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Secondary metabolites: A review

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Abstract
Plants secondary metabolites are unique sources for pharmaceuticals food additives, flavors and others industrial values. Commercial importance of these secondary metabolites has resulted in a great interest in its production and in exploring possibilities of enhancing its production by means of tissue culture technology in the recent years. Plants cell culture technologies were introduced at the end of 1960's as a possible tool for both studying and producing plant secondary metabolites. The focus of the present review is the application of tissue culture technology for the production of some others important plant secondary metabolites. The present communication deals the brief introduction, classification, recent improvements of production, anti-microbial properties and importance of plant secondary metabolites.

Keywords: secondary metabolites, alkaloids, essential oil, saponins

Introduction

The term metabolite is usually restricted to small molecules. Metabolites have various functions, including fuel, structure, signaling, stimulatory and inhibitory effects on enzymes, catalytic activity of their own (usually as a cofactor to an enzyme), defense, and interactions with other organisms. Plant produce a vast and diverse assortment of organic compounds the great majority of which do not appear to participate directly in growth and development. These substances, traditionally referred to as secondary metabolites, often are differentially distributed among limited taxonomic groups within the plant kingdom. The evolving commercial importance of secondary metabolites has in recent years resulted in a great interest particularly in the possibility of altering the production of bioactive plant metabolites by means of tissue culture technology [1]. Plant cell and tissue culture technologies can be established routinely under sterile conditions from explants, such as leaves, stems, roots, and meristems for both the ways for multiplication and extraction of secondary metabolites. *In vitro* production of secondary metabolite in plant cell suspension cultures has been reported from commercial medicinal plants.

Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavors, and other industrial materials[1] and the use of plant cell cultures has overcome several inconveniences for the production of these secondary metabolites. Organized cultures, and especially root cultures, can make a significant contribution in the production of secondary metabolites. Secondary metabolites are described as an organic compounds which were not directly involved in the normal growth, development, or reproduction of an organism [2]. These metabolites are those metabolites which are often produced in a phase of subsequent to growth, have no function in growth (although they may have survival function), are produced by certain restricted taxonomic groups of microorganisms, have unusual chemicals structures, and are often formed as mixtures of closely related members of a chemical family.

Unlike primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism's survivability, fecundity, or aesthetics, or perhaps in no significant change at all. These are often restricted to a narrow set of species within a phylogenetic group. And these are also play an important role in plant defense against herbivory[4] and other interspecies defenses [5]. Humans use secondary metabolites as medicines, flavorings, and recreational drugs in the recent past.

Srivastava *et al.* (2007) [51] also added that use differentiated cultures instead of cell suspension cultures have focused on transformed (hairy) roots. Smetanska (2008) [47] has summarized the process for obtaining the secondary metabolites from plant cell cultures is represented as a multi-stage strategy, and each link should be described according to specifications of cell cultures or products [7]. For the establishing of high-producing and fast-growing cell lines, the parent plants should be selected. The expression of synthetic pathways can be influenced by environmental conditions, the supply of precursors, and the application of elicitors, and it can be altered by special treatments such as biotransformation and immobilization.

Kossel was the first to define these metabolites as opposed to primary ones. It has been clearly demonstrated that secondary products play a major role in the adaptation of plants to their environment (Kossel *et al.*, 1981) [1]. Due to antibiotic, antifungal, and antiviral, activities have been recorded to ability to protect plants from pathogens [8].

Classification

Secondary metabolites can be classified on the basis of chemical structure (for example, having rings, containing a sugar), composition (containing nitrogen or not), their solubility in various solvents, or the pathway by which they

are synthesized (e.g., phenylpropanoid, which produces tannins). And usually classified according to their biosynthetic pathways. Three large molecule families are generally considered: Phenolics, Terpenes and Steroids, and Alkaloids, Flavanoids[10].Some of them can have severe consequences.

Alkaloids
Alkaloids are originally defined as pharmacologically active, nitrogen-containing basic compounds of plant origin. And they can block ion channels, inhibit enzymes, or interfere with neurotransmission, producing hallucinations, loss of coordination, convulsions, vomiting, and death.

Phenolics
Phenolics interfere with digestion, slow growth, block enzyme activity and cell division, or just taste awful.

Terpenes
Terpenes are among the most widespread chemically diverse groups of natural products. Terpenes are a unique group of hydrocarbon-based natural products whose structures may be derived from isoprene. Terpenes are classified by the number of 5-carbon units. The function of terpenes in plants is generally considered to be both ecological and physiological: Allelopathy, Insecticidal, Insect pollinators, Plant hormone (Abscisic acid, gibberellins).

Flavonoids
With more than 4500 different representatives known thus far, the flavonoids constitute an enormous class of phenolic natural products. Present in most plant tissues, often in vacuoles, flavonoids can occur as monomers, dimmers and higher oligomers.
Flavonoids comprise a diverse set of compounds and perform a wide range of functions. Specific flavonoids can also function to protect plants against UV-B irradiation. The flavonoids consist of various groups of plant metabolites which include chalcones, aurones, flavanones, isoflavonoids, flavones, Flavonols, leucoanthocyanidins, catechins, and anthocyanins.

Coumarins and Stilbenes
Coumarins belong to a widespread family of plant metabolites called the benzopyrones, with more than 1500 representative in more than 800 species. In plants these compounds can occur in seed coats, fruits, flowers, roots, leaves, stems, although in general the greatest concentration are found in fruits and flowers. Their roles in plants appear to be mainly defense-related, given their antimicrobial antifectant, UV-screening and germination inhibitor properties. Stilbenes are present in bryophytes, pteridophytes, gymnosperms and angiosperms, with more than 300 different stilbenoids known today. The stilbenes play important roles in plants, particularly in heartwood protection and also have significance in pharmacology and human health.
Due to their large biological activities, plant secondary metabolites have been used for centuries in traditional medicine. Nowadays, they correspond to valuable compounds such as pharmaceutics, cosmetics, fine chemicals, or more recently nutraceuticals. Recent surveys have established that in western countries, where chemistry is the backbone of the pharmaceutical industry, 25% of the molecules used are of natural plant origin.

Production of secondary metabolites and recent improvements of production process
Plant secondary metabolites are unique resources for pharmaceuticals, food additives, and fine chemicals. They also provide original materials used in other areas. Besides direct extraction from plants, and chemical synthesis to provide those compounds or derivatives with similar uses, plant cell culture has been developed as a promising alternative for producing metabolites that are difficult to be obtained by chemical synthesis or plant extraction. However, in spite of decades of efforts, production of plant secondary metabolites by plant cell culture technology is still facing many biological and biotechnological limitations. One of the major obstacles is the low yield of plant secondary metabolites in plant cell cultures. Since the major roles of plant secondary metabolites are to protect plants from attack by insect, herbivores and pathogens, or to survive other biotic and a biotic stresses, some strategies for culture production of the metabolites based on this principle have been developed to improve the yield of such plant secondary metabolites and they include treatment with various elicitors, signal compounds, and a biotic stresses. Many such treatments indeed effectively promote the production of a wide range of plant secondary metabolites, both *In vivo* and *In vitro*. However, the productivity is still rarely competitive for industrial application.
A recent work for improving production of plant secondary metabolites was mainly focused on the following aspects: 1) manipulation of plant cell cultures to improve productivity of target compounds, through improving chemical processing and bioreactor performance or employing elicitors, a biotic stresses, and other approaches, regardless of their mechanisms; 2) studying signal transduction pathways underlying various effective strategies leading to biosynthesis of target secondary metabolites; 3) studying transcription factors and their regulation mechanisms, including genetic manipulation of regulator genes to improve production of target secondary metabolites; 4) cloning of secondary metabolite biosynthetic genes, and genetic modification of key genes to engineer the metabolic flux to target compounds; 5) studying metabolic flux and profiling metabolic intermediates to understand whole pathways and overall regulation of target compound accumulation; and 6) studying gene transcripts for plant secondary metabolism by profiling and analyzing global gene expression under different conditions to understand the regulation of plant secondary metabolism in a whole sense.
Plant cell culture are able to produce secondary metabolites came quite late in the history of *In vitro* techniques. It had been considered for a long time that undifferentiated cells, such as callus or cell suspension cultures were not able to produce secondary compounds, unlike differentiated cells or specialized organs. Zenk and co-workers experimentally demonstrated that this theory was wrong, as they could observe dedifferentiated cell culture of Morinda citrifolia yielding 2.5 g of anthraquinones per litre of medium [26]. This finding opened the door to a large community of vitro culturists who extensively studied the possible use of plant

cultures for the production of secondary compounds of industrial interest and the possible use of plant cell cultures for the specific biotransformation of natural compounds has been demonstrated.

The major advantages of cell culture system over the conventional cultivation of whole plants are:

1. Useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions;
2. Cultured cells would be free of microbes and insects.
3. The cells of any plants, tropical or alpine, could easily be multiplied to yield their specific metabolites.
4. Automated control of cell growth and rational regulation of metabolite processes would reduce of labor costs and improve productivity.
5. Organic substances are extractable from callus cultures. Due to these advantages, research in the area of tissue culture technology for production of plant chemicals has bloomed beyond expectations.

Transgenic hairy root cultures also have revolutionized the role of plant tissue culture in secondary metabolite production. They are unique in their genetic and biosynthetic stability, faster in growth, and more easily maintained. Using this methodology a wide range of chemical compounds has been synthesized. Advances in tissue culture, combined with improvement in genetic engineering, specifically transformation technology, have opened new avenues for high volume production of pharmaceuticals, nutraceuticals, and other beneficial substances. Recent advances in the molecular biology, enzymology, and fermentation technology of plant cell cultures suggest that these systems will become an important viable source for the secondary metabolites.

Large-scale plant tissue culture is found to be an attractive alternative approach to traditional methods of plantation as it offers a controlled supply of biochemical‘s independent of plant availability. Kieran *et al.* (1997) [27] detailed the impact of specific engineering-related factors on cell suspension cultures. Current developments in tissue culture technology indicate that transcription factors are efficient new molecular tools for plant metabolic engineering to increase the production of valuable compounds.

Once interesting compounds are identified from plant extracts, the first part of the work consists in collecting the largest genetic pool of plant individuals that produce the corresponding substances. This work allows the screening of hyper-producing plants that present the most valuable secondary metabolites. However, a major characteristic of secondary compounds is that their synthesis is highly inducible. After choosing the most promising individual plants, begins the real work of *In vitro* cultures with callus initiation. This work consists mainly in determining the medium that will be best adapted for cultivation. And when calli are obtained, it is well known that they can undergo somaclonal variation, usually during several subculture cycles. This is a critical period where, due to this vitro-variation, secondary metabolite production is often variable from one subculture cycle to another. When genetic stability is reached, it is necessary to screen the different callus lines according to their aptitudes to provide an efficient metabolite production. Hence, each callus must be assessed separately for its growth speed as well as intracellular and extracellular metabolite concentrations. This allows an evaluation of the productivity of each cell line so that only the best ones will be taken to cell suspensions and reactor studies. When growth stops, carbon is no longer needed in large quantities for primary metabolism and secondary compounds are more actively synthesized. Bioreactor studies represent the final step that leads to a possible commercial production of secondary metabolites from plant cell cultures. This is an important phase as numerous problems arise when scaling up the work realized on Erlenmeyer flasks. After successful optimization of the biomass production in a bioreactor, plant cell cultures must undergo well-adapted processes to achieve a good production of secondary metabolites.

Antimicrobial properties of plant secondary metabolites

Plants produce a huge variety of secondary compounds as natural protection against microbial and insect attack. Some of these compounds are also toxic to animals, but others may not be toxic. Indeed, many of these compounds have been used in the form of whole plants or plant extracts for food or medical applications in man. The potential of essential oils and saponins as beneficial feed additives in ruminant production will be used here as an illustration of the potential benefits of plant compounds.

Essential Oil and its effects

Essential oils are steam-volatile or organic-solvent extracts of plants used traditionally by man for many centuries for the pleasant odour of the essence, its flavor or its antiseptic and/or preservative properties. Recently, for example, useful effects of essential oils have been demonstrated against pathogenic bacteria. Oils from *Cinnamomum osmophloeum* have been shown to possess antibacterial activity against *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* (including the *Clinically problematic* methicillin-resistant *S. aureus*), *Salmonella* sp. and *Vibrio parahaemolyticus*; *Cinnamaldehyde* is the main antibacterial component of the mixture. *E. coli* O157:H7 is inhibited by oregano oil, peppermint oil, and essential oils from other herbs. Helicobacter pylori are highly sensitive to spearmint oil. Essential oils are potent against a wide range of oral bacteria, and they are used widely in antiseptic mouthwashes.

With this range of antimicrobial activity, it was considered logical to evaluate essential oils for possible beneficial selective effects against rumen micro-organisms. Essential oils were examined many years ago in rumen bacteria in relation to their contribution to poor palatability in some plant species. Oh *et al.* (1967) [37] have demonstrated that individual oils have different effects on mixed rumen bacteria. Monoterpene hydrocarbons are less toxic and sometimes stimulatory to microbial activity compared with the corresponding oxygenated compounds, the monoterpene alcohols and aldehydes. R.J. Wallace (2004) [40, 41], stated that essential oils are not necessarily toxic to rumen bacteria, and their effects may be expected to persist.

Saponins and effects of saponins in ruminants

Saponins, like essential oils, cover a wide variety of chemical compounds and, also like essential oils, man has made use of

their properties for centuries. The word saponin‘ is derived from the Latin word sapo (soap) and traditionally saponin-containing plants have been utilized for washing. Chemically, saponins are high-molecular-weight glycosides in which sugars are linked to a triterpene or steroidal aglycone moiety.
Van Nevel & Demeyer (1990) have found no indication of any toxic effects or effects of sarsaponin on microbial growth or protein breakdown *In vitro*. In contrast, Lu *et al.* (1987) have reported that Lucerne (*Medicago sativa*) saponins appear to suppress fermentation in continuous culture. There are many consequences for the fermentation, and consequently for nutrition, that result from the removal of protozoa. A meta-analysis has recently demonstrated that the benefits of defaunation outweigh any disadvantages. Antiprotozoal agents, such as surface-active agents, that have been investigated in attempts to apply defaunation at the farm level have been hampered by problems with toxicity, either to other rumen micro-organisms or to the host. Lipids are toxic to protozoa, and also to fiber digestion. Thus, there has been, until now, no reliable safe on-farm method available for suppressing rumen protozoa

Importance and natural functions of secondary metabolites
Secondary metabolites, which are a characteristic feature of plants, are especially important and can protect plants against a wide variety of microorganisms (viruses, bacteria, fungi) and herbivores (arthropods, vertebrates). As is the situation with all defense systems of plants and animals, a few specialized pathogens have evolved in plants and have overcome the chemical defense barrier [63].
Secondary metabolites, including antibiotics, are produced in nature and serve survival functions for the organisms producing them. Secondary metabolites serve:

1. As competitive weapons used against other bacteria, fungi, amoebae, plants, insects, and large animals;
2. As metal transporting agents;
3. As agents of symbiosis between microbes and plants, nematodes, insects, and higher animals;
4. As sexual hormones; and
5. As differentiation effectors. Although antibiotics are not obligatory for sporulation, some secondary metabolites (including antibiotics) stimulate spore formation and inhibit or stimulate germination.

Formation of secondary metabolites and spores are regulated by similar factors. Thus the secondary metabolite can

1. Slow down germination of spores until a less competitive environment and more favorable conditions for growth exist;
2. Protect the dormant or initiated spore from consumption by amoebae; or
3. Cleanse the immediate environment of competing microorganisms during germination.

Conclusion
Secondary metabolites are the useful natural products that are synthesized through secondary metabolism in the plants. The production of some secondary metabolites is linked to the induction of morphological differentiation and it appears that as the cells undergo morphological differentiation and maturation during plant growth. It is observed that *In vitro* production of secondary metabolites is much higher from different tissues when compared to non-differentiated or less - differentiated tissues. There are lots of advantages of these metabolites like there is recovery of the products will be easy and plant cultures are particularly useful in case of plants which are difficult or expensive and selection of cell lines for high yield of secondary metabolites will be easy. Many other examples could be presented with plant metabolic engineering as this research area is developing actively. Metabolic engineering is probably a large step forward but playing on the genes will not solve all the problems that have prevented the development of commercial success in the field of plant secondary metabolites. And Advances in plant cell cultures could provide new means for the cost-effective, commercial production of even rare or exotic plants, their cells, and the chemicals that they will produce. Knowledge of the biosynthetic pathways of desired compounds in plants as well as of cultures is often still rudimentary, and strategies are consequently needed to develop information based on a cellular and molecular level. Because of the complex and incompletely understood nature of plant cells *In vitro* cultures, case-by-case studies have been used to explain the problems occurring in the production of secondary metabolites from cultured plant cells. Advance research has succeeded in producing a wide range of valuable secondary phytochemical in unorganized callus or suspension cultures till to date; in other cases production requires more differentiated micro plant or organ cultures.
Due to these advances, research in the area of tissue culture technology for production of plant chemicals has bloomed beyond expectations. The major advantages of a cell culture system over the conventional cultivation of whole plants are as follows:

1. Organic substances are extractable from callus cultures.
2. Cultured cells would be free of microbes and insects.
3. The cells of any plants, tropical or alpine, could easily be multiplied to yield their specific metabolites.
4. Useful compounds can be produced under controlled conditions independent of climatic, environmental changes or soil conditions due to over pollution.
5. Automated control of cell growth and rational regulation of metabolite processes would reduce labor costs and improve productivity.

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