A review on biotransformation

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ABSTRACT

Biotransformation means chemical alteration of chemicals such as nutrients, amino acids, toxins, and drugs in the body. It is also needed to render nonpolar compounds polar so that they are not reabsorbed in renal tubules and are excreted. Biotransformation of xenobiotics can dominate toxic kinetics and the metabolites may reach higher concentrations in organisms than their parent compounds. The conversion of molecules from one form to another within an organism often associated with change (increase, decrease, or little change) in pharmacologic activity; refers especially to drugs and other xenobiotics.

KEY WORDS: Biotransformation, Biotechnology, enzyme immobilization.

INTRODUCTION

In recent year, biotechnology has emerged as a frontier branch of science increasingly being used in several areas. Biotechnological approach is being employed to the production of secondary metabolites for pharmaceutical use. Plant biotechnology includes methods for tailoring plant resoures, plant cell and protoplast culture, manipulation of nuclear and plasmid genes, plant cell and enzyme immobilization and industrial scale production or biotransformation. The interest in the production of secondary metabolites using there techniques stems from there being commonly of high value but required in relatively small quantities Biotransformation is also a important technique for production of metabolite. Biotransformation is defined as The conversion of a small part of chemical molecule by means of biological system. The living plant may be considered as a bio-synthetic laboratory not only for the primary metabolites like sugar, amino acids but also for a sec. product like glycosides.

Secondary metabolite: Plants represent and unlimited source of phyto-chemicals such as the metabolites of primary and secondary origin. The secondary compounds which are biosynthetically derived from the primary metabolites are of measure interest because of their different functions and biological activities. They are sometimes considered to be waste or secretor products of plant metabolism. Examples of secondary metabolites found in plants glycosides, alkaloids, flavonoids, terpinoides, volatile oil.

Advantage of Plant tissue culture

1. Quick response to variability in demand.
2. Culture condition controlled easily.
3. Simplified downstream processing.

Definition: Biotransformation is an area of biotechnology that has gained considerable attention. It is the ability of plant cell culture to catalyze the conversion of readily available on inexpensive precursor into a more valuable final product. This precursor cannot be transformed effectively by chemical or microbial methods are an interesting area for commercial application of plant tissue culture. This is a process of chemical conversion of an exogenously supplied substance by living cell culture. Plant-cells can transform a wide range of substrate and; thus perform several reactions such as, oxidation, hydroxylation, reduction, methylation, amino-acylation, glucosylation-acylation. They can tolerate a number of compounds like steroids, alkaloids, phenolics etc Biotransformation is the resent technique for commercial exploitation of secondary metabolites from cell-culture. It can also be defined as-chemical transformation which is catalyzed by micro-organism or their enzymes. Enzymatically catalyzed biotransformation is supior to chemically catalyzed reactions. The production of digitoxin from digitoxigenin by Digitalis lanata culture is classical examples of utilizing plant cell culture for achieving a specific biotransformation on a large scale.

Advantage of Biotransformation

- More than one reaction can be accomplished using cell cultures that express a series of enzyme activities.
- In some instances even non producing cell culture can be used to synthesize the desired end product using appropriate precursor.
- The process of biotransformation may be simple where the process is mediated by one or more enzymes with many steps.
- Single step biotransformation is comparatively efficient, as the yield decreased with increase in steps.
- Natural or synthetic substrate is used for biotransformation.

Techniques of Bio- transformation:

Biotransformation by immobilized cells: It has been observed that immobilized plant cells may have higher production rate compared with freely suspended cells under same conversion conditions. Immobilization of plant cells as pioneered by this technology which enables entrapment of cells in a gel of calcium alginate, polyvinyl alcohol resin on fixed support of foam, fabric or hollow fibers. It creates for cells a situation which is to imitate membership in a tissue of whole plant. In this, cells are expected to crease to grow and accumulate metabolites.

Biotransformation by hairy root culture: Roots are good sources of variety of natural products like propane alkaloid, Catharanthus alkaloids, atropine, and hyoscyamine. Much attention is being given in recent years for obtaining secondary plants products by hairy root culture. The hairy roots are sub-cultured primarily on solid medium and then on rotary shaker at 1000 rpm at 250^0C in dark in liquid medium. Hairy root culture is potencially applicable to the production of all root-derived metabolites from dicotyledonous plant. Undifferentiated celli of Atropa belladonna did not produce the tropane alkaloids-hyoscyamine but the culture gained the ability to synthesize this compound with the differenciation of roots.

Capability to biotransform BHT into stilbenequinone has been found in several hairy root cultures of members of the family-Asteraceae. It has reported the biotransformation of 18-glycyrrhetinic acid in Panax ginseng hairy root culture, which were also able to glycosylate digitoxigenin,a precursor of cardiac glycosides.Whitakar produced Onion culture with high levels of ethylcysteine sulfoxide which does not occurs naturally in Allium's species.

Table.1.Examples of biotransformation by plant-cell culture

Substrate Biotransformed	Plant	Cell culture product
Carrosine	Nicotiana tabacum	Cavaxone
Codeinone	Papaver somniferum	Codiein
Ellipticin	Choisya ternata	5-formylelliptione
Solavetivone	Solonum tuberosum	Hydroxylated derivative
2-Succinylbenzoate	Galium Mallugo	Anthraquinone
Valencene	Citrus Sps	Noothatone

Table.2.Examples of biotransformation by immobilized plant-cells

Cell culture	Support	Precursor	Produce
Coffea	Polyurethane	Theobromine	Caffeine
Papaver somniferum	Calcium alginate	Codeinove	Codinove
Mentha Sp.	Polyacrylamide, Hydrazide	(-)menthone	(+)Neomenthal
Prdephyllotoxin	Calcium aginate	Phenyl propanoid	podophyllotoxin

Table.3. Examples of biotransformation using root cultures

Products	Compound added	Plant Species
Scopolamine	Hyoscyamine	Hyoscyamus niger
Quinine	Tryptophan	Chinchona ledgeriana
Nicotine	Lysin , Putresine	Nicotiana Species
Scopolamine	Putriscine, Spermidive	Duboisia
Hyoscyamine	Cadaverine and other amine	Myoporieds
Digitoxin	Digitoxgenin	Panax ginseng

Biotransformation by free cells: This is also a useful technique for biotransformation. Both free cells and immobilized cell system are useful. The kinds of biotransformation reaction include oxidation, hydroxylation, methylation, and acylation. Following appear important in evolving strategies for maximal release of secondary metabolites by cultured cells.

a) Selection of clones with high efficiency.
b) Permeabilization of cells
c) Selective removal of metabolites from media.
d) Optimization of media for maximization of production and excretion.

These cells are growing in different types of cultures of which two are given here:

1. Callus culture
2. Suspension culture.

Callus culture: Callus culture is a mass of cells or tissue resulted subsequent to initiation & continued proliferation of the undifferentiated parenchyma cells from parent tissue on a clearly defined semi solid media. When an explants from a differentiated tissue is cultured on a medium. The quiescent cells undergo changes to achieve meristematic state. This phenomenon of mature cells reversion back to the meristematic state leading to the formation of callus growth is called differentiation. Moreover, the cells from callus are capable of generating into whole plant, a phenomenon referred to as redifferentiation. The callus formation is controlled by the endogenous auxin & cytokinin. Organogenisis can be initiated & regulated in the callus culture by the manipulation of the ratio of auxin & cytokinins.

Suspension culture: This culture essentially contains homogenous individual plant cells in its liquid medium. The suspension cultures are generally initiated by transferring an established callus tissue to an agitated liquid nutrient medium in Erlenmeyer culture vessels. The composition of the medium for the establishment of suspension cultures could be the same as defined for callus cultures except for the addition of agar. The soft callus generally forms in a suspension culture without much difficulty. The suspension culture is usually incubated at 25^0C in darkness or in low intensity fluorescent light. A cell suspension is generally formed within 4 to 6 weeks. The cells grown in culture meristematic & usually undifferentiated & there is no evidence that cells of shoot or root origin is metabolically different. The suspension culture is sub cultured by the transfer at regular intervals of untreated or fractionated aliquots of the suspension to fresh medium.

Several forms of suspension cultures are commonly utilized as follows

1. Batch suspension cultures
2. Semi continuous culture
3. Continuous culture.

Elicitation: Elicitors are compounds of biological origin involved in plant microbe interaction. But in the context of product accumulation by cell cultures, elicitors are considered as mediator compounds which induce secondary metabolites formation in cells cultures. Varieties of elicitors have been used for production of secondary metabolites. Polysaccharides in alginate are known to act like elicitor for shikkonin production in lithospermum erythrorhizen cells & for echinatin production by glycyrrhiha echinatin cells.

Elicitation improves the efficiency of Sec. Product accumulation in plant cell culture by:

a) Minimizing up on time
b) Avoiding change of media
c) Induction of enzymes involved in biosynthetic pathway.
d) Inducing excretion of metabolites into the medium.

Apart from using elicitors of biotic origin a biotic elicitors have also been used. They are either physical or chemical in nature. Elicitation involves the usage of microbial pathogens for triggering various reactions in cell cultures.

Precursor feeding: It is another diverse, but contradictory tool for enhancing secondary metabolite production. Even precursors to increase die synthesis of various phytochemicals invitra.There are two distinct methods of increasing the precursor supply within the cell, firstly by addition to the medium in which care the uptake mechanism may not be limiting. Secondly, by selecting for resistance to precursor analogues in which cares the intracellular level may be modified for e.g. shikonin production increase three fold when cultured cells of Lithospeimum species are fed with L-Phenylalanin. Similarly Datura sp. Cell suspension cultures are supplemented with hydroquinone, in traces, the arbutin synthesis increase considerably.

Application of biotransformation: There is biotransformation have many applications in various fields. It is used to production of secondary metabolites. The genetic information required for the manufacture of sec. Metabolites is also present in the undifferentiated cells.

There are various applications. Some examples are described here.

Biotransformations of steroids:

Digitoxin: In which digitoxigenin and digitoxin and their derivatives can be extracted from the leaves of Digitalis lanata. In the process of biotransformation of Digitoxin many compounds are produced, out of which purpurea glycoside A is mainly produced and methyl digitoxin is usable substrate. In biotransformation purpurea glycoside A is a main product and deacetyl lanotoside C and lanatoside C is minor product.

Table.4.Some of the biotic elicitor- induced products are given below

Elicitor	Preparation	Cell culture	Product
Aspergillus Niger	Homogenate	Cinchona ledgeriana	anthraquinones
Botrytis SP.	Homogenate	Papaver somniferum	sanguinarine
Chry sosporium	Filtrate & extracts	Catharanthus roseus	Tryptamine, catharantine
Yeast	Carbohydrate	Glycine max	glyceoline

Biotransformation of alkaloids: A variety of alkaloids have been used, as pharmaceuticals & most of them are plant metabolites. Research of production of useful alkaloids by plant cell cultures has been carried out for more than 25 years. Industrial production has not yet succeeded because of low producing ability of the cultured of the cultured cells. Plant used for their studies are mainly, *Atropa belladonna*, *Hysocyamus niger*, *Datura metalloids.*

Morphine alkaloids: Codeine is an analgesic and cough-suppressing drug and *Papaver somniferum* L. is a traditional commercial source of codeine & Morphine which can be converted to codeine. Mature capsule of *P.bracteatum* accumulates up to 3.5 % of the baine, which also can be converted to codeine. Although many researchers have tried to produce codeine by undifferentiated cells of their plants, little success has been achieved.

Berberine: Berberine is an isoquinoline alkaloid, which is distributed in roots of coptis Japonica, & cortex of philodendra's anurans. Addition of a polyamine, sperm dine, was found to stimulate the production of berberine by Thalictrum minus cell suspension cultures.

Tropane alkaloids: Scopolamine & hyosyamine are tropane alkaloids. The cone of scopolamine & hyoscyamine in cultured cells are generally very low in spite of many efforts of increase the field using various approaches

Vincristine: It is reported biotransformation of a hydro vincritin to vindolin by a cell extract of Catharanthus-roseus cell suspension culture. Their species produce high-value specialist; phytochemicals, highly valuable drugs like vinblastin & Vincristine that are used for the treatment of cancer are extracted from Catharanthus roseus plants.

Biotransformation of glycoside: The rue plant (*Ruta graneolens*) ads its normal tissue cultures contain a no. of constituents, including furanecoumarins derived from 7 hydroxy coumarin (A) stack & constable showed that two chemical mammies of 7 hydroxy coumarin precursor, the 4 methyl & 8 methyl derivatives, when fed to the Ruta cell culture, give rise to a no. of the comespanding unnatural analogues (B,C)

Biotransformation of terpenoids: Monoterpenes Biotransformations have been demonstrated with mentha cell lines capable of transforming pulegon to iromenthone, & (-)- Mentone to (+) neomenthal.

Biotransformations of paclitaxel (Taxel) by plant cell culture: Incubation of eucalyptus citriodera, Azadirachata indica & capsicum annum cell cultures with piclitaxel was carried in a refrigerated shaker incubator at 120 rpm & 25^{o}C (±1 ^{o}C) for 48 hr. The culture were extracted for Texans & analyzed by HPLC. Only E. citriodora cultures were able to biotransform paclitaxel into two known compound (baccatin III & deacetyl baccatin III) & an unknown compound.

Biotransformation of diatpenoid: Isosteviol Stevioside is a sweet glycoside extracted from the leaves of Stevia rebaudiana. Upon hydrolysis it gives isosteviol. The biotransformation of isosteviol by Aspergillus niger produced the B-OH derivative,, ent-7- alpha-hydroxy –16- Ketobeyeran –19-oic, & the 1-alpha, 7-beta-dioxy derivatives, and –1-beta 7-alpha- dihydroxy –16-ketobeyenan –19-oic acid.

Biotransformation of constituents of essential oils by germinating wheat seed: Wheat seeds when exposed to essential oils are able to metabolite certain monoterfoene. The actual amount of compound & their derivatives is the endosperms & embryo of wheat seeds after exposer to the monoterpenes more determined.Many other biochemical transformations by cell cultures have been demonstrated, and include epoxidation, ester formation and saponification, glycosylation, hydroxylation, isomarization, methylation and oxidation. For this technique to be commercially viable, the product must be sufficiently important the substrate must be available in reliable amount.

Factors affecting biotransformation: Biotransformation is longely depends an various factors like physiological, biochemical aspects & environmental conditions of cell culture. Source of origin of plant tissue, culture media formulation, carbon sources, plant growth regulator, physical

condition of cultures like oxygen, co2 influence the production of sec.

Origin of plant tissue: Production of secondary product in cultures in under the control of genetic nature of the explants. Scientist proved that culture derived from high fielding cultivars, produced high nicotine content, where as cultures established from low fielding cultivate showed less potential for nicotine production.

Culture Media: Chemical composition of culture media for establishing callus or suspension culture influences the production of biomass & also the synthesis of sec. Metabolites. An ideal culture conditions are to be maintained to achieve plant products in culture by manipulating an optimum balance between biomass production & secondary metabolite production.

Growth regulators: Growth regulators affect growth & synthesis of sec. Metabolites of cultured cells. Sometime combination of auxins & cytokinins had synergistic effect while other had antagonistic influence on steroidal synthesis. Taxol producing plant, Taxus cuspidate was significantly promoted by addition of gibberellic acid into the solid medium.

Carbon source in the medium: Sucrose is the most commonly used carbon source in the culture medium for the growth of tissue, & also drastically influences, the biosynthesis of sec. Metabolites in culture. Many other carbon compounds like glucose, fructose, and galactose also influence product accumulation in cultures. Out of all the carbon smokes used so for in cultures, sucrose & glucose gave the most encouraging results in terms of biomass production of product field.

Temperature: Plants are frequently able to exist in a considerable range of tem. In general, the formation of volatile oils appeases to be enhanced at higher temperature.

PH: A medium PH is usually adjusted to between 5 & 6 before autoclaving & extreme of PH are avoided.

Light intensity: Light intensity stimulate the in vitro process of enzyme action. High light intensity suppressed the production of nicotine in Nicotiana tabacum, where as continuous growth in darkness enhanced nicotine synthesis & its accumulation. Light is a factor, which helps to detamine the amount of glycosides, or alkaloids produced with belladonna, Stramonium & cinchona ledgeriana full Sunshine gives a higher content of alkaloid than does shade.

High cell density culture: To increase the productivity of sec. Metabolites, high cell density culture have been investigated, using a nearly designed fermented & optimized culture medium, copies Japonica cells were grown up to 759\L of cell mass.

CONCLUSION

Production of medicinal & aromatic substances through the application of cell & tissue culture technique is a novel approach to obtain their substances in large scale. Several research groups have paid an attention to suing Recombinant DNA technology as a toll for improvement of cultured cells although it is not an easy task in secondary metabolism. This technique used to obtain secondary metabolite. For example in the case of shikonin production from Lithospermum erythrorhizen the natural plant roots contain 1.2% of shikonin, & the plants take 3-4 years after seed formation before the roots can be utilized for industrial exploitation. But the cultured cells produce 15.20 % of shikonin & the cells are ready for harvest after 23-daysof culture. By the use of immobilized cells, hairy root culture & precursor feeding we can improve the production of secondary metabolites.

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