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Review

식물 조직 배양의 생물공학적 응용

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The Biotechnological Application of Plant Cell Culture Engineering

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Plant tissue culture techniques have been less utilized in the area of biotechnology except for mainly improving crops, compared to other applications of biotechnology (1-3). Commercial production of specialty chemicals such as codenine, jasmine and pyrethrines from plant cell cultures has never been achieved, even though these chemicals are well known of their food and pharmaceutical applications. Fig. 1 illustrates overall applications of plant tissue culture. Up to now only shikonin, which is used for dye and pharmaceutical and a secondary metabolite in *Lithospermum erythrorhizon* cells, is commercially produced from plant tissue cultures by Mitsui Petrochemical Industries Ltd. (Japan) (6). Generally, plant secondary metabolites are commercially useful because they often have physiological affects on human and animals to combat infectious diseases. However, there have been several technical difficulties in cultivating large quantities of plant tissues. Because plant cells are different from microorganisms and mammalian cells in scaling-up processes and suspension cultures, and most pharmaceutically important products are secondary metabolites which do not play important role in physiological functions and only exist in certain locations of the plant cell (7,8). Table 1 is the list of potential market products for plant secondary metabolites. Therefore, the selection of plant tissues to produce desirable metabolites is extremely important, and continuous production of these chemicals in *in vitro* system is also a key factor for the industrialization of plant tissue cultures. Differentiation of plant cells is also an important factor in producing secondary metabolites since the plant cells can only produce these chemicals in this phase. Conventional fermentation techniques can not regulate secondary metabolite pathways in differentiated cell cultures. In additon, slow growth rates and entrapped products within the cells are another potential problems. In this article, currently developed plant cell culture techniques will be reviewed for the commercial production of plant secondary metabolites.

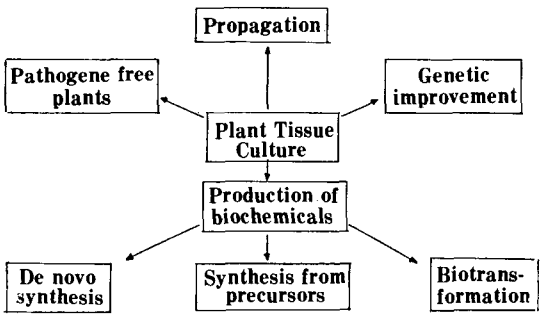

Fig. 1. Diagram of plant tissue culture applications (15).

Tissue Culture and Secondary Metabolites

It is obvious that suspension culture technique

Table 1. Markets for Plant Secondary Metabolites(9)

Compounds	Use	Price* (U.S. dollar)
Ajmalicine	circulatory problems	1500.00
Digitalis	heart disorders	3000.00
Jasmine	fragnance	5000.00
Pyrethrins	insecticide	300.00
Quinine	malaria fever	100.00

* Whole sale prices per kilo gram of each compounds.

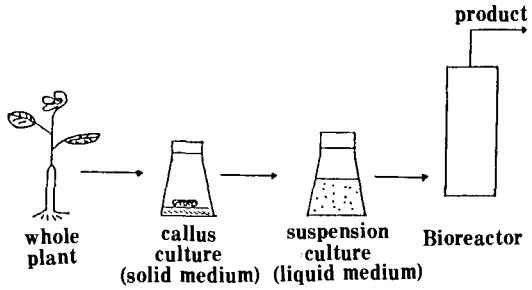

Fig. 2. Schematic diagram of establishing cell line from an intact plant.

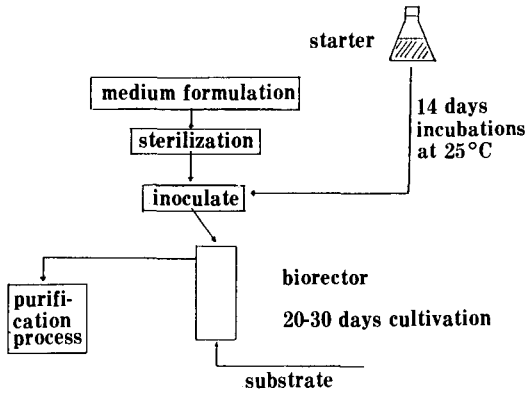

Fig. 3. The flow diagram of cultivating plant cells up to the bioreactor (26).

has advantages over the extractions of interesting compounds from agricultural products. However, it is absolutely necessary to establish cell lines capable of producing high yields of secondary compounds in suspension cultures (10). It is also demonstrated that the screening of cell strains which can produce the desired product can be applied for the industrialization (10). Fig. 2 illustrates a general procedure of establishing a cell line from whole plant. The recent development of establishing plant cell culture has been reported by following four-step strategy (11): 1 establish a suitable selection method; 2 select a plant to contain secondary products; 3 develop a growth medium; 4 select variant strains. However, the establishment of desired cell line is not the only solution to produce useful natural compounds from plant cells. Because secondary metabolites have not been directly involved in forming desired chemicals within the plant cell (8).

It would be speculated that secondary product can be produced from recombinat plant cells. Unfortunately, success is years behind due to the weak knowledge of plant gene expression, and the fact that secondary metabolites are not products of single gene (26). Therefore, plant tissue culture offers good potential for the production of secondary products since tissue cultures are more easily developed and more boradly applicable than genetic engineering techniques (12,13). One of ways to improve secondary metabolite productions is the direct production of chemicals from the culture. It is apparent that useful compounds in culture broth have advantages over those obtained by conventional plantations (14). Supplies would be reliable, the quality of products would be uniform, and in that case where the plant is rare or difficult to grow the supply could meet the demand with higher profit.

Cell Culture Technology

As described above, the suspension culture has obvious merit in maintaining large amounts of plant cells and producing secondary metabolites. However, it must be stressed to consider several important factors in scaling-up cell cultures to suspension bioreactors; they will be media composition, biochemical engineering parameters and reactor configurations, etc.

Besides developing productive cell lines, the task of designing appropriate media is also important. Media used for plant tissue culture are less complicated and expensive than those used for animal cell cultures, but formulating the optimal medium for a particular application seems more or less to depend on the luck than scientific approach. That is, defining the best medium for a special purpose can be achieved by a trial-and-error method. Fujita *et al.* developed their own medium by modifying LS and White media, which eventually increased the productivity almost twelve times.

Then, the scale-up of tissue culture to pilot plant level is next task to be accomplished. Fig. 3 shows common sequences of scaling-up cell cultures to the bioreactor. Unlike animal cells, plant cells are easily grown as suspension culture because they are not anchorage-dependent. Mixing is the most critical factor in maintaining high density of plant cells in the bioreactor, since plant cells are extremely sensitive to shear stress due to fragile cell wall and they tend to stick together to form large buoyant aggre-

gates. Therefore, delicate mixing, to break cells and effectively supply nutrients, must be employed. Methods to overcome these problems have been developed. Tanaka introduced several types of bioreactors to efficiently supply oxygen and nutrients with less shear stress (16) by sparging air bubbles from the bottom of the reactor. It is an effective method in transporting oxygen, but it can also generate many foams which is a source of contaminations and his system requires much power to agitate the media.

Therefore, air-lift type bioreactor would be a suitable method for plant cell cultures because this reactor employs the characteristics of fluidized bed; the culture is well mixed by the action of air bubbles rising into the reactor and aggregates are less formed because of less space of sticking together due to fluidized bed polymers. Therefore, efficient oxygen transfer with least shear forces can be achieved by this type of the reactor. Immobilized cell culture technique is also one of possible alternatives in growing plant cells, whose method can be carried out by physical entrapment of plant cells inside the matrix (17,18). This method has several advantages; easy control of metabolic pathway (19), less shear stress and contaminations by least formations of aggregates (20), and high yields of secondary products because of continuous flow system (17). However, this technique has also a disadvatage that the most products are stored intracellurly in vacoules. It must be accomplished to effectively release desired products out of the vacoules. Recently, techniques to release these metabolites from the vacoules have been improved by changing pH and using permeabilizing ageants (21).

Differentiation of plant cells may be another technical problem in producing valuable drugs. Cells from plants simply can not make products even though all the genes found in the plant are pressent in cultivated cells (22). Because cells do not metabolize as they would in the plant. Therefore, it is required to find the keys for regulations of secondary metabolisms in completely differentiated cultures. Currently developed techniques have solved these problems by the induction of cell differentiations using precursors or the accumulation of extractable forms of secondary metabolites in the plant cells (23). Moreover, the automated plant cell culture system is developed for mass propagation (27).

The Futher of Plant Cell Culture

Plant tissue cultures have great potentials for producing pharmaceutically active drugs and chemicals by cultivating plant cells through industrial scale reactors. Because this technique can significantly reduce plantation areas by employing suspension cultures and process time to obtain valuable products, regardless of the fluctuations of weather, season and politics. It is believed that plant secondary metabolites are highly profitable products by the results of economic asssesment of current technology and increasing demand. For example, one kilo gram of ajmalicine which is used for circulatory problems runs about $ 3214.00 (U.S. dollar) (25). It is a good evidence why Japan and European countries have pushed this technology to the commercialization for two decades: The Japan Salt & Tobacco Public Corporation (JS&T) to develop 20,000 liter reactor, Kibun Corp., Meiji Seika and Nippon Shinyaku Co. s (Japan) to expand R&D activities in plant tissue culture, Boehringer Mannheim and Gesellschaft fur Biotechnology Forshung mbH (GBF) (West German) to actively involve in plant tissue cultures, and Plant Science Ltd. (Sheffield, UK) to establish Wolfson Institute for plant cell technology.

It should also be emphasized that current status of plant cell culture techniques is very primitive and needs to be improved for the commercial production of valuable products. However, with more of a systematic long-term commitment on plant cell culture techniques, the future of plant tissue culture will be promising

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