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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).

Table 1
Biotransformation of compounds through different hairy root systems and the type of reactions involved in transformation.

Sl	Type of reaction	Plant system	Substrate	Biotransformed Product with conversion rate*	Reference
1	Esterification	*Panax ginseng*	Digitoxigenin	**Esters:** Digitoxigenin stearate, **(0.6%)** Digitoxigenin palmitate, **(2.4%),** Digitoxigenin myristate, **(0.9%)** Digitoxigenin laurate (**0.6%**), 3-epidigitoxigenin **(2.4%)**, Periplogenin (**3.5%),** 3-epiperiplogenin (**1.4%).**	Kawaguchi et al. (1990)
	Glycosylation	,,	,,	**Glycosides:** 3-epidigitoxigenin β-D-gentiobioside, (**0.6%**) Digitoxigenin β-D- sophoroside, **(2.2%),** 3-epidigitoxigenin β-D-glucoside (**1.3%),** Digitoxigenin β-D-glucoside (**12%),** Periplogenin β-D-glucoside **(8.3%).**	
2	Glycosylation	*Panax ginseng*	(RS)-2-phenylpropionic acid	(RS)-2-phenylpropionyl β-D-glycopyranoside, **(71%)** (2RS)-2-0-(2-phenylpropionyl) D-glucose**, (8%)** (2RS)- 2-phenylpropionyl) 6-0-β-D-xylopyranosyl β-D-glycopyranoside, **(10%)** Myo-inositol ester of (R)-2-phenylpropionic acid, **(5%)**	Yoshikawa et al. (1993)
3	Glycosylation	*Panax ginseng*	18 β-glycyrrhetinic acid	30-O-[β-D-glucopyranosyl (1→2) β-D- glucopyranosyl] 18 β-glycyrrhetinic acid, 30-O-[β-D-glucopyranosyl] 18 β-glycyrrhetinic acid, 3-O-[β-D-glucopyranosyl -(1→2) β-D- glucopyranosyl] 18 β-glycyrrhetinic acid, 3-0-[β-D-glucopyranosyl -(1→2) -β-D- glucopyrano syl] -30-0-(β-D-glucopyranosyl) 18 β-glycyrrhetinic acid**,**	Asada et al. (1993)
	Malonylation	,,	,,	30-O-[6-O-malonyl-β-D-glucopyranosyl]18 β-glycyrrhetinic acid, 3-O-[6-O-malonyl-β-D-glucopyranosyl -(1→2) β-D-glucopyranosyl]18 β-glycyrrhetinic acid,	
4	Glycosylation	*Panax ginseng*	*p*-hydroxybenzoic acid	**Glycosides**: *p*-carboxyphenyl β-D-glycopyranoside, **(15%)** **Glycosyl esters:** *p*-hydroxy benzoic acid β-D-glycopyranosyl ester, **(15%)**	Chen et al. (2008)
		,,	*m*-hydroxybenzoic acid	**Glycosides**: *m*-carboxyphenyl β-D-glycopyranoside, **(47%)** **Glycosyl esters:** *m*-hydroxy benzoic acid β-D-glycopyranosyl ester, *m*-hydroxy benzoic acid β-D-xylopyranosyl (1→6)-β-D-glycopyranosyl ester,	
		,,	*o*-hydroxybenzoic acid	No transformation.	
5	Glycosylation	*Brassica napus*	**Ketone:** 1-(5-acetyl-2-hydroxyphenyl)-3-methylbutan- 1-one	4-acetyl-2-(3-methylbutanol)-phenyl- O-β-D-glucopyranoside	Orden et al. (2006)
6	Glycosylation	*Polygonum multiflorum* Thunb	Furannoligularenone	3-oxo-eremophila 1,7(11)-dien-12,8-olide (**27.2%**) 3-oxo-8-hydroxy-eremophila 1,7(11)-dien-12,8-olide	Yan et al. (2008a)
7	Glucosylation	*Lobelia sessilifolia*	(+)- catechin Protocatechuic acid (-)- epicatechin	(+)- catechin 7-O-β-D-glucopyranoside, protocatechuic acid 3-O-β-D-glucopyranoside, (-)- epicatechin 7-O-β-D-glucopyranoside, (-)- epiafzelechin 7-O-β-D-glucopyranoside,	Yamanaka et al. (1995)
		,,	Gallic acid	β – glucogallin, gallic acid 3-O-β-D-glucopyranoside,	
8	Glucosylation	*Lobelia sessilifolia*	Protocatechuic acid	protocatechuic acid 3-O-β-D-glucopyranoside, **(0.38%)**	Ishimaru et al. (1996)
		L.cardinalis	,,	,,	
		Campanula medium	,,	Gallic acid,	
		Ocimum basilicum	,,	,,	
		Fragaria xananassa	,,	,,	
		Lobelia sessilifolia	Gallic acid	β – glucogallin,	
		L.cardinalis	,,	,,	
		Campanula medium	,,	gallic acid 3-O-β-D-glucopyranoside,	
		Ocimum basilicum	,,	,,	
		Fragaria xananassa	,,	,,	
		Lobelia sessilifolia	Trans-cinnamic acid	1-O-(*p*-coumaroyl) -β-D-glucopyranoside, **(6.11%)**	
		L.cardinalis	,,	,,	
		Campanula medium	,,	,,	
		Fragaria xananassa	,,	,,	
		Ocimum basilicum	,,	No transformation	
		Lobelia sessilifolia	*p*-coumaric acid	*p*-coumaric acid 1-O-(*p*-coumaroyl) -β-D-glucopyranoside,	
		L.cardinalis	,,	1-O-Caffeoyl -β-D-glucopyranoside,	
		Campanula medium	,,	1-O-(p-hydroxybenzoyl) -β-D-glucopyranoside	
		Ocimum basilicum	,,	Trans-cinnamic acid 1-O-(trans-cinnamoyl) -β-D-glucopyranoside, **(6.19%)**	
		Fragaria xananassa	,,	,,	
		Lobelia sessilifolia	Caffeic acid	No transformation	
		L.cardinalis	,,	,,	
		Campanula medium	,,	,,	
		Ocimum basilicum	,,	,,	
		Fragaria xananassa	,,	,,	

(continued on next page)

Table 1 (*continued*)

Sl	Type of reaction	Plant system	Substrate	Biotransformed Product with conversion rate*	Reference
9	Glucosylation	*Plantago lanceolata*	(E)-cinnamic acid	(E)-p-coumaroyl-1-O-β-D-glucopyranoside,	Fons et al. (1999)
10	Glycosylation	*Coleus furskohlii*	Methanol	Methyl β-D-glucopyranosides, Methyl β-D-ribo-hex-3-ulopyranosides,	Li et al. (2003)
		„	Ethanol	Ethyl β-D-glucopyranosides, Ethyl β-D-ribo-hex-3-ulopyranosides,	
		„	2-propanol	2-propyl β-D-glucopyranosides,	
11	Glucosylation	*Pharbatis nil*	**Coumarin Derivatives**:		Kanho et al. (2004)
			Umbelliferone	Skimmin	
			4-methylumbelliferone	4-methylskimmin	
			Scopoletin	Scopolin	
			3,4,8-tri- methylumbelliferone	3,4,8-tri methylskimmin	
			Aesculetin	Scopolin, Aesculin, Eichoriin,	
	Hydroxylation	„	4- methyl aesculetin	6,7-di-0-glucopyranosylaesculetin 4- methyleichoriin, 4- methylaesculin, 6,7-di-0-glucopyranosyl -4-methylaesculetin, 4- methyl scopolin	
		„	7,8-dihydroxy-6-methoxycoumarin	6,7-dimethoxy-8-glucopyranosyl oxycoumarin (or) 7-dimethoxy-8-glucopyranosylcoumarin	
			4- hydroxycoumarin		
		„	4- hydroxyl-6-methylcoumarin	2-glucopyranosyl oxyacetophenone 2-glucopyranosyl oxy-5-methylacetophenone	
			Flavones Derivatives:		
		„	3-hydroxyflavone	3-hydroxyflavone glucopyranoside 3-0-(6-0-oxalyl-glucopyranosyl)flavones	
			3,6-dihydroxyflavone	3-glucopyranosyl oxy-6- hydroxyflavone	
			3,7-dihydroxyflavone	3-glucopyranosyl oxy-7- hydroxyflavone	
12	Glucosylation	*Pharbitis nil*	Vanillin	Vanillin-4-O-β-D-glucopyranoside, **(1.4%)**	Kanho et al. (2005)
				Vanillyl alcohol-4-O-β-D-glucopyranoside, **(12%)** Vanillyl alcohol-7-O-β-D-glucopyranoside, **(3.9%)**	
		„	Vanillyl alcohol	Vanillyl alcohol-4-O-β-D-glucopyranoside, **(13.5%)** Vanillyl alcohol-7-O-β-D-glucopyranoside, **(2.6%)**	
		„	Isovanillin	Isovanillyl alcohol-3-O-β-D-glucopyranoside, **(10.8%)**	
			Isovanillyl alcohol	Isovanillyl alcohol-3-O-β-D-glucopyranoside, **(13.3%)** Isovanillyl alcohol-7-O-β-D-glucopyranoside, **(2.1%)**	
		„	3,4-dihydroxybenzaldehyde	3,4-dihydroxybenzyl alcohol-3-O-β-D-glucopyranoside, **(12%)** 3,4-dihydroxybenzyl alcohol-3-O-β-D-glucopyranoside, **(1.4%)**	
		„		Vanillyl alcohol-4-O-β-D-glucopyranoside, **(0.9%)**	
			3,4-dimethoxybenzaldehyde	3,4-dimethoxybenzyl alcohol-7-O-β-D-glucopyranoside, **(21.9%)**	
		„		3,4-dimethoxybenzyl alcohol, **(33.5%)**	
			3,4,5-Trimethoxybenzaldehyde	3,4,5-trimethoxybenzyl alcohol-7-O-β-D-glucopyranoside, **(30.3%)**	
		„	Salicylaldehyde	Salicyl alcohol -2-O-β-D-glucopyranoside, **(14.7%)** Salicyl alcohol -7-O-β-D-glucopyranoside, **(21.4%)**	
			Acetophenone Derivative:		
		„	4-hydroxy-3-methoxyacetophenone	4-hydroxy-3-methoxyacetophenone-4-O-β-D-glucopyranoside, **(6%)** 5,4'-diacetyl-3,2'-dimethoxy-2-β-D-glucopyranosyloxy biphenyl ether, **(1.9%)** 5,5'-diacetyl-3,3'-dimethoxy-2-β-D-glucopyranosyloxy-2'-hydroxy biphenyl, **(1.8%)**	
		„	Acetophenone	1-β-D-glucopyranosyloxy-1-phenylethane, **(3.9%)** 2-β-D-glucopyranosyloxyacetophenone, **(2%)**	
13	Glucosylation	*Polygonum multiflorum* Thunb	**4-hydroxybenzen derivatives:**		Yan et al. (2007)
			1-4-benzendiol	4-hydroxyphenyl β-D- glucopyranoside (arbutin) (**81.91%**)	
		„	4-hydroxybenzaldehyde	4-hydroxyl methylphenyl β-D- glucopyranoside, (**4.90%**)	
		„	4-hydroxybenzyl alcohol	4-hydroxyl methylphenyl β-D- glucopyranoside, (**4.71%**)	
		„	4-hydroxybenzoic acid	4-carboxyphenyl α-D- glucopyranoside (**6.36%**)	
14	Glucosylation	*Polygonum multiflorum* Thunb	furannoligularenone	2 new compounds	Yan et al. (2008b)
		„	1,4-benzenediol	Arbutin	
		„	Artemisinin	2 new compounds	
15	Glucosylation	*Datura taluta* L	*p*-hydroxybenzyl alcohol	*p*-hydroxy-methyl phenol-β-D-glucoside (Gastodin) (**59.75%**)	Peng et al. (2008)
16	Glucosylation	*Brugmansia candida*	Hydroquinone	4-hydroxyphenyl β-D- glucopyranoside (arbutin)	Casas et al. (1998)
17	Glucosylation	*Physalis ixocarpa*	Hydroquinone	Arbutin **(67.6 – 70.6%)**	Bergier et al. (2008)
18	Glucosylation	*Polygonum multiflorum*	Thymol	5-methyl-2-(1-methylethyl) phenyl-β-D-glucopyranoside	Dong et al. (2009)
19	Reduction	*Cyanotis arachnoidea*	Artemisinin	Deoxyartemisinin	Zhou et al. (1998)
		Panex japonicus var. *major*	„	No transformation	
20	Reduction	*Daucus carota*	Acetophenone and its drivatives	(S)-1-phenyl ethanol (10 more compounds) (**96%**)	Caron et al. (2005)

Table 1 (*continued*)

Sl	Type of reaction	Plant system	Substrate	Biotransformed Product with conversion rate*	Reference
21	Reduction	*Brassica napus*	**Diketones:**		Orden et al. (2006)
			1-(5-acetyl-2-hydroxyphenyl)-3-methylbut-2-en-1-one	6-(1(S)-hydroxyethyl)-2,2-dimethyl-2,3-dihydro-4H-chromen-4-one (>**78%**)	
			6-acetyl-2,2-dimethyl-2,3-dihydro-4H-chromen-4-one		
			Acetate Derivative		
	Hydrolysis	,,	4-(acetyl-2-(3-methylbut-2-enoyl)-phenylacetate	6-(1(S)-hydroxyethyl)-2,2-dimethyl-2, 3-dihydro-4H-chromen-4-one	
			O-methyl Derivative:		
		,,	1-(5-acetyl-2-methoxyphenyl)-3-methylbut-2-en-1-one	No biotransformation	
22	Reduction	*Raphanus sativus*	Acetophenone and its drivatives	(S)-1-phenyl ethanol (10 more compounds) (**26%**)	Orden et al. (2009)
23	Other Types of Reaction (Condensation)	***Asteraceae:***			Flores et al. (1994)
		Bidens sulphureus,	Butylated hydroxytoluene (BHT)	Stilbenequinone **(50- 90%)**	
		Tagetes erecta	,,	,,	
		Acmella oppositifolia	,,	,,	
		Gaillardia pulchella	,,	,,	
		Ambrosia trifida	,,	,,	
		Rudbeckia hirta	,,	,,	
		Non-Asteraceae:			
		Datura innoxia	,,	,,	
		Hyoscyamus muticus	,,	,,	
		Glycyrrhiza glabra	,,	,,	
		Hibiscus abelmoschus	,,	,,	
		Trichosanthes cucumeroides	,,	,,	
24	Oxidation	*Anisodus tanguticus*	Dehydroepiandrosterone	Androst-4-ene-3,17-dione 6 α-hydroxy androst-4-ene-3,	Liu et al. (2004)
				17-dione 6 α-hydroxy androst-4-ene-3, 17-dione androst-4-ene-3,6,17-trione, 17 β-hydroxy androst-4-ene-3-one,	
25	No Reaction	*Levisticum officinale*	Menthol	No conversion	Nunes et al. (2009)
	Isomerization		Geraniol	linalool, (**0.1 -1.2%**), nerol, (**15.0%**)	
	Oxidation		,,	neral , (**15.0%**)	
	Cyclization		,,	α terpineol, (**0.2 -3.0%**)	
	Reduction		,,	citronellol (**15.0%**)	
	Acetylation		,,	geranyl acetates (**2.0%**)	
	Glycosylation		,,	Geraniol glycosides	
26	Acetylation	*Anethum graveolens*	Menthol	Menthyl acetate (**55.0%**)	Faria et al. (2009)
	Reduction		Geraniol	**Alcohols**: linalool, α -terpineol, citronellol (< **10%**)	
	Isomerisation		,,	**Aldehyde**: neral, geranial,	
	Transacetylation		,,	**Esters:** citronellyl, neryl and geranyl acetates, **(47%)**	
				Traces: linalool, nerol oxides	

* No data in parenthesis means conversion rate is not available in the cited literature.

Similar kind of glucosylation activities against several phenolic compounds had been reported through the use of *Pharbitis nil* hairy root cultures (Table 1). Glucosylation and other types of conversions of seven coumarin and flavone derivatives were carried out by *P. nil* hairy root cultures and the biotransformed products could only be detected in the root mass but not in the media (Kanho et al., 2004). This report clearly indicated preferential glucosylation of C-7 hydroxyl group over decreased glucosylation of C-8 methyl group of coumarin skeleton (Kanho et al., 2004). Similarly, in case of flavones derivatives. *P. nil* hairy root culture predominantly glucosylated 3-hydroxy group while hydroxyl groups on A and B rings of flavone hampered glucosylation (Kanho et al., 2004). In a later study, hairy root cultures of *P. nil* were further explored for biotransformation of benzaldehyde- and acetophenone-type derivatives (Table 1) and various biotransformation potentials including glucosylation of phenolic and benzylic hydroxyl groups were testified (Kanho et al., 2005).

Brassica napus hairy root cultures also demonstrated biotransformation of 1-(5-acetyl-2-hydroxyphenyl)-3-methylbutan-1-one to its glucosides, 4-acetyl-2-(3-methylbutanol)-phenyl-O-β-D-glucopyranoside (Orden et al., 2006).

Hairy root cultures *Polygonum multiflorum Thunb.* further extends the list of hairy root mediated glucosylation reactions where four 4-hydroxybenzene derivatives were used which were transformed to their corresponding glucosides (Yan et al., 2007). The biotransformation reactions involved conversion of 1–4-benzendiol into 4-hydroxyphenyl β-D-glucopyranoside (arbutin); 4-hydroxybenzaldehyde and 4-hydroxybenzyl alcohol into 4-hydroxyl methylphenyl β-D-glucopyranoside (Gastrodin) and 4-hydroxybenzoic acid into 4-carboxyphenyl α-D-glucopyranoside (Table 1). Both arbutin and gastrodin were reported to have diverse desirable bioactivities than the parent substrates (Yan et al., 2007, 2008b). *Polygonum multiflorum Thunb.* hairy root cultures further illustrated biotransformation of substrate furannoligularenone into two products, i.e. 3-oxo-eremophila 1,7(11)-dien-12,8-olide and 3-oxo-8-hydroxy-eremophila 1,7(11)-dien-12,8-olide through glycosylation reactions(Yan et al., 2008a). Furthermore, *Datura tatula L.*, hairy root cultures demonstrated analogous glucosylation reaction and converted p-hydroxybenzyl alcohol to Gastrodin, i.e. p-hydroxy-methyl phenol-β-D-glucoside (Peng et al., 2008). *Polygonum multiflorum* hairy root was also capable to transform thymol to its glucoside-5-methyl-2-(1-methylethyl) phenyl-β-D-glucopyranoside (Dong et al., 2009).

Brugmansia candida and *Physalis ixocarpa* hairy root culture also produced arbutin through biotransformation of hydroquinone (Bergier et al., 2008; Casas et al., 1998). In case of *B. candida* hairy root mediated biotransformation of hydroquinone, the efficacies of two different free-radical scavengers (sodium benzoate and gallic acid)

were evaluated where sodium benzoate reduced biotransformation competence and gallic acid proved non-effective (Casas et al., 1998).

2.2. Reduction

Regio- and sterioselective reduction is another notable biotransformation reaction demonstrated by hairy root cultures of several plant systems (Table 1). *Cyanotis arachnoidea* hairy roots converted artemisinin to deoxyartemisinin through reduction reaction (Zhou et al., 1998).

Daucus carota hairy root cultures reduced aromatic ketones, keto esters and a simple ketone with proficient stereoselectivity (Caron et al., 2005). However, the reduction potential of the *D. carota* hairy roots was noted to be highly substrate specific and no reduction occurred to several ketones, such as *p*-hydroxyacetophenone, 1-acetonaphthone, 3-acetylindole, 6-methoxy-1-tetralone and estrone, whereas best reductions occurred with substrates bearing a carbonyl function conjugated with an aromatic or heteroaromatic ring (Caron et al., 2005).

Likewise, *Pharbitis nil* hairy root cultures demonstrated not only strong potential to glucosylate the phenolic hydroxyl group but also to reduce the formyl and carbonyl groups of several benzaldehyde- and acetophenone-type derivatives (Kanho et al., 2005).

In the same way, *Brassica napus* hairy root cultures depicted enantio-selective bioreduction of natural prochiral diketones into the corresponding (S)-1′-hydroxy compound besides its proficiency to carry out glycosylation of ketone and hydrolysis of acetate derivative. However, in case of *O*-methyl derivatives, the same root culture proved to be non-effective (Orden et al., 2006). Concurrently, the stereoselective reduction potential of *Raphanus sativus* hairy root culture had also been reported involving prochiral-alkylaryl-ketones which had clearly depicted the strong influence of substituent position both on the conversion rates as well as on the stereoselectivity of the entire process (Orden et al., 2009).

2.3. Other types of reactions

Hairy root cultures of several other plant systems demonstrated potential to perform other distinct kinds of biotransformation reactions such as oxidation and condensation (Table 1).

The hairy root cultures of six *Asteraceae* and four *non-Asteraceae* plant systems produced a dimeric quinone derivative through condensation, i.e. stilbenequinone owing to the use of butylated hydroxytoluene (BHT) as the substrate (Table 1). The biotransformation of BHT to stilbenequinone was strongly stimulated through the use of *Pythium aphanidermatum* as fungal elicitor (Flores et al., 1994). *Armoracia rusticana* hairy root cultures demonstrated potential for biotransformation of food antioxidants (Sudhir and Mukundan, 2001).

Anethum graveolens hairy root cultures, showed effective biotransformation ability with regard to two oxygen-containing monoterpene substrates, i.e. menthol and geraniol, resulting in conversion of menthol to menthyl acetate and geraniol to ten new products in the form of alcohols, aldehydes, esters and oxides (Faria et al., 2009). *Levisticum officinale* hairy roots, on the other hand, demonstrated discrete biotransformation reactions in response to the same two substrates, i.e. menthol and geraniol. In response to geraniol as substrate, *L. officinale* hairy roots brought about five different kinds of reactions (Table 1), namely: acetylation, reduction, cyclization, isomerization and oxidation, whereas no conversion was obtained by addition of menthol (Nunes et al., 2009). The divergent bioconversion response towards the same substrates by the hairy root cultures of two different plant systems noticeably indicated exclusive role of source plant species which implicitly determines the enzymatic profile of the concerned hairy root system used.

Hairy root cultures of *Anisodus tanguticus* showed oxidation reactions for bioconversion of dehydroepiandrosterone into five products, namely androst-4-ene-3,17-dione, 6 α-hydroxy androst-4-ene-3,17-dione , 6 α-hydroxy androst-4-ene-3,17-dione , androst-4-ene-3,6,17-trione and 17 β-hydroxy androst-4-ene-3-one (Liu et al., 2004).

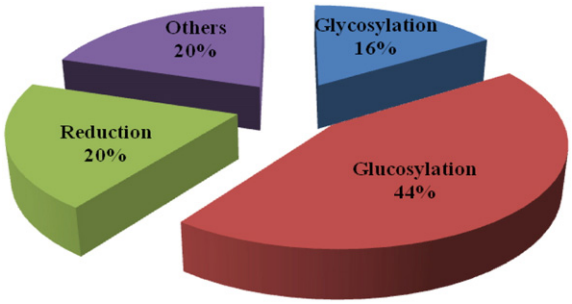

Fig. 1. Hairy root mediated major biotransformation reactions.

Biotransformation of artemisinin into two new compounds had also been reported through the use of *P. multiflorum* hairy root cultures (Yan et al., 2008b).

To sum up the reported investigations into the process of hairy root mediated biotransformations, the major representative reaction categories have been represented in Fig. 1. This comprehensive literature survey clearly depicted the maximum report of glucosylation (44%) + glycosylation (16%) reactions, both of which bestowed desired modifications of the exogenous substrates in terms of water solubility, bioavailability and more effective biotransformed products. Regio- and sterioselective reduction is the next prominent reaction type followed by other distinct kinds of biotransformation reactions (Fig. 1).

3. Impact of other factors

A detailed literature survey indicated that many aspects, other than reaction types, played vital roles in hairy root mediated biotransformation events. Including the major role of the hairy root generating starting plant systems and their families, the diversities of the used *A. rhizogenes* strains, media composition, reaction durations etc. spoke about the relevant role of each component which consequently elucidated the correct perspective of the concerned reaction type. Although neither of these illustrated parameters proved obvious for any given reaction type and did not interfere amongst themselves, yet preponderance or infrequency of certain components seemed worth reviewing for conveying the realistic perspective of this review.

So far as the hairy root generating starting plant families are concerned, it was interestingly noted that out of 14 reported plant families, three (i.e. *Asteraceae*, *Solanaceae* and *Campanulaceae*) predominantly dominated the entire progression of hairy root mediated biotransformation events (Fig. 2). Apart from this observation, the preferred utilization of the hairy roots of *Panex ginseng* of the *Araliaceae*

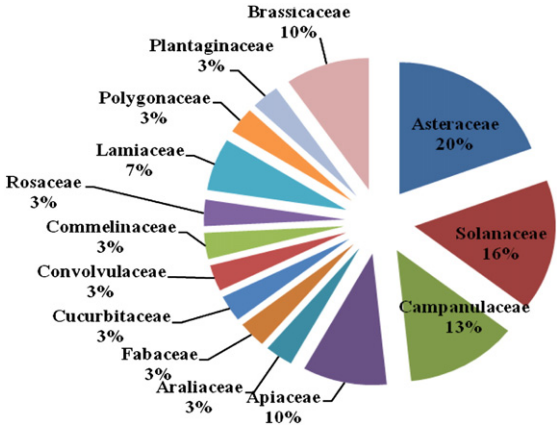

Fig. 2. Comparative involvement of plant families in hairy root mediated biotransformations.

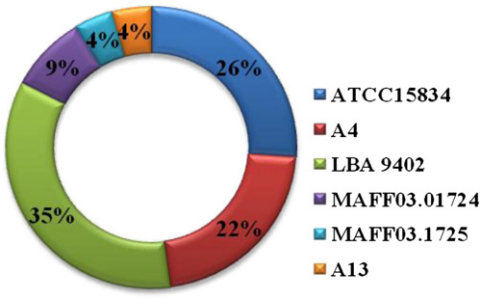

Fig. 3. Hairy root generating *Agrobacterium rhizogenes* strains utilized for biotransformation.

family by different research groups for successful biotransformation of diverse substrates justified special mention. It was even more worthy to note divergent bioconversion response by the hairy root cultures of two different plant systems (i.e. *Anethum graveolens* and *Levisticum officinale*) belonging to the same family (*Apiaceae*) towards identical substrates (Faria et al., 2009; Nunes et al., 2009) which indicated exclusive role of the source plant species with characteristic enzymatic profile, rather than the entire plant family, on the biotransformation propensity towards external substrates. With regards to the utilization of hairy root generating *A. rhizogenes* strains, three strains (LBA 9402, A4 and ATCC 15834) showed prominence than the rest (Fig. 3) possibly due to precisely specific susceptibilities of the targeted plants towards these strains. Likewise, in view of the types of media used, Murashige and Skoog's (1962) medium at different concentrations and with/without plant growth hormones acquired maximum applications, even though diversity ruled the situations (Fig. 4). So far as the rate of bioconversion is concerned, diverse results could be noted as indicated in Table 1.

Sucrose had commonly been used as the carbohydrate source for hairy root growth. However, the efficacies of different carbohydrate sources (sucrose, glucose, mannitol, sorbitol) were evaluated in case of *B. candida* hairy root mediated biotransformation of hydroquinone, where sucrose proclaimed superior to others (Casas et al., 1998). The effects of different carbohydrates (sucrose, glucose, fructose and mannitol), and anti-oxidants (gallic acid and ascorbic acid) had been evaluated in yet another study involving *Panex ginseng* hairy root cultures where the glycosylation potentials were enhanced towards the carboxylic acid moiety in case of biotransformation of *p*- amd *m*-hydroxybenzoic acid. (Chen et al., 2008). One more glucosylation reaction carried out by *Datura tatula* hairy root cultures reported addition of salicylic acid for biotransformation of *p*-hydroxybenzyl alcohol into Gastrodin (Peng et al., 2008). So far as the duration of reactions are concerned, the time range varied from 20 h (Flores et al., 1994) to 25 days (Peng et al., 2008) and in majority of the reported hairy root mediated biotransformation events, the transformed products were recovered both from the root biomass as well as from the media.

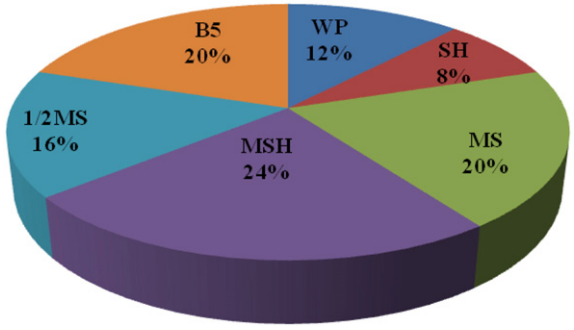

Fig. 4. Media specification utilized for hairy root mediated biotransformation.

4. Conclusion

The detailed literature survey clearly indicated that hairy root cultures are entering into a new phase of applied research in generating pharmaceutical lead compounds by bringing about chemical transformations aided through these unique biological systems. Although hairy root mediated *in-vitro* biotransformation studies are less investigated than those using plant cells and enzymes, but their genetic/biochemical stability, hormone-autotrophy, multi-enzyme biosynthetic potential and relatively low-cost cultural requirements instilled new dynamism to hairy root cultures as model system for *in-vitro* biotransformations of exogenous substrates. In reality, the variations in biosynthetic abilities among hairy root clones due to differences in insertion sites/copy number of Ri T-DNA ensure diversification of the pathway enzymes through activation or suppression of the functional genes. This leads to appreciatively pragmatic, illimitable opportunities to explore and utilize the operating pathway related enzymes of hairy root clones of a variety of medicinal plants for biotransformation mediated diversification of wide range of chemical moiety for their value addition. Indisputably, establishment of effective and economical scaled-up culture systems would bring about biggest benefits out of the success stories of hairy root mediated *in-vitro* biotransformations. The cost-effectiveness and clinical performances of the biotransformed products currently being developed may well establish the future of this fascinating technology.

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