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Precursor feeding for enhanced production of Secondary metabolites: A review

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ABSTRACT

Medicinal plants are unique sources of pharmaceutically significant secondary metabolites which include alkaloids, glycosides, flavonoids, volatile oils, tannins, resins etc. Demands of these secondary metabolites are not readily accomplished because of their low yield in intact plants, environmental, geographical and/or governmental restrictions. Chemical synthesis or semi-synthesis of these metabolites are either extremely difficult or economically infeasible because of their highly complex structures and stereospecific chemical nature. Plant cell culture is an attractive alternative, but to date this has had only limited commercial success. Precursor feeding has been a successful approach for enhanced production of secondary metabolites from plant cells grown *in vitro*. Present review summarizes the application of precursor feeding for enhanced production of secondary metabolites from plant cells.

KEY WORDS: Pharmaceuticals, precursor, precursor feeding, secondary metabolites.

INTRODUCTION

Medicinal plants are the most exclusive source of life saving drugs for the majority of the world's population. Apart from primary metabolites (i.e. carbohydrates, lipids and amino acids), plants synthesize a wide variety of secondary metabolites like alkaloids, glycosides, steroids, flavonoids, volatile oils, tannins, resins etc. Figure 1 represents the biosynthetic origin of some important plant derived secondary metabolites. These compounds may represent chemical adaptations to environmental stress, or they may serve as defensive, protective or offensive chemicals against microorganisms, insects and higher herbivorous predators. They are some times considered to be waste or secretary products of plant metabolism (1-3).

Possible modes of obtaining these secondary metabolites are: extraction from natural sources, partial synthesis from structurally similar compounds, total synthesis and through plant biotechnology. Direct isolation from plants involves cultivation of plants, harvesting the desired parts, extraction and purification of desired product. This conventional method has a number of limitations such as plants are often not readily available because of geographical or governmental restrictions, requires huge area of land, which otherwise can be utilized for primary food crop. The process is labor intensive and time consuming, the quality may be affected by unforeseen environmental conditions and demands and supplies are difficult to manage. Total or partial synthesis of secondary metabolites proved to be extremely difficult and very expensive.

Plant tissue culture technique has become a powerful tool in plant biotechnology. The potential of plant tissue culture for plant propagation and production of secondary metabolites has itself provided substantial impetus for research (4). However, the use of plant cell or organ cultures has had only limited commercial success. This is explained by the empirical nature of selecting high-yielding, stable cultures and the lack of understanding of how secondary metabolites are synthesized or how their synthesis is regulated (5, 6). Many biotechnological strategies have been hypothesized and experimented for enhanced production of secondary metabolites from medicinal plants. Some of these include screening of high yielding cell line, media modification, precursor feeding, elicitation, large scale cultivation in bioreactor system, hairy root culture, plant cell immobilization, biotransformation, genetic manipulations and others (1, 7-10). Present review highlights precursor feeding as one of the strategies for enhanced production of secondary metabolites through plant cells cultured *in vitro*.

PRECURSOR FEEDING

On the basis of the knowledge of the biosynthetic pathways, several organic compounds have been added to the culture medium in order to enhance the synthesis of secondary metabolites. The exogenous supply of a biosynthetic precursor to culture medium may increase the yield of the desired product. This approach is useful when the precursors are inexpensive. The concept is based on the idea that any compound, which is an intermediate, in or at the beginning of a secondary metabolite biosynthetic route, stands a good chance of increasing the yield of the final product. Attempts to induce or increase the production of plant secondary metabolites, by supplying precursor or intermediate compounds, have been effective in many cases (11-13). For example, amino acids have been added to cell suspension culture media for production of tropane alkaloids, indole alkaloids etc. Addition of phenylalanine to *Salvia officinalis*

Table 1. Effect of precursor feeding on the production of secondary metabolites in various medicinal plants.

Sl. No.	Culture	Precursor	Product	Reference
1	*Aesculus californica*	Isoleucine	2-Amino-4-methylhex-4-enoic acid	(24)
2	*Ageratum houstonianum*	^{14}C-labelled precocene I	Precocene II.	(25)
3	*Allium cepa*	S-alk(en)yl—cysteine sulphoxides	Γ-Glutamyl peptides	(26)
4	*Atropa belladonna*	Tropic acid, tropinone and tropanoltropic acid, tropinone, tropanol	Tropane alkaloid	(27)
5	*Azadirachta indica*	Sodium acetate, Squaline, IPP, GPP	Azardirachtin	(28)
6	*Camptotheca acuminata*	[1′-^{14}C]-tryptophan, [Ar-$^{3}H_4$]-tryptophan, [Ar-$^{3}H_4$,1′-^{14}C] tryptophan,	Camptothecin.	(29)
7	*Capsicum annuum*	L-tyrosine, DL-DOPA	Capsaicin	(30)
8	*Capsicum frutescens*	Phenyalanine, Valine, Vanillyl amine, Isocapric acid, Coumaric acid, Cinnamic acid, Ferulic acid	Capsaicin	(31-33)
9	*Capsicum frutescens*	Ferulic acid and vanillylamine	Vanillin and capsaicin	(34)
10	*Catharanthus roseus*	Secologanin, loganin or loganic acid	Ajmalicine and strictosidine	(11)
11	*Catharanthus roseus*	Tryptophan	Ajmalicine	(3)
12	*Catharanthus roseus*	Methyl jasmonate	Terpenoid indole alkaloid (TIA)	(35)
13	*Ceanothus americanus*	Amino acids	Macrocyclic alkaloids	(36)
14	*Celosia plumosa*	Betanidin and betanin	Amaranthin	(37)
15	*Cephaelis ipecacuanha*	L-tyrosine, Phenyalanine, Shikimic acid	Cephaeline	(38), (39)
16	*Cicer arietinum*	4,2′,4′-trihydroxychalcone [-carbonyl-^{14}C] and (–)-7,4′-dihydroxyflavanone-[T]	Flavonoids	(40)
17	*Cistanche deserticola*	l-Phenylalanine, l-tyrosine, sodium acetate, phenylacetic acid	Phenylethanoid glycosides (PeG)	(41)
18	*Coleus blumei*	Phenyalanine	Rosmaric acid	(42)
19	*Convallaria majalis*	(R,S)-[1-14C, 15N]Methionine	Azetidine-2-carboxylic acid (A-2-C)	(43)
20	*Coreopsis tinctoria*	Phenylalanine	1′-Isobutyryloxyeugenol-4-isobutyrate	(44)
21	*Coryphantha macromeris var. runyonii*	N-methyltyramine	N-methyl-3,4-dimethoxy-β-hydroxyphenethylamine	(45)
22	*Datura tabula*	Tropic acid	Tropic acid	(46),(47)
23	*Datura stramonium*	Cinnamoyl-[2-^{14}C]-tropine-[N-methyl-^{14}C]	Atropine , scopolamine	(48)
24	*Daucus carota*	Sinapic acid	Anthocyanin	(49)
25	*Delonix regia*	A,γ-Diaminobutyric acid	L-Aazetidine-2-carboxylic acid	(50)
26	*Digitalis lanata*	Cholesterol, sitosterol and stigmasterol	Pregnane derivatives	(51)
27	*Digitalis purpurea*	Glucose	Digitoxose	(52)
28	*Dioscorea deltoidea*	Cholesterol	Diosogenin	(46), (47)
29	*Duboisia leichhardtii*	Phenylalanine	Tropane alkaloid	(53)
30	*Eschscholtzia californica*	(S)-reticuline, L-tyrosine	Benzophenanthridine, alkaloids	(54)
31	*Eucomis bicolor*	Phenylalanine-[1-^{14}C], -phenylalanine-[U-^{14}C], sodium acetate-[2-^{14}C]	3-Benzylchroman-4-one eucomin	(55)
32	*Ginkgo biloba*	Terpenoid	Bilobalide and ginkgolides	(56)
33	*Glycine max*	^{14}C-labelled phenylalanine, daidzein, 7,2′,4′-trihydroxyisoflavone, 3,9-dihydroxypterocarpan and glycinol	Phytoalexins glyceollins I, II and III	(57)
34	*Hebenstretia dentata*	8-Epi-iridodial, 8-epi-iridotrial	Ipolamiide and lamiide, the iridoid glucosides	(58)
35	*Heimia salicifolia*	Phenylalanine	Cryogenine	(59)
36	*Helianthus annuus*	$CuCl_2$ and sucrose	Coumarin phytoalexins, scopoletin and ayapin	(60)
37	*Holarrhena*	Cholesterol	Conessine, a steroidal alkaloid	(61)

	antidysenterica			
38	*Hypericum perforatum*	l-[U-(13)C(5)]Valine and l-[U-(13)C(6)]isoleucine	Hyperforin and adhyperforin	(62)
39	*Lithospermum erythrorhizon*	L-Phenyalanine	Rosmaric acid	(46), (47)
40	*Lunaria annua*	Phenylalanine	Alkaloid lunarine	(63)
41	*Mentha piperita*	Mevalonate-2-^{14}C	Mono- and sesqui-terpenes	(64)
42	*Morinda citrifolia*	O-succinyl benzoic acid	Anthraquinons	965)
43	*Nicotiana tabacum*	Ornithine	Nicotine	(65)
44	*Nicotiana tabacum*	Spermidine	P-Coumaroylspermidin , cinnamoylputrescines	(66)
45	*Nicotiana tabacum*	Nornicotine	nicotine.	(67)
46	*Oryza sativa*	[26,27- 13C2]-24-methyl-gD24-cholesterol and [26,27- 13C2]-24-ethyl-Δ24-cholesterol	(24R)- and (24S)-24-methylcholesterols (24R)-24-ethylcholesterol	(68)
47	*Oryza sativa*	D-glucose or L-galactono-gamma-lactone	L-ascorbic acid	(69)
48	*Papaver bracteatum*	Tetrahydropalmatine , tetrahydropalmatine methiodide	Bnzazepine alkaloid alpinigenine	(70)
49	*Phaseolus vulgaris*	2′,4′,4-Trihydroxychalcone, daidzein, 7,2′,4′-trihydroxyisoflavone, 3,9-dihydroxypterocarpan and phaseollidin	Pytoalexin phaseollin	(71)
50	*Podophyllum hexandrum*	Phenylalanine, cinnamic acid and ferulic acid	Pdophyllotoxin and 4′-demethylpodophyllotoxin	(72)
51	*Portulaca grandiflora*	3,4-Dihydroxyphenylalanine	Bexanthin	(73)
52	*Rhodiola sachalinensis*	l-Phenylalanine, l-tyrosol and l-tyrosine	Salidroside	(74)
53	*Ricinus communis*	Glycerol ,succinic acid	Ricinine	(75)
54	*Ruta graveolens*	4-Hydroxy 2-Quinoline	Dictamine	(65)
55	*Scopilia japonica*	Tropic acid	Scopolamine	(46), (47)
56	*Securinega suffruticosa*	Tyrosine	Securinine alkaloid	(76)
57	*Strawberry cells*	L-Phenylalanine	Anthocyanin	(77)
58	*Taxus chinesis*	Phenyalanine, Benzoyl glycine	Taxol	(78)
59	*Taxus wallichiana*	Sodium acetate, Benzoyl glycine, Phenyalanine Luicine	Taxol, baccatin DBA	(79)
60	*Taxus cuspidata*	Phenyalanine, Benzoic acid, Serine, Benzoyl glycine, Glycine	Taxol	(16), (80)
61	*Taxus cuspidata*	Carboxylic acid and amino acid	Taxol	(16)
62	*Thalictrum minus*	Reticuline, isoboldine	Thalicarpine	(81)
63	*Trifolium pratense*	7,2′-Dihydroxy-4′-methoxy-isoflavone, isoflavanone	Phytoalexin demethylhomopterocarpin	(82)
64	*Trifolium pratense*	Chalone and isoflavone	Demethylhomopterocarpin , maackiain	(83)
65	*Vanilla planifolia*	Cinnamic acid and ferulic acid	p-Hydroxy benzoic acid and vanillic acid	(84)
66	*Vigna unguiculata*	Phenylalanine	2-Arylbenzofuran phytoalexin vignafuran	(85)

cell suspension cultures stimulated the production of rosmarinic acid (14). Addition of the same precursor resulted stimulation of taxol production in *Taxus* cultures (15, 16). Feeding ferulic acid to cultures of *Vanilla planifolia* resulted in increase in vanillin accumulation (17). Furthermore, addition ofleucine, led to enhancement of volatile monoterpenes in cultures of *Perilla frutiscens*, where as addition of geraniol to rose cell cultures led to accumulation of nerol and citronellol (18).

Precursor feeding has been a successful approach. In particular feeding of loganin, secologanin and tryptamine has been studied extensively. In most *C. roseus* cell lines the availability of secologanin is a limiting factor for alkaloid accumulation. Only by selection, a cell line was obtained, which accumulated secologanin in addition to tryptamine, strictosidine and ajmalicine. An increase of the inoculum size from 40 to 160 g fresh weight per liter medium favored the accumulation of secologanin and alkaloids (19). Extensive precursor feeding experiments were performed using transgenic *C. roseus* cell lines overexpressing Tdc and/or Str (20, 21). Loganin feeding to these cell lines efficiently increased alkaloid accumulation. Precursor feeding studies with hairy root cultures revealed also a limitation in the terpenoid pathway. After feeding geraniol, 10-hydroxygeraniol or loganin resulted in a significant increase of tabersonine accumulation (22). Similarly, Namdeo (3) reported that the effect of tryptophan addition on ajmalicine

in *C. roseus* cells cultured in Zenk's production medium, maximum ajmalicine (310 μg g^{-1} dry weight) was recorded in medium with 100 mg l^{-1} tryptophan followed by 292 and 140 μg g^{-1} dry weight ajmalicine in medium with 250 and 50 mg l^{-1} tryptophan respectively. Higher concentrations of tryptophan inhibited ajmalicine production. In contrast, suspension cultures of *C. roseus* synthesized higher concentrations of ajmalicine in 20 l airlift bioreactor by addition of tryptophan as a precursor in culture medium (23). Moreno et al., (11) studied the effect of feeding different terpenoid precursors on alkaloid production. They observed that the addition of secologanin, its precursor loganin and loganic acid increased the accumulation of ajmalicine and strictosidine. Table 1 summarizes the effective utilization of precursor feeding for the production of secondary metabolites.

CONCLUSION

Secondary metabolites of pharmaceutical significance are widely employed either directly or indirectly by a large number of pharmaceutical industries. The regular supply of these secondary metabolites has many limitations. It is well recognized that cultured plant cells represents a potential source of phyto-pharmaceuticals but very few cell cultures synthesize secondary metabolites in comparison to those produced in intact plants. Many strategies have been tried to synthesize the desired secondary metabolites in appreciable quantity and at competitive economic value but fail to gain commercial exploitation. This may reflects the poor understanding of basic secondary metabolic regulation in plant cell cultures. Precursor feeding to plant cell culture system may be promising as it showed favorable results. The key to successful protocol using precursor feeding lies in identification of cheapest by product of other process which can be converted to desired secondary metabolite by selected plant cell line. Though, precursor feeding enhances secondary metabolism, the exact mechanism of biosynthesis is still not exactly understood. An intensive research in this field for exploitation of plant cells for the production of secondary metabolites is envisaged.

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