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CURRENT APPROACHES IN IN-VITRO PRODUCTION OF SECONDARY METABOLITES FROM MEDICINAL PLANTS

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ABSTRACT

Plants are a vital source for producing drugs of medicinal value. Today many unique chemicals which are extracted from plants are important drugs, that are currently used in many countries across the globe. Majority of the drugs we see today are simple synthetic modifications or artificial copies of the naturally obtained substances. As of today, pharmaceutically significant secondary metabolites are isolated from wild or cultivated plants because their production is not feasible be it economically or in sense of the efforts needed for it. The ever growing commercial importance of secondary metabolites in recent years has lead to a great interest in secondary metabolism, particularly in the possibility of using Plant tissue culture technology,which was put forth in the latter half of the 20th century for research purposes. Different strategies, especially the idea of using an in-vitro system, has been extensively studied to improve the production of plant chemicals and secondary metabolites. In this paper, we will focus upon and review different processes to obtain some of the secondary metabolites from medicinal plant tissue/cell cultures.

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INTRODUCTION

Medicinal plants are undoubtedly the most exclusive and important source of drugs for most of the world's population. Many higher plants are major sources of natural products used as pharmaceuticals, agrochemicals, flavour and fragrance ingredients, food additives, and pesticides. In order to find alternatives to produce various desirable medicinal compounds from plants, various approaches, specifically PTC (plant tissue cultures), has many potential options as a supplement to traditional agriculture in the industrial production of plant metabolites. The utilization of plant cells for the production of natural and recombinant compounds of various commercial interest has gained a lot of attention over past years. These secondary metabolites play a major role in the adaptation of plants to their respective environment and also act as an important source in pharmaceuticals.

PTC represents a source of essential secondary metabolites that is an option to be used as food additives and pharmaceuticals. The synthesis of phytochemicals by the cell cultures in contrast to those in plants is independent of environmental conditions and quality fluctuations. Many a times, the chemical synthesis of metabolites becomes difficult due to many reasons, especially the financial aspect. Moreover, the artificial food additives are not readily accepted by the consumers when compared to natural food additives. There are various advantages of a cell culture system when compared to the conventional cultivation of whole plants like the useful compounds which can be produced under proper, well-maintained and suitable conditions independent of climatic changes. The cultured cells should have no participation of microbes and insects. The cells of any plants, can be easily multiplied to yield specific metabolites with the help of automated control of cell growth that would reduce of cost of labour and thus improve the productivity of organic substances that can be extracted from callus cultures.

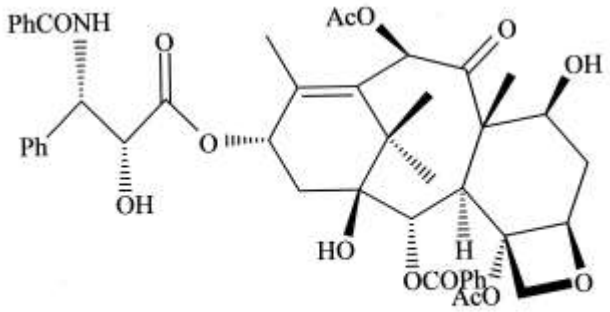
Due to the recent advances, research in the areas of tissue culture technology for production have bloomed immensely beyond expectations. The combined research in various fields of establishment of in-vitro cultures, development of technologies for product recovery and targeting the process of metabolite synthesis can exploit the potential of the plant cells as a source of secondary metabolites. Major important findings have been reported for a huge variety of medicinally valuable substances, some of which could be produced on an industrial scale in few years.

Principal Review

Tissue Cultures Producing Pharmaceutical Products of Interest with the immense research work done in the area of plant tissue culture, the production of various pharmaceutical substances for new therapeutics is possible in large quantities. The pharmaceuticals like alkaloids, phenolics, steroids, saponins, terpenoids, and amino acids can be produced in desired quantities by cell cultures. Successful attempts to produce some of these precious pharmaceuticals are illustrated.

Taxol

Taxol (plaxitaxol), which is found in the bark of the Taxus tree is a complex diterpene alkaloid. It is used as an anticancer agent and is considered one of the most promising known because of its unique mode of action on the microtubular cell system. Researches are still going on to find better methods of production of taxol various Taxus species cells in cultures because of its high commercial value and the



Taxol

scarcity of the Taxus tree.

In 1989, Christen et al. were the first to report the production of taxol (paclitaxel) by Taxus cell cultures. Fett-Neto et al. (1995) did a study on nutrients and other factors on paclitaxel production by T. cuspidata cell cultures and got a 0.02% yield on a dry weight basis.

Diosgenin

Diosgenin is very important to the pharmaceutical industry as it is a precursor for the chemical synthesis of steroidal drugs. In 1983, Tal et al. use the cell cultures of Dioscorea deltoidea to produce diosgenin. They found that nitrogen and carbon levels considerably influenced diosgenin accumulation in one cell line.

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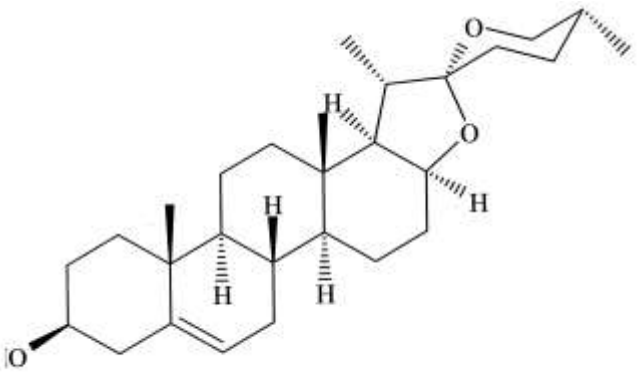


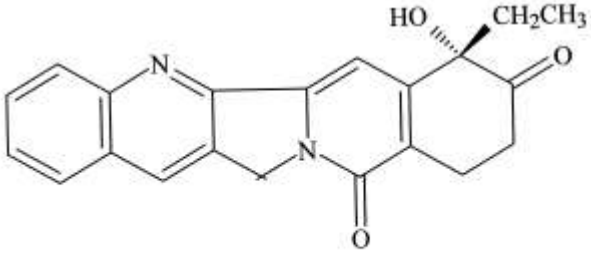
Diosgenin

Camptothecin

Camptothecin is a potent antitumor alkaloid. It was isolated from Camptotheca acuminata. 10-Hydroxycamptothecin is a promising derivative of camptothecin and it is in clinical trials in the US.
Sakato and Misawa (1974) produced camptothecin at about 0.0025% on a dry weight basis by inducing C. acuminata callus on MS medium that contained 0.2 mg/l 2,4-D and 1 mg/l kinetin. They developed liquid cultures in the presence of L-tryptophan, gibberellin, and conditioned medium.

Studies on In Vitro Cultures and Production of Important Secondary Metabolites in a Laboratory:

There are several types of cell culture methods that are used for producing the important bioactive secondary metabolites, mostly cell suspension cultures are preferred for industry-scale production



Camptothecine

because of their rapid growth cycles. Therefore, cell suspensions are used to generate large amounts of cells for qualitative or quantitative analysis of metabolism and growth responses of novel chemicals. Based on the great results in the production of medical compounds which were reported above by using cell suspension cultures, this method was successfully used for producing taxol from Taxus mairei, imperatorin from Angelica dahurica, and diosgenin from Dioscorea doryophora along with Diosgenin from Dioscorea doryophora in the lab. The work which was carried out at the lab is summarized below.

Taxol Production from *Taxus mairei* by Cell Suspension Cultures

Taxol is a complex diterpene alkaloid. It is an anticancer drug that was found in 1971, by Wani et al. It was found from the Pacific yew tree, *Taxus brevifolia.* At present this drug is approved for the clinical treatment of breast and ovarian cancer by the FDA, USA. It is also effective against lung cancer, malignant melanoma, and other solid tumors. However, its supply is limited as it depends on extraction from the bark of yew trees. On a dry weight basis, the thin bark of the yew tree has only 0.001% taxol. A century-old tree yields 3kgs of bark on an average, which gives around 300 mg of taxol which is just a single dose in the cancer treatment course. Because of its scarcity of slow-growing trees and relative slow taxol content there raised a need to look for alternate sources or methods to meet the increasing demand for the drug. The industrial-scale production of this drug seemed impossible because of the complexity in the chemical structure of this molecule. The plant cell culture of *Taxus* spp. is considered to be one of the possible approaches for providing a stable supply of taxol and other related taxane compounds.

To exploit the source of taxol, different tissues of *Taxus mairei* were used. *Taxus mairei* is a species found in Taiwan at 2,000 m above sea level. The extracts of leaf and bark tissues were analyzed using HPLC to find the content of taxol and taxol-related compounds. It was found that the amounts of taxol and taxol-related compounds vary in individual plants and the principal components in leaf extract were higher than those in bark extracts such as baccatin III, docetaxel, and 10- deacetylbaccatin. *Taxus mairei* calli were induced from the stem and needle explants on Gamborg's B5 medium which was supplemented with 2 mg/l NAA or 2,4-D. Different cell lines were established using a needle and stem-derived callus. After 6 weeks of incubation and the precursor feeding, one of the cell lines produced 200 mg taxol per liter of cell suspension culture.

Diosgenin Production from *Dioscorea doryophora* by Cell Suspension Culture:

In Chinese traditional medicine, Dioscorea spp. (Dioscoreaceae) are frequently used as a tonic. *Dioscorea doryophora* Hance tubers have high demand as they are used as crude drugs and food. The most active ingredient which was discovered in the tuber was diosgenin. It can be used as a precursor for a lot of important medicinal steroids, like prednisolone, norethisterone, dexamethasone, and methenolone,

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For increasing the diosgenin yield and facilitating the purification process, a cell suspension culture of *Dioscorea doryophora* Hance was established. Cell suspension cultures were acquired from stem node and micro tuber derived callus in liquid culture medium supplemented with 0.1 mg/l 2,4-D, 3% sucrose and was incubated on a rotary shaker at 120 rpm. Although for the growth of cell suspension culture, 6% sucrose was found to be optimum, cells cultured in a 3% sucrose medium were observed to produce more diosgenin. HPLC analysis revealed that both microtuber and stem-node derived suspension cells had diosgenin. The micro tuber derived cell suspension culture had 3.2% diosgenin per gram dry weight while the stem-node derived cultures had only 0.3%. As the amount of diosgenin obtained from a tuber-derived cell suspension is high and comparable with that found in the intact tuber, a cell suspension culture can be utilized for the production of diosgenin.

CONCLUSIONS & FUTURE PERSPECTIVES

Recent advances in biological science, specifically methods for culturing plant cells culture provides new means for the commercial processing of rare plants and the chemicals they lay out. Basically the pros of such methods is that it can eventually provide an uninterrupted , reliable source of standard products . Also the important advantages of cell cultures include synthesis of bioactive secondary metabolites , running in controlled environment, independently from climate and soil conditions.

In recent years ,the application of plant cell culture is due to an improved understanding of the secondary metabolite pathway . Also in our review paper we have basically understood the nature of plant cells in in vitro cultures, case by case studies has been used to describe the problems occurring in the production of secondary metabolites from plant cell culture. Within last few years , considerable progress has been improving in secondary metabolite production from cultured plant cell. Such new technologies will supply to extend and to continue usefulness of higher plants as sustainable sources of chemicals, especially medicinal compounds.

In future , we wish that intensification efforts will be made in this field and thus would lead to successful biotechnological production of particular, valuable , and would also yet to discover unknown plant chemical.

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