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MICROBIAL BIOSTIMULANTS INCREASE BIOACTIVE COMPOUNDS IN MEDICINAL PLANTS - A REVIEW

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Abstract

Functional foods are gaining popularity among consumers because they contain biologically active compounds involved in human health and wellness. Although some plant species are already known for their beneficial properties if properly consumed, certain cultivation technologies can increase their content of bioactive compounds. For this purpose, plant probiotics could be applied as biostimulants, which are approved in organic agriculture. Plant probiotics are beneficial microorganisms that can be found in the rhizosphere, or as endophytes. They can be applied as single strains or as consortia. These beneficial microorganisms could increase the production of biologic active compounds in their host plants, such as: flavonoids, organic acids, phenols, tannins, and vitamins. This review focuses on various plant probiotics and their role in the bioactive compounds synthesis in their hosts. Among plant probiotics mediating bioactive compounds synthesis in plants are mentioned Azospirillum, Azotobacter, Bacillus, Phyllobacterium, Pseudomonas, Rhizobium and several other genera of plant beneficial microorganisms.

Key words*: plant probiotics, beneficial bacteria, endophytes, medicinal bioactive compounds, functional foods.*

INTRODUCTION

Functional foods, nutraceuticals, and designer foods are edible products containing supplements which complete diets and improve consumer's health. The functional foods, beside their nutritional value, provide unexpected health benefits for the consumer. In comparison, designer foods are fortified with health promoting ingredients, while nutraceuticals are derived from food sources and used as additional health improvers to complete the basic nutritional value found in foods. All these became increasingly popular in our times, due to the fact they bring additional fiber, vitamins, minerals, biologic active compounds, prebiotics and probiotics in our diets. Nowadays, some spontaneous herbs also acquired unexpected credentials. Less popular plant species, such as amaranth (*Amaranthus* spp.), sorrel (*Rumex* spp.), purslane (*Portulaca oleracea*). dandelion greens (*Taraxacum* spp.), watercress (*Nasturtium officinale*) and others, are gaining popularity as functional foods, as they contain health beneficial bioactive compounds, vitamins, minerals or essential oils.

Other supplements with proven health benefits are the prebiotics and probiotics. The prebiotics are indigestible fibers that stimulate the intestinal microbiota in humans and animals. And the probiotics are viable microorganisms, mostly bacteria and some yeast, which are helping digestion and food transformation, with various beneficial traits for their host organisms. Although most studied probiotics are those for human and animal health, there is a special category of probiotics associated to plants, which bring beneficial attributes to these hosts. These plant probiotics are beneficial microorganism living in symbiotic relationship with the plant hosts or in free-living association with plants. They can act in different ways to increase plant development, to protect plant against different stress factors or increase their production of biologically active compounds (de Souza Vandenberghe et al., 2017). Therefore, selected plant probiotics could be applied as bioprotectants, biocontrollers, biofertilizers or biostimulants, especially in organic gardening. These plant probiotic microorganisms could be found on plant surfaces, as epiphytes, root surface, as rhizospheric microorganisms, or inside plant tissues, as endophytes (Whipps et al., 2008; Dutta & Bora, 2019). The contact between plants and microorganisms is made at the rhizosphere, endosphere and phillosphere level. Plant roots penetrate various layers of soil substrate in search of nutrients and water. During soil exploration, they encounter millions of different microorganisms and have developed advanced genetic and metabolic mechanisms to both recruit and defend against microorganisms. Root colonization involves a complex molecular communication between microorganism and roots. Attracted by root exudates, the microorganisms migrate towards roots via chemotaxis and may colonize the root surface, and soil aggregates that form around roots, or both. Best studied microbial colonizers of the root system, with beneficial effects on their hosts, are PGPR (plant growth promoting rhizobacteria) and mycorrhiza. Most beneficial interactions between plants and their symbionts begin at the rhizosphere level and should be considered the first application management of plant probiotics (Walker et al., 2020). The phillosphere is considered a hostile environment for microorganisms due to the limited nutrient sources, and UV radiation, but microorganisms found at this level were able to regulate various processes in plants, such as production of biologic active compounds including organic acids, ascorbic acid (vitamin C), and diverse phenolic compounds, flavonoids and essential oils (Lindow & Leveau, 2002; Flores-Felix et al., 2008). Endophytes are more involved in plant metabolic activity than other plant associated microorganisms. Thanks to their intra-tissue colonization they have an intimate relationship with their host. Although plants are not revealing any symptomatology due to endophytic colonization, many processes in plants are improved (Fadiji & Babalola, 2020). This review focuses on various plant probiotics and other plant associated microorganisms with beneficial traits for their host. Mostly, the role of plant associated microorganisms in the bioactive compounds synthesis in their hosts.

MATERIALS AND METHODS

This review, is presenting plants probiotics effect, as single population or consortia of microorganisms, on bioactive compounds production in their hosts.

Data collected in this study derives from research articles published since 1995, featuring the importance of plant associated microorganisms for their hosts (Table 1).

The beneficial aspects documented in this review are focused on the improved plant quality, regarding their nutritional and medicinal value, if properly colonized by certain microorganisms. The most studied bioactive compounds are the antioxidants, especially ascorbic acid (vitamin C); flavonoids, such as anthocyanin; chlorophyll; alkamides and essential oils.

Table 1. Plant probiotics used in agriculture
(adapted after Jiménez-Gómez et al., 2017)

Inoculated plants	Microbial inoculants & Plant probiotics	Triggered effects on bioactive compound composition	Reference
Begonia malabarica	*Glomus mosseae,* *Bacillus coagulans,* *Trichoderma viridae*	Increased various secondary metabolites, such as total phenols, ortho-dihydroxy phenols, tannins, flavonoids, and alkaloids	Selvaraj et al., 2017
Borago officinalis	AMF inoculated soil	Increased content of carotenoids and chlorophyll	Rahimi et al., 2017

Inoculated plants	Microbial inoculants & Plant probiotics	Triggered effects on bioactive compound composition	Reference
Brassica oleracea	*Bacillus megaterium, Pantoea agglomerans* and *B. subtilis*	Increase chlorophyll content	Turan et al., 2014
Capsicum annuum	*Rhizobium leguminosarum* PETP01	Increased antioxidant activity	Silva et al., 2014
Cymbopogon citratus	*Azotobacter* sp. and *Pseudomonas* sp.	Increased total flavonoid and total phenol content, improved the antioxidant capacity of the essential oils and helped lemongrass plants to tolerate better the abiotic stress	Mirzaei et al., 2020
Echinacea purpurea	*Glomus intraradices*	Increased production of secondary phytomedicinal metabolites	Araim et al., 2009
	Bacterial endophytes of *E. purpurea*	Increased concentration of alkamides	Maggini et al., 2017
Fagopyrum esculentum	*Azospirillum* spp. *and Azotobacter* spp. *inoculants* applied to buckwheat plants	Increased grain yield and concentrations of total flavonoid and phenol content in buckwheat	Singh et al., 2015
Fragaria x ananassa	*Bacillus* sp. RC23*, B. cereus* RC18, *B. megaterium* RC01 or *Paenibacillus polymyxa* RC05	Vitamin C enhancement	Erturk et al., 2012
	Phyllobacterium sp. PEPV15		Flores-Félix et al., 2015
Hibiscus sabdariffa	AMF or PGRP	Increased content of total chlorophyll and carotenoids in roselle	Sanayei et al., 2021
Hyoscyamus niger	*Pseudomonas putida* 168 or *P. fluorescens* 187 strains	Simulated antioxidant enzymes activity, increased proline accumulation, improved tropane alkaloid production and yield of root and shoot organs.	Ghorbanpour et al., 2013
Lactuca sativa	Endophytic plant growth promoting selenobacteria with or without mycorrhizal fungi	Increased content of total chlorophyll and carotenoids in lettuce	Durán et al., 2016
Lycopersicon esculentum	*Bacillus amyloliquefaciens* FZB42	Vitamin C enhancement	Gül et al., 2008
	Bacillus amyloliquefaciens and *B. megaterium*		Shen et al., 2016
	Pseudomonas sp. 19Fv1T		Bona et al., 2017
	Mixture of PGPR (*Pseudomonas putida* 41, *Azotobacter chroococcum* 5, and *Azospirillum lipoferum* OF strains) and AMF (*Glomus intaradics*, *G. mossea*, and *G. etanicatum*)	Increased antioxidant activity and lycopene content	Ordookhani et al., 2010
	Mixture of PGPR (*A. chroococcum* 5, and *A. lipoferum* OF strains) and AMF (*G. intaradics*, *G. mossea*, and *G. etanicatum*)		
	Mixture of the PGPR strains: *P. putida* 41, *A. chroococcum* 5, and *A. lipoferum* OF strain		
	Bacillus licheniformis	Improved total flavonoids content	Ochoa-Velasco et al., 2016
Mentha piperita	*Pseudomonas fluorescens*	Increased essential oils amount	Banchio et al., 2008

Inoculated plants	Microbial inoculants & Plant probiotics	Triggered effects on bioactive compound composition	Reference
Mentha piperita	Plant growth promoting rhizobacteria	Increased amount of essential oils	Santoro et al., 2011
Ocimum basilicum	*Bacillus subtilis* GB03	Elevated α-terpenol and eugenol accumulation	Banchio et al., 2009
	Mixture of *P. putida* 41 strain, *A. chroococcum* 5 strain, *A.lipoferum* OF strain	Increased levels of essential oils antioxidant activity and microelements content	Ordookhani, 2011
	Bacillus lentus, Pseudomonas sp., *Azospirillum brasilense*	Increased antioxidant activity and chlorophyll leaf content	Heidari & Golpayegani, 2012
	Diazotrophs	Increased carotenoids and chlorophyll content in purple basil	Mariotti et al., 2021
Origanum majorana	*Pseudomonas fluorescens Bradyrhizobium sp.*	Increased amount of essentials oils	Banchio et al., 2008
Nasturtium officinale	*Bacillus subtilis*	Increased antioxidant capacity and total phenols of watercress	Pignata et al., 2016
Pelargonium graveolens	*Bacillus subtilis, Pseudomonas fluorescens*	Enhanced essential oil yield	Mishra et al., 2010
Pelargonium species	*Glomus intraradices* AMF and phosphate solubilizing bacteria	Enhanced yield and composition of essential oil (citronellol, geraniol, geranial, and eudesmol) in rose-scented geranium	Prasad et al., 2012
Ribes nigrum	ProbioHumus (Baltic Probiotics, Latvia) based on *Saccharomyces cerevisiae* yeast, *Bacillus subtilis* sporulated bacteria, *Bifidobacterium animalis, B. bifidum, B. longum, Lactobacillus casei, L. diacetylactis, L. delbrueckii, L. plantarum, Lactococcus lactis, Streptococcus thermophiles* lactic acid bacteria, *Rhodopseudomonas palustris,* and *R. sphaeroides* phototropic bacteria	Ascorbic acid and anthocyanins increased content	Jurkonienė et al., 2021
	NaturGel containing also microorganisms of *Azotobacter, Bacillus, Rhizobium, Bradyrhizobium, Lactobacillus,* and *Trichoderma* genera		
	Probiotic strains and mycorrhizal fungi	Enhanced antioxidant activity of berries and increased level of anthocyanins in fruits	Lingua et al., 2013
Rubus sp.	*P. fluorescens* N21.4	Increase and stabilize total flavonoid content in blackberry	Ramos-Solano et al., 2014
Spinacia oleracea	*Rhizobium* sp. PEPV12	Increase chlorophyll content	Jiménez-Gómez et al., 2016
Stevia rebaudiana	PGPR (such as *Azotobacter chroococcum, Bacillus polymixa, Pseudomonas putida)* with or without *Glomus intraradices* AMF	Increased chlorophyll content and augmented stevioside amount	Vafadar et al., 2014
Tagetes minuta	*Pseudomonas fluorescens, Azospirillum brasilense*	Increased monoterpene and phenolic compounds	del Rosario Cappellari at al., 2013

RESULTS AND DISCUSSIONS

The interaction between medicinal plants and their endophytic community was studied in order to review the increased production of biologic active compounds in microbial fortified plants. A significant increase of vitamin C level in plants was observed if microbial inoculated. The vitamin C is also known as ascorbic acid.

The results obtained by Flores-Félix et al. (2015) on strawberries showed that vitamin C levels were significantly higher in the fruits of plants inoculated with *Phyllobacterium* sp. PEPV15 strain, with approximately 79% more, compare to the uninoculated control. Previous studies performed by Erturk et al. (2012) showed high levels of vitamin C content in strawberry fruits after plants were inoculated with *Paenibacillus polymyxa* RC05 strain, as well as with *Bacillus megaterum* RC01, *Bacillus cereus* RC18 and *Bacillus* RC23 single strains, but not significant when treated with *Bacillus* RC03. Less significant differences regarding vitamin C content were observed in strawberry after foliar, and/or root application of *Pseudomonas* BA-8, *Bacillus* OSU-142 and *Bacillus* M-3 strains (Pirlak et al., 2009). Although these studies are revealing a wide variation regarding vitamin C levels in fruits of microbial inoculated strawberry plants, all bacteria used stimulated plant growth and increased different parameters of growth and productivity. This means that proper inoculants should be properly selected in order to increase the levels of bioactive compounds in plants.

Beside strawberries, blackcurrant berries are also known for their beneficial impact on human health (Hannum, 2004; Gopalan et al., 2012). Blackcurrant berries are rich in anthocyanins, polyphenolic substances, antioxidants, vitamin C and gamma-linolenic acid. A recent study performed on blackcurrant grown in organic farming system showed that commercial plant probiotic products, like ProbioHumus and NaturGel increased the contents of ascorbic acid and anthocyanins. Moreover, when sprayed as single treatments or in combination, they significantly improved fruit yields with 38, 25, and 16%, respectively, compared with uninoculated control (Jurkonienė et al., 2021).

Increased levels of vitamin C are also detected in vegetables after bacterial inoculation. In tomato fruits, the vitamin C content varies depending on production system (autumn or spring), nutrient system (open or closed-loop fertigation), concentrations of nutrient solution (full or half amount) and microbial treatments (PGPR inoculated or control). For instance, Gül et al. (2008) obtained best levels of vitamin C in tomato fruits (19.43 mg/100 ml) produced in spring growing season, when plants grown in perlite pots were inoculated with *Bacillus amyloliquefaciens* FZB42, in closed-loop fertigation system, with half amount of the normally used concentration of nutrient solution. Additionally, it was shows that vermicompost combined with plant probiotics *Bacillus megaterium* and *B. amyloliquefaciens* inoculation also increases vitamin C contents in fruits and tomato yield (Shen et al., 2016). Inoculation with *Pseudomonas* sp. 19Fv1T strain not only enhanced yield of tomato plants but also increased vitamin C concentration in fruits compared with the control treatment (Bona et al., 2017).

Beside vitamin C content, the antioxidant activity was also analyzed. On basil (*Ocimum basilicum* L.) studies were performed with various mixtures of *Pseudomonas putida* 41, *Azotobacter chroococcum* 5, and *Azospirillum lipoferum* OF strains presented increased levels of essential oils antioxidant activity and microelements content, compared to the control treatment (Ordookhani, 2011). Inoculants based on *Pseudomonas* sp., *Bacillus lentus* and *Azospirillum brasilense* on water stressed basil also increased antioxidant activity. Catalase and guaiacol peroxidase activity were higher as well as leaf chlorophyll content (Heidari & Golpayegani, 2012). The antioxidant activity was higher also in tomato fruits when plants were treated with the PGPR mixture of *Pseudomonas putida* 41, *Azotobacter chroococcum* 5, and *Azospirillum lipoferum* OF strains, with and without AMF (arbuscular mycorrhizal fungi) colonized soil (Ordookhani et al., 2010).

Bacterial inoculation with *Bacillus subtilis* increased the antioxidant capacity and total phenols of watercress (*Nasturtium officinale* R. Br.) medicinal plant (Pignata et al., 2016)

The antioxidant activity of berries can also be increased by plant microbial inoculation. Probiotic strains and mycorrhizal fungi are able to increase the level of anthocyanins in fruits (Lingua et al., 2013).

In addition to vitamin production and antioxidant activity, some plant probiotics are able to stimulate also carotenoids production. Such pigments are involved in photosynthesis, photoprotection, and act as stress hormones and signaling molecules in plants (Shumskaya & Wurtzel, 2013). Carotenoids are also revealing human health, nutrition and wellbeing attributes and some are precursors of vitamin A or act as antioxidants (Park et al., 2017). Beside the very well-known beta-carotene, lycopene is another known carotenoid. In tomato fruits, lycopene amount can be increased by proper PGPR inoculation of tomato plants. The combined treatment of PGPR and AMF on tomatoes also revealed higher levels of lycopene and potassium in tomato fruits (Ordookhani et al., 2010). Plants inoculation with *Pseudomonas putida* 41, *Azotobacter chroococcum* 5, and *Azospirillum lipoferum* OF strains of PGPR along with a mixture of *Glomus lipoferum, G. mossea* and *G. etunicatum* AMF increased not only lycopene amount in fruits but also antioxidant levels and potassium content in fruits and shoots (Ordookhani et al., 2010).

Carotenoids and chlorophyll content was increased in lettuce plants inoculated with endophytic plant growth promoting selenobacteria with or without mycorrhizal fungi (Durán et al., 2016). Similar effects were seen also in the medicinal plants *Borago officinalis* grown in AMF inoculated soil (Rahimi et al., 2017), in roselle (*Hibiscus sabdariffa*) inoculated either with AMF or PGRP (Sanayei et al., 2021), in purple basil (*Ocimum basilicum* L. cv. Red Rubin) inoculated with diazotrophs (Mariotti et al., 2021).

In *Stevia rebaudiana*, not only the chlorophyll content was increased but also the stevioside amount was augmented in biofertilised plants. Best results were obtained with the combined treatment of *Azotobacter chroococcum* PGPR + *Glomus intraradices* AMF, followed by other mixed treatments, such as *Bacillus polymyxa* + *G.intraradices*, or *A. chroococcum* + *Pseudomonas putida*. Although, the other nine microbial biofertilizers also revealed improved NPK, chlorophyll and steviosede content compared to the untreated control (Vafadar et al., 2014).

Azospirillum spp. and *Azotobacter* spp. inoculants applied to buckwheat plants (*Fagopyrum esculentum*) increased grain yield and concentrations of total flavonoid and phenol content (Singh et al., 2015). In water stressed lemongrass (*Cymbopogon citratus*), *Azotobacter* sp. and *Pseudomonas* sp. inoculants not only that increased total flavonoid and total phenol content, but also improved the antioxidant capacity of the essential oils and helped plants to tolerate better the abiotic stress (Mirzaei et al., 2020).

Pseudomonas fluorescens N21.4 inoculation in *Rubus* sp. var. Lochness increase and stabilize total flavonoid content in blackberry (Ramos-Solano et al., 2014).

Effect of root colonization with selected microorganisms was studied to different medicinal plants in order to determine the composition and amount of essential oils. *Pseudomonas fluorescens* inoculation of peppermint (*Mentha piperita*), as well as *P. fluorescens* + *Bradyrhizobium* sp. inoculation of oregano (*Origanum majorana*) increased total essential oil content without modifying the composition (Banchio et al., 2008). Later studies on microorganisms' effect on peppermint showed that the volatile organic compounds of rhizobacteria can increase biosynthesis of essential oils and plant growth parameters (Santoro et al., 2011).

Phytosterols are essential biologic active compounds present in high concentrations in vegetable oils (Granado et al., 1995). Some studies suggest that application of plant probiotics can increase the levels of sterols in plants. Silva et al. (2014) inoculated two strains of *Rhizobium* (TVP08 and PEPT01) in pepper (*Capsicum annuum*) and evaluated their effect on sterols. The rhizobia inoculation produced a positive effect on the ripening of the pepper fruit, in addition to an improvement in several primary and secondary metabolites, which improved the nutritional value of the plant. Moreover, the aqueous extracts of *Capsicum* *annuum* leaves, after bacterial inoculation with *Rhizobium* TVP08 strain, presented a significant acetylcholinesterase (AchE) inhibitory activity, with an important relevance in the treatment of Alzheimer's disease (Silva et al., 2014).

Alkamides from *Echinacea* have significant anti-inflammatory and immunomodulatory properties. Maggini et al. (2017) reported an increased concentration of alkamides in bacterial colonized plants of *Echinacea purpurea*.

Microbial inoculants perspectives

Studies on microbial inoculants are in continuous process, either for new strains selection, analyzing their mechanisms of action, or understanding their implications on plants or environment. Microbial inoculants development is taking place at a global level. In agriculture, most of them are used as microbial fertilizers, biostimulants or plant protection products. However, in most countries, commercialization depends on specific established standards, and government approval. However, according to policy initiatives addressed at the global level (e.g. Horizon 2020), it is absolutely necessary that the quantities of chemical fertilizers used be kept to a minimum and that "green products" be introduced (García-Fraile et al., 2015). Many countries have developed government policies for the use of biofertilizers, and biostimulants, although, the regulations are still in progress (García-Fraile et al., 2017).

In this context, collaboration between researchers and industry is a key factor in establishing the basis of a global biofertilizer market (García-Fraile et al., 2015).

Microbial inoculants or plant probiotics must meet a number of characteristics, but most importantly they must be safe for the environment and humans. Another important feature is their stability, and viability, also in terms of survival in certain biotic or abiotic conditions. Inoculants should include the most effective microbial strains that promote plant growth and productivity (Glick, 2012). Selected microbial strains should survive in wide range environmental conditions, as well as in certain abiotic stress factors (Chauhan et al., 2015). Various formulation types are available for microbial inoculant used in agriculture most commons being the granules, powders, liquids, suspensions, emulsions and effervescent products (Kamilova et al., 2015; Lesueur et al., 2016; Zamfiropol-Cristea et al., 2017; Macik et al., 2020).

CONCLUSIONS

Microbial inoculants are having an important role in agriculture, stimulating plant growth and development. Their beneficial traits were demonstrated in both normal and stressful environmental conditions of plant production. This review documented important knowledge regarding microbial inoculants and their role in plant stimulation to produce phytomedicinal metabolites and nutritional compounds.

Such microbial inoculants can be considered plant probiotics, as they are able to improve plant physiological status in various environmental conditions. The applications of plant probiotics in agricultural practices enhance plants yield and quality.

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