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Research review paper

Biotransformation studies using hairy root cultures — A review

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ABSTRACT

Agrobacterium rhizogenes induced hairy root cultures are entering into a new juncture of functional research in generating pharmaceutical lead compounds by bringing about chemical transformations aided through its inherent enzyme resources. Rational utilization of hairy root cultures as highly effective biotransformation systems has come into existence in the last twenty years involving a wide range of plant systems as well as exogenous substrates and diverse chemical reactions. To date, hairy root cultures are preferred over plant cell/callus and suspension cultures as biocatalyst due to their genetic/biochemical stability, hormone-autotrophy, multi-enzyme biosynthetic potential mimicking that of the parent plants and relatively low-cost cultural requirements. The resultant biotransformed molecules, that are difficult to make by synthetic organic chemistry, can unearth notable practical efficacies by acquiring improved physico-chemical properties, bioavailability, lower toxicity and broader therapeutic properties. The present review summarizes the overall reported advances made in the area of hairy root mediated biotransformation of exogenous substrates with regard to their reaction types, plant systems associated, bacterial strains/molecules involved and final product recovery.

Contents

1. Introduction

Compounds derived from natural reserves have long been sources of medicines and have always made immense impact on the pharmaceutical industry through the process of drug discovery (Newman and Cragg, 2007). However, in recent years, a second generation of phyto-pharmaceuticals with altered molecular structures has gained world-wide recognition due to their improved pharmaceutical properties, such as lower toxicity, improved solubility and pharmacokinetics. These are primarily natural product analogs. This new generation of natural products relies upon the structural diversification of complex phyto-molecules through different means, one of which is biotransformation.

Biotransformation can be defined as regio-selective and stereo-specific chemical transformations that are catalyzed by biological systems through their effective enzyme structures. Biotransformation has been progressively utilized as a means to create notable therapeutic compounds by doing “what nature hasn't done yet” (Ji-Hua and Bo-Yang, 2010). It allows changes in areas of phytomolecules that are not realistically attainable by chemical semi-synthesis. A wide variety of natural products have been biotransformed to generate libraries of analog compounds by the enzymes derived from plant cell/organ cultures (Suga and Hirata, 1990). Reaction types carried out by such cultures include hydroxylation, glycosylation, glucosylation, oxido-reduction, hydrogenation, hydrolysis, methylations, acetylations,

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isomerization, glycosylations and esterfications of various exogenous substrates (Giri et al., 2001; Ishihara et al., 2003).

The biotransformation procedures undoubtedly offer the power to re-investigate the well examined natural products, not only with the intention to find improved versions of already utilized phytomolecules but also to upgrade the efficacies of known molecules with broader applications through creation of analogs. Such procedures carried out by plant cell/organ cultures generate libraries of analog compounds with unique structural modifications and also ensure sustainable use of resources under defined culture conditions independent of seasonal fluctuations and pathological constraints. The resulting compounds, in addition to maintaining the prominent characteristic potency of parent natural molecule, can also acquire improved selectivity, safety/physico-chemical properties and lower toxicity profiles which can be more suitable for some new therapeutic functions. Interestingly, the entire process of biotransformation can lead to the discovery of a completely novel group of therapeutically and commercially advantageous phytomolecules. This method is also gaining considerable attention as a step towards green chemistry by reducing the usage of hazardous chemicals.

In recent years, genetically transformed hairy root cultures are gaining preferential advantage as biocatalysts over cell suspension cultures mostly because of their genetic/biochemical stability, multienzyme biosynthetic potential similar to that of the parent plant and relatively low-cost culture obligations (Banerjee et al., 2002; Giri et al., 2001). Rational utilization of hairy root cultures of diverse medicinal plants as highly effective biotransformation systems has come into existence in the last twenty years involving a wide range of exogenous substrates as well as divergent chemical reactions. The present review summarizes the overall reported advances in the biotransformation of exogenous substrates by hairy root cultures in terms of their reaction types, plant systems, *A. rhizogenes* strains, media compositions and molecules involved and final product recovery.

By and large, the genetically transformed hairy root cultures of different plant systems exemplify rich repositories of enzymes as they mimic their respective parent plants. Consequently, the natures of biotransformation reactions differ depending upon the availability of the plant enzymes as biocatalysts as well as on the structure and functional group of the exogenous substrate. So far, seven distinct kinds of biotransformation reactions have been reported through the use of hairy root cultures of seventeen different plant systems which have been represented in Table 1. Elucidation of each representative reaction category reflected by the hairy root system of specific plant species will be elaborated in this review for complete depiction of the relevant insights into the process of "hairy root mediated biotransformation".

2. Type of reactions

2.1. Glycosylation/glucosylation

Glycosylation is the enzymatic progression that links saccharides to produce glycans, either free or attached to proteins or lipids. It involves the coupling of a sugar moiety to a glycosyl acceptor forming glycosides.

In context to the hairy root mediated biotransformation studies, ginseng hairy roots attracted major attention owing to its prominent glycosylation ability. It is worth referring to the production of glycosides of digitoxigenin namely 3-epidigitoxigenin β-D-gentiobioside and digitoxigenin β-D-sophoroside by the constitutive gentiobiose and sophorose ginsenoside sugars of *Panex ginseng* hairy root cultures (Table 1). Esterification of the same substrate into different esters such as Digitoxigenin stearate, Digitoxigenin palmitate and Digitoxigenin myristate was also accomplished using *ginseng* hairy root culture (Kawaguchi et al., 1990).

The glycosylation potential of *P. ginseng* hairy root cultures has successfully been utilized in another study for continuous biotransformation of (RS)-2-phenylpropionic acid in a bioreactor leading to the formation of (RS)-2-phenylpropionyl β-D-glycopyranoside, (2RS)-2-O-(2-phenylpropionyl) D-glucose, (2RS)-2-phenylpropionyl) 6-O-β-D-xylopyranosyl β-D-glycopyranoside, myo-inositol ester of (R)-2-phenylpropionic acid (Yoshikawa et al., 1993)

In a later investigation, glycosylation of 18 β-glycyrrhetinic acid by *P. ginseng* hairy root cultures led to the formation of three glycosylated products, i.e. 30-O-[β-D-glucopyranosyl (1→2) β-D-glucopyranosyl] 18 β-glycyrrhetinic acid, 30-O-[β-D-glucopyranosyl] 18β-glycyrrhetinic acid, 3-O-[β-D-glucopyranosyl-(1→2) β-D-glucopyranosyl] 18β-glycyrrhetinic acid and 3-O-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-30-O-(β-D-glucopyranosyl) 18β-glycyrrhetinic acid (Asada et al., 1993). Additionally, *P. ginseng* hairy root cultures also demonstrated for the first time its ability to make malonyl conjugates at the C-30 carboxyl and C-3 hydroxyl group of 18 β-glycyrrhetinic acid to form 30-O-[6-O-malonyl-β-D-glucopyranosyl]18β-glycyrrhetinic acid and 3-O-[6-O-malonyl-β-D-glucopyranosyl-(1→2) β-D-glucopyranosyl] 18β-glycyrrhetinic acid (Asada et al., 1993). *P. ginseng* hairy root cultures had further demonstrated regioselective glycosylation potential with regard to two isomers of hydroxybenzoic acid leading to the conversion of *p*-hydroxy benzoic acid and *m*-hydroxy benzoic acid to their corresponding glycosides and glycosyl esters (Chen et al., 2008).

Glucosylation is also an enzymatic reaction that links glucose to produce glucosides. It is another important desired form of biotransformation which enhances the bioavailability and bioactivity of the transformed products. Hairy root cultures of diverse plant systems have demonstrated glucosylation potentials which deserve special attention.

Hairy root cultures of *Lobelia sessilifolia* demonstrated strong capability for glucosylation following treatment with four phenols, i.e. (+)-catechin, (−)-epicatechin, protocatechuic acid and gallic acid and resulted in the formation of (+)-catechin 7-O-β-D-glucopyranoside, (−)-epicatechin 7-O-β-D-glucopyranoside, (−)-epiafzelechin 7-O-β-D-glucopyranoside, protocatechuic acid 3-O-β-D-glucopyranoside, β-glucogallin and gallic acid 3-O-β-D-glucopyranoside respectively (Yamanaka et al., 1995). Another contemporary report corroborates this finding with regard to glucosylation of protocatechuic acid to protocatechuic acid 3-O-β-D-glucopyranoside through the use of hairy root cultures of *Lobelia sessilifolia* and *L. cardinalis* (Ishimaru et al., 1996). However, according to this later report, it is interesting to note that biotransformation of gallic acid by *Lobelia sessilifolia* and *L. cardinalis* hairy root cultures led to the formation of a single glucosylated product i.e. β-glucogallin, whereas hairy root cultures of *Campanula medium*, *Ocimum basilicum* and *Fragaria xananassa* biotransformed it into gallic acid 3-O-β-D-glucopyranoside (Ishimaru et al., 1996). The same study also reported hydroxylation of trans-cinnamic acid to produce *p*-coumaric acid followed by site-specific glycosylation to get 1-O-(*p*-coumaroyl)-β-D-glucopyranoside by hairy root cultures of *L. sessilifolia*, *L. cardinalis*, *Campanula medium* and *Fragaria xananassa* (Ishimaru et al., 1996). There was one more report regarding the bioconversion of E-cinnamic acid to (E)-*p*-coumaroyl-1-O-β-D-glucopyranoside by the hairy root culture of *Plantago lanceolata* (Fons et al., 1999).

The glucosylation ability of the hairy root cultures of *Lobelia sessilifolia*, *L. cardinalis* and *Campanula medium* had further been demonstrated through multistep reactions when *p*-coumaric acid was used as substrate, which led to the production of 1-O-(*p*-coumaroyl)-β-D-glucopyranoside, Caffeic acid, 1-O-Caffeoyl)-β-D-glucopyranoside, *p*-hydroxybenzoic acid and 1-O-(*p*-hydroxybenzoyl)-β-D-glucopyranoside. On the other hand, biotransformation of the same substrate, i.e. *p*-coumaric, to different products, *viz* trans-cinnamic acid and 1-O-(trans-cinnamoyl)-β-D-glucopyranoside had been noted when the hairy root lines of *Ocimum basilicum* and *Fragaria xananassa* were used (Ishimaru et al., 1996).

Coleus furskohlii hairy root cultures also demonstrated glucosylation potentials rendering biotransformation of methanol and ethanol to their corresponding β-D-glucopyranosides, β-D-ribo-hex-3-ulopyranosides and of 2-propanol into 2-propyl β-D-glucopyranosides (Li et al., 2003).