



Biotransformation of salicylaldehyde to salicin using *Varthemia persica* cell suspension cultures

Gholamreza Asghari*, Maryam Mosaeybi

Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Cell cultures of *Varthemia persica* DC. have been studied to evaluate their abilities in biotransformation of aromatic and aliphatic precursors. *V. Persica* (Asteraceae) is an aromatic plant growing in Iran. *V. persica* contain different terpens but its cell culture does not posses these compounds. Callus cultures of *V. persica* was established from seedlings and healthy suspensions were grown using Murashige and Skoog (MS) medium supplemented with 2,4-dichlorophenoxy acetic acid (2 mg/l) and kinetin (0.2 mg/l). Then exogenous precursors were fed to *V. persica* cell suspension cultures. Biotransformation reactions were monitored after 24 h of incubation. The cultures then extracted with dichloromethane or methanol and concentrated within nitrogen stream. The extracts subjected to gas chromatography (GC) or thin layer chromatography (TLC) analysis. *V. persica* cultured cells in this study seem to exhibit ability in glucosylation of salicylaldehyde to salicin. No conversion was observed with several precursors fed to the cultures. The ability of cultured plant cells in biotransformation of the precursors appears to be depending on the substrate structure and active enzymes available in the cultures. It seems that in cultured cells of this plant only glucosylation enzymes are active.

Keywords: Biotransformation; Cell suspension culture; Salicin; Salicyladehyde; *Varthemia persica*.

Received: September 12, 2008; **Accepted:** December 20, 2008

1. Introduction

Salicin, salicylic acid-O-p-D-glucopyranoside, is a naturally occurring glycoside found in the bark of willow trees (*Salix* spp.) and used as a template for the synthesis of acetylsalicylic acid, known as aspirin. Ancient

man used it as an analgesic and antipyretic. Salicin plays an important role in plant-herbivore interactions[1, 2].

Varthemia persica DC. (Asteraceae) is an aromatic plant growing wild in central parts of Iran [3]. The essential oil of *V. persica* has been reported to have several mono- and sesquiterpenes, specially β -eudesmol and spathulenol [4]. There is no report on cell cultures of *V. persica*.

*Corresponding author: Gholamreza Asghari Isfahan, Pharmaceutical Sciences Research Center, School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, I.R.Iran. P.O. Box: 81745-359.
Tel (+98)311-7922644; Fax (+98)311-6680011
E-mail: asghari@pharm.mui.ac.ir

Plant cell cultures are capable of glucosylation of exogenously added phenols and their analogues. Several reports on biotransformation of salicylaldehyde, salicyl alcohol, and salicylic acid as precursors for the production of glycosides have been published in the literature. Mizukami *et al.* studied glucosylation of salicyl alcohol in several cultured plant cells [6]. High level production of salicin from salicylic acid has been achieved by cell suspension cultures of *Catharanthus roseus* [7, 8]. It was reported that salicylaldehyde is the optimal precursor of salicin in cultured cells of *Datura meteloides* and *Coronilla varia* [9]. Also salicin was enzymatically synthesized from salicylic acid and salicyl alcohol using glucanyltransferase of *Bacillus subtilis* macerans and sucrose dextranucrase of *Leuconostoc mesenteroides* [10]. So far, there is no report on using cultured cells of *V. persica* for salicylaldehyde biotransformation studies.

2. Materials and methods

2.1. Cell cultures

Seeds of *V. persica* were surface sterilized in 30% w/v hydrogen peroxide containing 1% tween 80 for 2 min., then germinated on wet filter paper in Petri dishes in the dark at 25 °C. The cotyledons were then transferred onto Murashige and Skoog (MS) media containing 5 ppm ascorbic acid, 2 ppm 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.2 ppm kinetin (kin) [11]. Calli were maintained by subculturing every 4 weeks, and suspension cultures were formed by agitation of 5 g callus to liquid medium until a suspension of free cells was formed. The suspensions were then placed on a rotary shaker running at 100 rpm, and were maintained by subsequent subculturing, using a dilution of 1 to 2, into new fresh liquid media.

The callus and suspensions were maintained in a 16 h light / 8 h dark cycle at 27 °C and subcultured every 4-6 weeks.

Suspension cultures grown over more than twenty generations were used for substrate feeding and bioconversion studies.

2.2. Substrate feeding and product extraction

The salicylic acid, salicylaldehyde, benzaldehyde, anisaldehyde, citral, eugenol, and safrole obtained from Sigma. Chemical purity (greater than 98%). Substrates were dissolved in a water-miscible solvent (ethanol 70%), which resulted in good mixing of the substrate upon addition to the aqueous medium. The substrate was added to suspension cultures to make a final concentration of 100 ppm, cell volume (50% p.v.). Control readings were made without addition of substrate to cultures and with addition of substrate to cell-free medium. The cultures were incubated under the conditions mentioned above. After the incubation period, the flask was swirled to ensure good mixing and two samples were

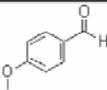
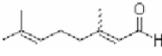
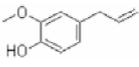
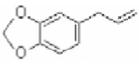
Precursor	Structure
Anisaldehyde	
Benzaldehyde	
Citral	
Eugenol	
Safrole	
Salicylic acid	
Salicylaldehyde	

Table 1: Precursor fed to *Varthemia persica* cells suspension.

removed with a 10-ml pre-sterilized, glass-tipped pipette. A new pipette was used for each sample [12].

The substrates and products were extracted from the dried cells which had been filtered from suspension cultures. The extract was reduced to a final volume of 1 ml and subjected to thin layer chromatography (TLC).

2.3. Analysis

TLC analysis were carried out as following: the concentrated methanolic extracts obtained were spotted on precoated silicagel plates (Merck) and chromatographed in a saturated chamber containing EtO-AC: MeOH: H₂O (77:13:10) solvent mixture. Visualisation of the separated bands was carried out under U.V. light (365 nm) [13]. The glycosides were detected as a brown and red individual bands on extract obtained from salicylaldehyde feeding. The dried plates were then sprayed with vanillin-glacial acetic acid reagent.

Gas chromatography (GC) analysis were carried out as following: After 24 h of substrates feeding both the cells and the media were extracted using dichloromethane. The extract was reduced to a volume of 100 µl under nitrogen, then 0.1 µl was analyzed by gas liquid chromatography (GLC). GC analysis was carried out on a Perkin-Elmer 8500 GC with FID detector and a BP-1 capillary column (39 m×0.25 mm; film thickness 0.25 µm). The carrier gas was He with a flow rate of 2 ml/min., the oven temperature for first 4 min. was kept at 60 °C and then increased at a rate of 4 °C /min. until reached to the temperature of 280 °C, injector and detector temperatures were set at 280 °C. Confirmation of peak identity was effected by co-chromatography with standards [14].

3. Results and discussion

Several substrates including; two aromatic aldehydes, an aliphatic aldehyde, an aromatic

acids, a phenol and an ether phenol were fed to *V. persica* cell suspension cultures to study the possible enzymatic conversion. No metabolism was detected over 48 h of incubation when the cultures were extracted with dichloromethane and methanol and extracts subjected to GC and TLC analysis, except for salicylaldehyde in which case salicin were detected as biotransformation products (Table 1).

V. persica cell suspension cultures were able to produce salicin from salicyl aldehyde when non-quantitative TLC analysis was viewed. No biotransformation was detected over 24 h when salicylic acid was administered to the cells. Zenk (1967) suggested that salicyl alcohol is not a direct precursor of salicin, these findings may suggest the non necessity of alcohol formation for salicin production in these cultured cells [15]. Terao *et al.* (1984) described a novel enzyme catalyzing a position-specific glycosylation of salicyl alcohol to form salicin in *Gardenia jasminoides* cell cultures [16]. No salicyl alcohol formation was observed in our study. It has been reported that cell suspensions of some plant species glucosylate salicyl alcohol to form the phenolic glycoside salicin whereas cultures of other species form the corresponding alcohol glycoside, isosalicin, when they are fed with salicyl alcohol [6, 17]. It appears that the glycosylation reaction may vary with culture strains as well as chemical structures of substrates. No conversion was achieved when

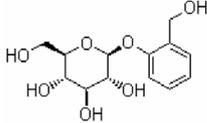
Precursor	Product
Salicylaldehyde	Salicin
	

Table2: Salicin bioproduced using *Varthemia persica* cells suspension.

we administered benzaldehyde, anisaldehyde, citral, eugenol, and safrole to cultured cells of *V. persica*. Similarly, Tabata *et al.* (1988) have shown that from cell suspension cultures from ten different plant species, only six were able to glucosylate salicyl alcohol [18]. Dombrowski and Alfermann (1992) found that suspensions from 12 different plant species converted salicyl alcohol to salicin and isosalicin or only isosalicin [19, 20].

V. persica cell cultures were not able to glucosylate benzaldehyde, anisaldehyde, and citral. The position of glycosylation in the substrate molecules seems to be specific. In this connection it has been reported that *Bupleurum falcatum* failed to glucosylate *m*-hydroxybenzoic acid, whereas some other culture strains glucosylated *m*- and *p*-hydroxybenzoic acid [18].

Interestingly, the salicylaldehyde administered was converted to the salicin within 24 h (Table 2), whereas other culture strains derived from different plant species produced salicin only within four days [21]. It seems the successful glycosylation of an exogenous substrate requires a strong glucosyltransferase activity. The interesting point to note is that the reverse reaction, conversion of salicin to salicylaldehyde, can be seen in plant defensive situation [22].

In plant cells, glucosylation reactions are of special interest because they facilitate the conversion of water-insoluble compounds to water-soluble compounds. Plant systems typically retain secondary metabolites intracellularly, therefore their accumulation might interfere with basic physiological functions, especially if the compounds are toxic or precipitate due to low solubility. Moreover, the investigation of phenolic glycosylation is of great importance in view of the possible application of plant cell culture to an efficient production of useful compounds.

Acknowledgment

This research was supported by a grant

from the research council of the Isfahan University of Medical Sciences.

References

- [1] Samuelsson G. *Drug of natural origin*. 4th ed. Stockholm: Swedish Pharmaceutical Press, 1999; pp. 283-5.
- [2] Teija M.R. Maija-Riitta K. Julkunen T. Salicylates of intact *Salix myrsinifolia* plantlets do not undergo rapid metabolic turnover. *Plant Physiol* 2000; 122: 895-906.
- [3] Gahraman A, Attar F. *Biodiversity of Plant species in Iran*. Tehran: Tehran University Press, 1998; p. 327.
- [4] Ghasemi N, Asghari G, Shams Ardakani A, Siahpoush A. Characterization of volatile constituents from aerial parts of *Varthemia persica* DC. (var. *persica*). *IJPR* 2003; 2: 241-3.
- [5] Suresh B, Ravishankar GA. Methyl jasmonate modulated biotransformation of phenylpropanoids to vanillin related metabolites using *Capsicum frutescens* root cultures. *Plant Physiol Biochem* 2005; 43: 125-31.
- [6] Mizukami H, Terao T, Miura H, Ohashi H. Glucosylation of salicyl alcohol in cultured plant cells. *Phytochemistry* 1983; 22: 679-80
- [7] Hirata T, Koya K, Sarfo KJ, Shimoda K, Ito DI, Izumi S, Ohta S, Lee YS. Glucosylation of benzyl alcohols by the cultured suspension cells of *Nicotiana tabacum* and *Catharanthus roseus*. *J Molecul Catal B: Enzymatic* 1999; 6: 67-73.
- [8] Inomata S, Yokoyama M, Seto S, Yanagi M. High level production of salicin from salicylic acid in suspension cultures of *Catharanthus roseus* plant cell. *Appl Microbiol Biotechnol* 1991; 36: 315-9.
- [9] Duskov J, Dusek J, Jahod L, Poustka F. Arbutin, salicin: The possibilities of their biotechnological production. *Ceska Slov Farm* 2005; 54: 78-81.
- [10] Yoon SH, Bruce Fulton D, Robyt JF. Enzymatic synthesis of two salicin analogues by reaction of salicyl alcohol with *Bacillus macerans* cyclomaltodextrin glucanyltransferase and *Leuconostoc mesenteroides* B-742CB dex-transucrase. *Carbohydr Res* 2004; 339: 1517-29
- [11] Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 1962; 15: 437-9.
- [12] Asghari G, Lockwood GB. Stereospecific biotransformation of phenylethyl propionate by cell culture of *Peganum harmala*. *Iranian Biomed J* 2002; 6: 43-6.
- [13] Wagner H, Bladt S. *Plant drug analysis*. New York: Springer-Verlag, 1996: pp. 204-30.
- [14] Davies NW. Gas chromatographic retention

- indices of monoterpenes and sesquiterpenes on methyl silicon, and Carbowax 20 m phases. *J Chromatogr* 1990; 503: 1-24.
- [15] Zenk MH. Pathways of salicyl alcohol and salicin in *Salix purpurea* L. *Phytochemistry* 1967; 6: 245-52.
- [16] Terao T, Ohashi H, Mizukami H. Position-specific glucosylation of salicyl alcohol with an enzyme preparation from *Gardenia jasminoides* cultured cells. *Plant Sci Lett* 1984; 33: 47-52.
- [17] Tabata M, Ikeda F, Hiraoka N, Konoshima M. Glucosylation of phenolic compounds by *Datura innoxia* suspension cultures. *Phytochemistry* 1976; 15: 1225-9.
- [18] Tabata M, Umetani Y, Ooya M, Tanaka S. Glucosylation of phenolic compounds by plant cell cultures. *Phytochemistry* 1988; 27: 808-13.
- [19] Dombrowski K, Alfermann AW. Glucosylation of salicylic acid and its derivatives on cultured *Salix matsudana* cells. *40th Ann. Cong. on Medicinal Plant Res.* Trieste, 1992; pp. 1-5.
- [20] Julkunen-Tiitto R, Petersen M, Alfermann AW, Sorsa S, Roininen H. The biotransformation of salicyl alcohol, helicin and salicin by *Salix* spp. cell cultures. *GA-Symposium*. Prague, 1996; pp. 2-6.
- [21] Misukami H, Terao T, Amano A, Ohashi H. Glucosylation of salicyl alcohol by *Gardenia jasminoides* cell cultures. *Plant Cell Physiol*. 1986; 27: 645-50.
- [22] Pasteels JM, Rowell-Rahier M, Braekman JC, Dupont A. Salicin from host plants as precursor of salicylaldehyde in defensive secretion of *Chrysomelinae* larvae. *Physiol Entom* 1983; 8: 307-14.

ONLINE SUBMISSION

ijps.sums.ac.ir