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REVIEW ARTICLE

INTERACTION OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) AND ENDOPHYTES WITH MEDICINAL PLANTS – NEW AVENUES FOR PHYTOCHEMICALS

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SUMMARY

Plant growth promoting rhizobacteria (PGPR) are usually applied to a wide range of agricultural crops for the purpose of growth enhancement, including increased seed germination, plant weight, and harvest yields. PGPR colonization triggers plant growth by bacterial synthesis of plant hormones including indole-3- acetic acid, cytokinin, and gibberellins as well as by increased mineral and nitrogen availability in the soil. Some of them were also known to protect their host plant from pathogenic microorganisms. The role played by PGPR in relation to medicinal plants and their effect on the production of botanicals is an area remaining naive. This paper brings out the possible PGPR – medicinal plant interactions which could improve the potency of the medicinal plant, particularly the cultivated one. Endophytic microorganisms of medicinal plants and their role in relation to bioactive potentials in the generation of phytochemicals also have been discussed.

Key words: Biotization, Endophytes, Medicinal Plants, PGPR, Phytochemicals, Secondary Metabolites

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1. Introduction

The World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs (Farnsworth, 1990). Also, modern pharmacopoeia contains at least 25% drugs derived from plants. Many other are synthetic analogues built on prototype compounds isolated from plants. Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural products, being non-toxic, having no side-effects and affordable prices. Allopathic medicine also owes a tremendous debt to medicinal plants: one in four prescriptions filled in a country like the United States is either a synthesized form of or derived from plant materials. Habitat loss and deforestation coupled with over harvesting has resulted in dwindling population of important medicinal plants around the world. So, direct extraction of natural products from wild medicinal plants to satisfy the current requirement is fast becoming an unrealistic goal. Domestic cultivation of medicinal plants was thought as a viable alternative. But, certain drawbacks including variability in yield and difference in phytochemical profile over wild one are making it as a last resort (Kala, *et al*., 2006). Hence, this article is aimed to throw light on the alternative approaches like investigating plant microbe interactions with medicinal plants and to produce desired or enhanced levels of phytochemicals exploiting this relationship or to use the interacting microorganism *per se* as a source of phytochemical.

Problems associated with the production of phytochemicals through tissue culture

Compounds derived from primary pathways make up the bulk of a plant. These are polysaccharides, sugars, proteins and fats, which are the building blocks for plant growth. Present at a much lower concentration are the secondary products, which include alkaloids, terpenoids, phenolics, steroids and flavonoids, and these have a wide diversity in structure and size and are found in very large numbers throughout the plant kingdom. It is estimated that there are approximately 100,000 different plant-derived compounds, with a large number of new ones being added to the list every year (Verpoorte *et al*., 1998). Besides direct extraction from plants, and chemical synthesis to provide those compounds or derivatives with similar uses, plant cell culture has been developed as an alternative for producing metabolites that are difficult to be obtained by chemical synthesis or plant extraction (Table 1).

However, in spite of four 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. When, *Gloriosa superba* L. was raised *in vitro,* the plant did not produce even nanogram quantity of the 24 or so alkaloids listed including colchicines and colchicoside (Sivakumar *et al*., 2004). The production of Shikonin by *Lithospermum erythrorhizon* Siebold & Zucc. cell cultures and of Taxol/Paclitaxel by *Taxus* cell cultures are the only successful commercial examples so far. The culture of plant cells on a large scale has been regarded as a convenient, reliable and potential source of secondary products than intact plants but the success list is small because only a few compounds harvested from tissue culture satisfy the commercial and biological criteria imposed on the product *i.e.* a high value and low volume, a strong commercial demand, a high yield in culture and the maintenance of a high yield in large-scale culture. 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 abiotic 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. These include treatment with various elicitors, signal compounds and abiotic stresses (Yukimune *et al*., 1996; Zhao *et al*., 2000, 2001[a,b,c] and Zhang *et al*., 2004). Constant efforts are being made by researchers in the following lines to improve the situation:

- improving chemical processing and bioreactor performance or employing elicitors, abiotic stresses and other approaches, regardless of their mechanisms (Zhong, 2001)
- studying signal transduction pathways leading to biosynthesis of target secondary metabolites (Zhao *et al*., 2005)
- studying transcription factors and their regulation mechanisms, including genetic manipulation of regulator genes to improve production of target secondary metabolites (Memelink *et al*., 2001)
- cloning of secondary metabolite biosynthetic genes and genetic modification of key genes to engineer the metabolic flux to target compounds (Verpoorte and Memelink, 2002)
- studying metabolic flux and profiling metabolic intermediates to understand whole pathways and overall regulation of target compound accumulation (Sumner *et al*., 2003)
- 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 (Goossens *et al*., 2003).

In addition to these approaches, employing microorganisms as co-cultures by biotization is tried. Biotization is a metabolic response of *in vitro-grown* plant material to a microbial inoculant(s), leading to the developmental and physiological changes enhancing biotic and

abiotic stress resistance of the derived propagules. When plantlets were co-cultured with PGPR it has been reported to produce more biomass and secondary metabolites. The *Origanum vulgare* L. plantlets when co-cultured with *Pseudomonas* spp. lowered the water content and contained more phenolics and chlorophyll than non bacterized controls (Nowak, 1998). Generally, biotization can be done as a bioassay experiment for short listing the PGPR isolates for their growth promoting properties.

Table 1: Problems associated with direct secondary metabolite isolation from some source plants

S. No.	Plant	Secondary Metabolite	Use	Problem	Reference
1	*Podophyllum hexandrum* Royle	Podophylloxin	Anticancer	Endangered species	Alfermann and Petersen, 1995
2	*Pilocarpus jaborandis* Holmes	Pilocarpine	Treatment of glaucoma	Endangered species	Alfermann and Petersen, 1995
3	*Catharanthus roseus (*L.*)* G. Don	Vinblastine, Vincristine	Anticancer drug	Low yield	Collin, 2001
4	*Catharanthus roseus* (L.) G. Don	Ajmalcine	Treatment of circulatory disorders	Low yield	Collin, 2001

Plant associated microorganisms and their use as biotic elicitors

Plant-associated microorganisms (PAMs) play essential roles in agricultural and food safety contributing to the environmental equilibrium. Both aerial and subterranean plant organs are constantly exposed to intimate contacts with diverse microorganisms. Plant microbe interactions occur at phyllosphere (aerial plant part), rhizosphere (zone of influence of the root system) and endosphere (internal transport system). Interactions involving plant roots in the rhizosphere include root-root, root-insect and root-microbe interactions. Rhizosphere, the layer of soil influenced by the root, is much richer in bacteria than the surrounding bulk soil (Hiltner, 1904). Studies based on culture independent molecular analysis have estimated more than 4,000 microbial species per gram of soil (Montesinos, 2003). These rhizosphere microbes benefit because plant roots secrete metabolites that can be utilized as nutrients. This rhizosphere effect is caused by the fact that a substantial amount of the carbon fixed by the plant, 5–21%, is secreted, mainly as root exudates (Marschner, 1995). Root exudation includes the secretion of ions, free oxygen and water, enzymes, mucilage and a diverse array of carbon-containing primary and secondary metabolites (Uren, 2000 and Bertin *et al*., 2003). The population dynamics of the rhizosphere microorganisms can change as the root structure and patterns of root exudation alter during development and as environmental conditions such as water availability and temperature alter. Adding to the complexity of the rhizosphere are the interactions among the members that take place including the competition for nutrients, colonization sites, scavenging and the production of antibiotics and bacteriocins that inhibit growth. When multiple bacterial species co-exist they do not colonize in distinct areas as pure cultures but as complex communities known as biofilms and this is thought to be the case also for rhizosphere bacteria living on plant roots (Pierson and Pierson, 2000). Rhizosphere microorganisms may also depend on other members of the community to provide nutrient sources as

one bacterium may convert a plant exudate into a form that can be used by another.

Plant–microbial interactions can be classified into three basic groups: (i) negative (pathogenic) interactions; (ii) positive interactions, in which either both partners derive benefits from close association (symbiosis), both partners derive benefits from loose association or only one partner derives benefits without harming the other (associative); and (iii) neutral interactions, where none of the partners derives a direct benefit from interaction and in which neither is harmed (Singh *et al*., 2004). Rhizobacteria that exert beneficial effects on plant growth and development are referred to as Plant Growth Promoting Rhizobacteria (PGPR). PGPR can affect plant growth either indirectly or directly; indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms; while direct promotion of plant growth by PGPR involves either providing plants with a compound synthesized by the bacterium or facilitating the uptake of certain nutrients from the environment. General mechanisms of plant growth promotion by PGPR include: associative nitrogen fixation, lowering of ethylene levels, production of siderophores, production of phytohormones, induction of pathogen resistance in the plant, solubilization of nutrients, promotion of mycorrhizal functioning, decreasing (organic or heavy metal) pollutant toxicity, etc (Glick *et al*., 1999).

Elicitors are chemicals or biofactors from various sources that can induce physiological changes of the target living organism. In a broad sense, elicitors, for a plant refer to chemicals from various sources that can trigger physiological and morphological responses and phytoalexin accumulation. It may include abiotic elicitors such as metal ions and inorganic compounds and biotic elicitors from fungi, bacteria, viruses or herbivores, plant cell wall components as well as chemicals that are released at the attack site by plants upon pathogen or herbivore attack. It is well known that treatment of plants with elicitors, or attack by incompatible pathogens, causes an array of defense reactions, including the accumulation of a range of plant defensive secondary metabolites such as phytoalexins in intact plants or in cell cultures. Signal perception is the first committed step of the elicitor signal transduction pathway and much effort has been put into isolation of effective elicitor signal molecules from fungal and plant cell extracts or other sources and identification of the corresponding receptors from plant plasma membranes. Thus, PAMs can produce elicitors which in turn will induce the synthesis of secondary products in plants. Elicitation is used to induce the expression of genes often associated with enzymes responsible for the synthesis of secondary metabolites. Jasmonic acid and its methyl ester are signal transducers in a wide range of plant cell cultures and these compounds accumulated rapidly and transiently when plant suspension cultures of *Rauvolfia canescens* L. and *Eschscholtzia californica* Cham. were treated with a yeast elicitor (Roberts and Shuler, 1997). Exogenously applied methyl jasmonate was shown to induce the production of secondary metabolites in 36 different plant species. In the past few years, jasmonic acid and methyl jasmonate have been shown to be inexpensive effective elicitors of secondary metabolite production in many other systems, including *Taxus.* Some PGPR or their components uses as biotic elicitor is given (Table 2).

Table 2. Some PGPRs reported as biotic elicitors

S. No	Plant	Treatment	Nature of the PGPR	PGPR Species	Metabolite induced in the plant	Reference
1	*Catharanthus roseus* (L.) G. Don.	Seedling Treatment by soil drenching	Non-native	*Pseudomonas fluorescens*	Ajmalicine	Jaleel *et al*., 2007
2.	*Catharanthus roseus* (L.) G. Don.	Seedling Treatment by soil drenching	Non-native	*Pseudomonas fluorescens*	Serpentine	Jaleel *et al*., 2009
3.	*Hyoscyamus niger* L.	Seedling radicle and culture media for *in vitro* growth	Non-native	*Pseudomonas putida* and *P. fluorescens*	Hyoscyamine and Scopolamine	Ghorbanpour *et al*., 2010
4.	*Crocus sativus* L.	Soaking corms and soil drenching	Non-native	*Bacillus subtilis*	Picrocrocin, Crocetin and Safranal compounds	Eldin *et al*., 2008
5.	*Calendula officinalis* L.,	Cell Suspension culture	Non-native	*Trichoderma viride* homogenate	Oleanolic acid	Wiktorowska *et al*., 2010
6.	*Salvia miltiorrhiza* Bunge	Hairy Root culture	Non-native	*Bacillus cereus* polysaccharide fraction	Tanshinone	Zhao *et al*., 2010

Endophytes as source of secondary plant products

The term endophyte (Gr. *endon*, within; *phyton*, plant) was first coined by De Bary (De Bary, 1866) and an endophyte is a bacterial or fungal microorganism, which spends the whole or part of its life cycle colonizing inter- and/or intra-cellularly inside the healthy tissues of the host plant, typically causing no apparent symptoms of disease (Sturtz *et al*., 2000; Wilson, 1995). The relationship between the endophyte and its host plant may range from latent phytopathogenesis to mutualistic symbiosis (Strobel and Long, 1998). Several endophyte species are usually associated with a single plant and among them, at least one species show host specificity. From the nearly 300,000 plant species in the globe, each one hosts several to hundreds of endophytes (Tan and Zou, 2001), creating an enormous biodiversity. Endophytic bacteria originate from the rhizosphere, seeds or plant material (Hallmann *et al.,* 1997). A subset of rhizobacteria may enter the interior of the root by hydrolysing wall-bound cellulose, through auxin-induced tumours, with water flow, through wounds or through lateral branching sites (Hallmann *et al*., 1997; Siciliano *et al*., 1998).

Evidence of plant associated microbes has been discovered in the fossilized tissues of stems and leaves and these endophytic relationships may have begun to evolve from the time that higher plants first appeared on the earth, hundreds of millions of years ago (Taylor and Taylor, 2000). As a result of these long-held associations, it is possible that some of these endophytic microbes devised genetic systems allowing for the transfer of information between themselves and the higher plant and *vice versa* (Stierle *et al*., 1993). Obviously, this would permit a more rapid and reliable mechanism for the endophyte to

deal with ever changing environmental conditions and perhaps allow for more compatibility with the plant host. Probably it may be for this reason they evolved biochemical pathways resulting in the production of plant growth hormones. Each of the five classes of these substances (auxins, abscisins, ethylene, gibberellins and kinetins) is, in fact, known to be synthesized from a list of a range of representative plant-associated fungi and bacteria (Goodman *et al*., 1986). However, the most fascinating nature of endophytes seems to be their adaption to their plant host by evolving to a point where they could contribute to the relationship by carrying out such functions as protection from pathogens, insects and grazing animals, leading to symbiosis and ultimately to host specificity (Fisher and Petrini, 1993).

The endophytes are now recognized as important sources of a variety of structurally novel and biologically active secondary metabolites, including terpenoids, steroids, alkaloids and isocoumarins derivatives. For example, Taxol, an effective antitumor drug produced by bark of the yew tree, *Taxus brevifolia*, could also be produced by endophytic fungi *Taxomyces andreanae* (Pezzato, 1996). These trees are rare, slow growing and a large amount of bark may have to be processed to obtain a small amount of the drug. The amount of taxol found in yews is relatively small, *ca* 0.01–0.03% dry weight and this has been a major factor in contributing to its high price in market (Stierle *et al*., 1993). Furthermore, *Pestalotiopsis microspora* (Strobel *et al*., 1996), *Periconia* sp. (Li *et al*., 1998), *Bartalinia robillardoides* and *Colletotrichum gloeosporioides* (Gangadevi and Muthumary 2008[a, b]) residing in plants other than *Taxus* species were also found to produce taxol. Fungal endophyte *Trametes hirsuta* isolated from *Podophyllum* sp. produces lignans (podophyllotoxin) with anticancer activity. Derivatives of podophyllotoxin, etoposide and teniposide are currently used in cancer chemotherapy. (Puri *et al*., 2006). The fungus isolated from inner bark of *Nothapodytes foetida* (Wight) Sleumer., produces the anticancer phytochemical camptothecin (Puri *et al*. 2005). Some other tropical plants that have been studied for endophytes include tropical palms (Frohlich and Hyde, 1999; Taylor and Crous, 1999; Rungjindamai *et al*., 2008), tropical fruit trees (Azevedo *et al*., 2000), banana (Photita *et al*., 2001), *Amomum siamense* Criab. (Bussaban *et al*., 2001), teak trees (Chareprasert *et al*.2006), *Aegle marmelos* (L.) Corr. Serr. (Gond *et al*., 2007), mangrove plants (Lin *et al*., 2005 and Xu *et al*., 2009), *Rhizophora apiculata* Blume. (Kumaresan and Suryanarayanan 2002), *Camptotheca acuminata* Decne. (Lin *et al*., 2007), three *Artemisia* species (Huang *et al*., 2009) and 29 traditional Chinese medicinal plant species (Huang *et al*., 2008). While literally hundreds of reports have appeared on many new endophytic microorganisms, the complex chemical and biochemical mechanisms that govern the biology of the endophytic processes are yet to be understood fully. In fact, it is becoming increasingly clear that host specificity is a bonafide phenomenon in endophyte higher plant relationships (Bacon and White, 2000). Knowledge of such interactions can provide guidance as to which endophytes might be selected in the search for novel medicinal natural products. The contribution of the endophyte to the plant may be to provide protection to it by virtue of antimicrobial compounds that it produces. Some of these compounds may be of interest medicinally, since they possess antifungal, antibacterial, antimalarial and a host of other biological activities.

2. Concluding Remarks

The process of medicinal plants cultivation thus creates the need for interdisciplinary studies on rhizosphere biology, microbiology, ecology and agricultural technology of medicinal plant species to develop effective methods of biomass production and obtaining quality material enriched with phytochemicals. If, phytochemials are prospected and produced from microorganisms the unwanted destruction of medicinal plants can be prevented protecting the green cover. The products can also be produced at large scale at economic pricings.

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