and, furthermore, triggers the killer cells to destroy infected cells. Since IL-12 activates the TH1 subset of CD4 cells as well, it has been shown to be a promising treatement for HIV disease progression.

With Dr. Yang’s invention pharmaceutical companies receive a new alternative to inexpensively produce and sell such therapeutical products.

To intdroduce the IL-12-genes into the plant cells, the researchers used the following method: Two recombinant vectors containing the genes for the two subunits were introduced into Agrobacterium tumefaciens, that subsequently was used to transform the tobacco plant, Nicotiana tabacum. Each plant expressing the highest level of corresponding IL-12 subunit RNA was cross-pollinated to obtain plants expressing both subunits. These plants were then used to make IL-12 producing plant cell cultures.

The high level of IL-12 secretion into the culture medium demonstrates that genes of animal origin can be expressed quite well in plant cell culture systems. However, after a while the proteases, natural enzymes that degrade proteins, start to break down the IL-12 proteins.

Seeking to fine tune the system, Dr. Yang and his team noticed that the addition of a small amount of gelatine to the medium resulted in an higher IL-12 level. This effect is due to the fact that the proteases spend more time degrading the gelatin proteins and less time breaking down IL-12. Unfortunately, the addition of gelatin makes the purification step more difficult and expensive, so Dr. Yang focused his search on other possible solutions.

Eventually, Dr. Yang's team landed upon using a stronger promoter system that increased the level of protein made by the plant cells. In this context, the team worked with GM-CSF, another protein of medical interest.

Commenting on the improved system, Dr. Yang said: "So far we found some promoters such as Amylase 3D and Amylase 3E which can produce reconbinant human GM-CSF as much as 150mg/L, which is equal to, or surpass, the yield of animal cell culture (High level of expression of recombinant human GM-CSF in transgenic rice cell suspension culture. Biotechnology and Bioengineering. In press). At the same time, we are trying to find other strong promoters working when degradation of recombinant proteins synthesized is minimazed."

Since plant cell suspension systems are in many ways beneficial, the technology has already been transferred to pharmaceutical companies for commercialization. "In term of technolgy, it is alredy set-up. It will take some time to get approval from the goverment for medical use. However, reconbinant proteins produced in plant cell culture system will be used for non-medical use such as reagents and cosmetics in a couple of years." asseverated Prof Yang.