Secondary Metabolites: A Review of Strategies used in the Synthesis of Secondary Metabolites

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Abstract: Secondary metabolites are the compounds synthesized by plants which are important in providing defense to the plants and also enhance their medicinal value. As the endogenous levels of these metabolites in plants are low, the present day research focusses on strategies for enhancement of these compounds. Biotransformation is a useful method which refers to chemical reactions which are catalyzed by cells or enzymes. This method can be used in plant cell and tissue culture systems in which different explants can be used to produce compounds which have a commercial potential. This method helps in production of secondary metabolites from plant sources. This review focusses on the different strategies which are used for synthesis of secondary metabolites.

Keywords: Biotransformation, secondary metabolites, agar cultures, agitated cultures, hairy roots.

Introduction:

Plants synthesize metabolites by primary metabolism and secondary metabolism. The primary metabolites are carbohydrates, amino acids, lipids etc. These are utilized by the plants for their growth and development. The secondary metabolites are the compounds involved in the protection of plants against various abiotic and biotic stresses[1]. Secondary products are synthesized from

primary metabolites. Secondary metabolites are sources for food additives, flavours, pharmaceuticals and industrially important pharmaceuticals[2,3]. In normal metabolic conditions the synthesis of secondary plant products is often low. The concentrations of various secondary plant products are strongly dependent on the growing conditions and physiology through altering the metabolic pathways responsible for the accumulation of the related natural products[4].

Biotransformation: Biotransformation in plants is the process in which the secondary metabolites are converted into a newly metabolite which is of more use than the former one[5]. The new compounds formed through biotransformation are considered as the modified compounds with improved or advanced molecular structure which consists of high stereo- and region-selectivity[6]. Secondary Metabolites are chemical compounds synthesized by the plants which do not directly aid in the growth and development of plants[7]. Biotransformation can be enzymatic or non-enzymatic conversion from one secondary metabolite to another novel and useful metabolite. The enzymatic reaction is further classified into microsomal and non-microsomal reactions[8].

Enzymatic Reactions

- Microsomal Reactions
- Non-Microsomal Reactions

Non-enzymatic Reactions

The most common enzymatic reactions taking place in biotransformation are Hydroxylation, Oxidation, Glycosylation, Alkylation, *O*-, *N*-dealkylation and Carbon-carbon fission[9]. The biotransformation capacity of the cell culture depends on pH, elicitation, permeabilization and substrate uptake and product release[10]. Any changes in the above factors can cause a change in the whole process so maintaining accurate and appropriate conditions is a must. Biotransformation provides a suitable environment to the *in vitro* cells, which allow the accumulation of secondary metabolites[11].

There are several advantages of biotransformation such as[12]

1. More than one reaction can be used in a single process of cell culturing.
2. The process of biotransformation is simple and efficient.
3. Non-useful compounds can be converted to compounds which are very important and can also be used as precursors.
4. Chemical and natural, both the compounds can be used as substrate.

Biotransformation has applications in the degradation of xenobiotics. The detoxification mechanism is usually done by glycosylation which often leads to the degradation of xenobiotics and the accumulation of different important metabolites and also some water soluble by-products[13].Biotransformation is also used in the transformation of several synthetic and toxic chemicals, some solutes and solvents, organic and inorganic compounds, natural and synthetic chemical compounds, steroids and non-steroidal compounds, sterols, pesticides and herbicides, antibiotics and different kinds of pollutants[14]. Some common substrates for the biotransformation are various phenols, alkaloids, steroids, coumarins, terpenoids and cardenolides[15]. Biotransformation is a very efficient process and is also environment friendly so, it is widely used for the accumulation of secondary metabolites[16]. Biotransformation has also been used to study the bioaccumulation potential and toxicity of the contaminants in aquatic animals[17]. This helps in the biotransformation of harmful toxic contaminants into less toxic compounds which is not lethal to the aquatic organisms. Biotransformation has been successively used in different plant species (Table 1)

Sr. No.	**Plant species**	**Secondary Metabolite**
1.	*Schisandra chinensis* (Turcz.) Baill[18]	Hydroquinone and 4-hydroxybenzoic acid
2.	*Origanum majorana* L.[19]	Hydroquinone
3.	*Astragalus vesicarius* L.[20]	Colchicoside
4.	*Catharanthus roseus* (L.) G. Don[21]	Betulin
6.	*Nicotiana tabacum* L.[22]	Betulin
7.	*Cucurbita pepo* L. Whole pumpkin plants[23]	Tetrabromobisphenol A

8.	*Rhodiola rosea* L. Rose root callus[24]	Cinnamyl alcohol glycosides
9.	*Withania somnifera* (L.) Dunal[25]	Phenol and Flavonoid
10.	*Cannabis sativa* L.[26]	Cannabinoid synthase and Osmoprotective metabolites
11.	*Citrus paradise* Macfad[27]	Citrus Flavonoid aglycones
12.	*Scutellaria lateriflora* L.[28]	Flavonoids and Verbascoside
13.	*Hypericum perforatum* L.[29]	Flavor compounds
14.	*Onobrychis viciifolia* Scop.[30]	Phenolic compounds
15.	*Micrococcus luteus* Cohn[31]	Oleic Acid
16.	*Eryngium borgatii* Gouan var. *borgatii*.[32]	Phenolic Acid and Flavonoids
17.	*Scutellaria baicalensis* Georgi[33]	Flavonoids and Verbascoside
18.	*Scutellaria baicalensis* Georgi[34]	Phenolic Acids, Flavonoids and Phenylethanoid glycosides
19.	*Verbena officinalis* L.[34]	Phenolic Acids and Phenylethanoid glycosides
20.	*Cistusin incanus* L.[34]	Phenolic Acids, Catechins and Flavonoids

Table 1: Secondary metabolites produced by biotransformation

Strategies for Biotransformation: Biotransformation can be carried out by various strategies such as[35]

1. Biotransformation using precursor feeding
2. Biotransformation using co-culturing techniques
3. Biotransformation using Nonspecific/Exogenous molecules

Biotransformation of secondary metabolites can also be done by using various plant cells, tissues and organ cultures. Biotransformation can also be carried out with the help of intact plants. For example, Biotransformation of tetrabromobisphenol-A dimethyl ether was done with the help of

whole pumpkin plants into tetrabromobisphenol-A[36]. Biotransformation can also be carried out by hairy root transformation which has been done by various researchers. Hairy root cultures have several advantages such as low cost and the yield obtained is higher than the conventional methods[37]. For example, sapogenins were produced from hairy root cultures of *Chlorophytum borivilianum* (Safed Musli)[38]. Silymarin flavolignans, phenolics, alkaloids and flavonoids were produced from *Silybum marianum* L. through hairy root culturing[39]. Hairy roots are produced by infecting the plants with *Rhizobium rhizigenes* which is responsible for causing hairy root disease in plants[40]. Hairy roots have been known to produce secondary metabolites in plants since a very long time[41]. The hairy root culture has several advantages due to which its application is high:

1. Biomass accumulation is very high.
2. Hairy roots can be cultured easily with varying mediums so; large number of variations and factors can be tested and studied.
3. Efficiency rate is comparatively higher than other techniques.
4. Effect of various elicitors on the production of secondary metabolites can easily be studied in hairy root cultured plants.
5. The amount of secondary metabolites produced through hairy root plant is higher and can be continuously produced, compared to the natural way.

Other Strategies for secondary metabolite synthesis:

Production of Secondary Metabolites through Hairy Root Culture: Hairy root cultures have been a widely adopted method wherein hairy roots of the selected plant can be cultivated with microbes like *Rhizobium* and the biomass increase is observed. The selected plant can also be subject to hairy root culture which has the potential for enhancement in levels of econdary metabolites(Figure1).

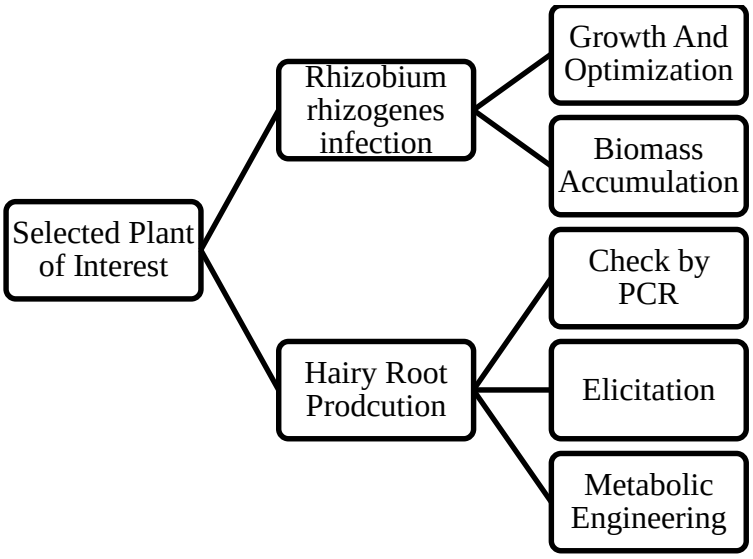

Figure 1: Production of Secondary Metabolites through Hairy Root Culture[42]

Production of Secondary Metabolites through Plant cell suspension cultures: The strategy adopted here includes an approach where the plants are acclimatized on the suspension culture media and using various elicitors, the secondary metabolites are enhanced. This method gives a good yield of industrially important compounds (Figure 2).

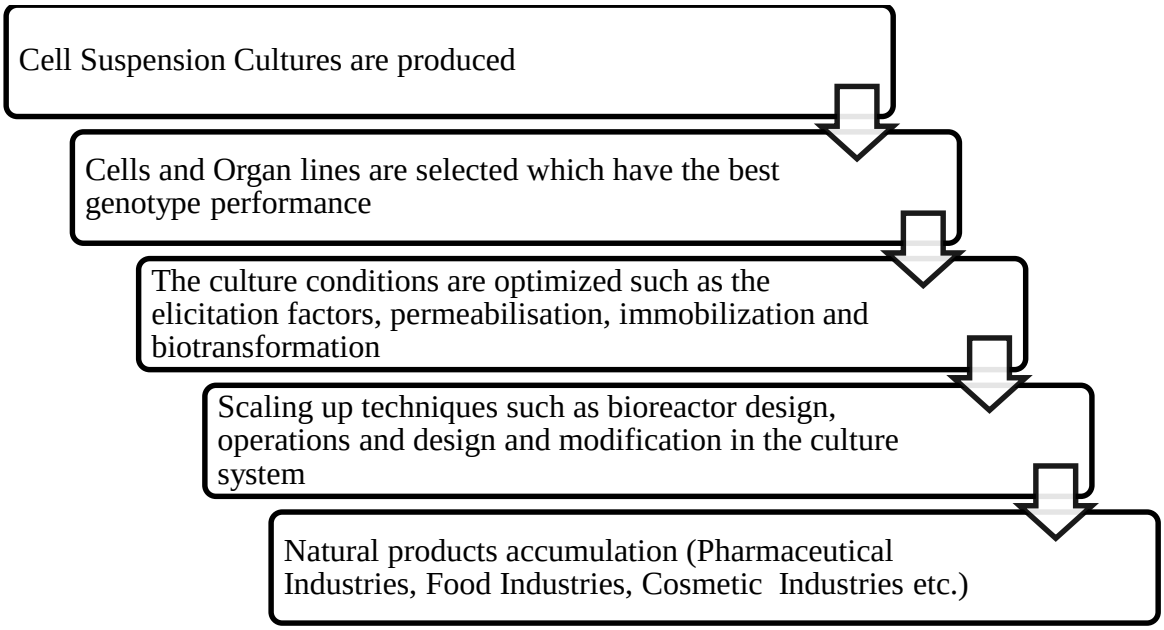

Figure 2: Production of Secondary Metabolites through Plant cell suspension cultures

Biotransformation of secondary metabolites by any of the process can lead to the discovery of various new compounds[43].

CONCLUSION:

The techniques discussed, provide a simple approach towards formation of novel compounds by introducing chemical changes which are normally not a part of their regular pathway of synthesis. Hairy root culture and suspension cultures are also a promising tool for synthesis of these metabolites. By conventional chemical methods, it is difficult to synthesize these compounds and expensive as well. These methods can be a promising tool for enhancement of many important medicinal components in plants.

FUTURE PROSPECTS:

The methods used for production of secondary metabolites can be subject to scale-up for getting good yield of metabolites. This will also help to extract useful metabolites for preparing different drug formulations with good quantities of the metabolites.

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Glossary

Precursor feeding- Addition of chemical substances or enzymes

Co-culturing- Culturing with another organism or two organisms together

Nonspecific/exogenous molecules- Externally supplied molecules

Permeabilization- The process of making a membrane/medium permeable

Xenobiotics- A chemical compound eg: a drug, found in an organism that is foreign to the organisms

Immobilization- Process of encasing the cells in some material to keep cells viable and useful for a longer duration

Microsomes- The components/structures derived from parts of endoplasmic reticulum obtained when the tissue is homogenized