Plants Probiotics as a Tool to Produce Highly Functional Fruits

63

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Contents

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Abstract

Plant probiotics are bacteria capable of improving crop yields reducing or even eliminating chemical fertilizers. During the last years, several studies show that many of these bacteria can improve not just production, but also food quality, through the increase of some nutrients as well as some plant bioactive compounds, which are beneficial to human health. This chapter compiles some of the recent research focused on the capabilities of several bacterial plant probiotics to enhance the production of more functional foods, and therefore benefiting our health.

Keywords

Food quality · Biofertilizers · Bacterial inoculants · Nutritional content · Horticultural crops

1 Introduction

According to Shahzad et al. [1], more than 175 million tons of chemical pesticides and fertilizers are applied every year to soils to improve crop yields. Nowadays, farming systems are mainly based on this type of products to satisfy worldwide food demand. However, chemical fertilizers are very costly and produce many environmental and human health problems [2, 3].

Moreover, an increasing percentage of consumers around the world are more aware of the production systems, food safety, and nutritional contents [4]. During last years, food quality and safety are one of the aim issues on the European political agenda [5]. Different production systems are being developed according to the new green politics, which many countries are implementing and including within their legislations.

One of the green new production systems is based on the use of bacterial inoculants, due to their ability to promote plant growth and development and therefor to produce healthier foodstuff. Currently, there are a substantial number of published studies which show significant increased yields and enhanced quality crops [3].

Haas and Keel [6] described the group of beneficial bacteria for plants as plant probiotic bacteria (PPB), according to their effectiveness in niche colonization, ability to induce systemic resistance in their host, improve plant nutritional content, and increase crop quality [7]. Many studies have focused on understanding how these bacteria interact with their plant host and measure the improvements in crop yields [8–10].

Interestingly, apart from the widely known capability to increase crop yields, several studies published in recent years show how bacterial plant probiotics can improve C and increase several plant compounds which augment human health and aid in the prevention of diseases [11]. This is a less-known potential of these bacterial biofertilizers, and therefore, this chapter is focused on the capabilities of plant probiotics to enhance not only crop yields, but also food quality, allowing the production of more functional products benefiting our health.

2 Effects of Microbial Inoculants in Bioactive Compounds Content in Legumes

Legume seeds are undoubtedly one of the most important sources of vegetable proteins in human nutrition [12]. Grains such as soya, beans, chickpeas, lentils, peas are the main source of proteins for many people in developing countries [13–16]. Apart from that, they are excellence source of nutrients such as fiber, vitamins, and minerals [12]. Moreover, several legume grains contain polysaccharides, such as fructooligosaccharides and starch which act as prebiotics [17–21].

Also, some legume seeds, mainly soybeans, but also peanuts, mung beans, lupine grains, green grams, pigeon peas, groundnuts, and bambara, are used to obtain milky drinks, after their extrusion and fermentation with bifidobacteria, lactic bacteria, or yeasts, which are excellent probiotics [22–28].

In addition to their nutritional value, several recent research studies show the importance of the presence of bioactive compounds in legumes, such as flavonoids, tocopherols, carotenoids, fatty acids, and anthocyanins, because of their multiple benefits for human health [12, 29, 30], having been reported how the consumption of legumes reduces the LDL cholesterol levels, preventing heart diseases [31, 32], gastrointestinal cancer [33], hypercholesterolemia [34], diabetes and stroke [32].

Legumes have the capability to establish symbiosis with nitrogen-fixing bacteria. These bacteria, which are currently distributed in several families and genera, are collectively known as rhizobia and have the capability to fix atmospheric nitrogen for the plant [24] inside some organs formed in the roots or stems of the legume plants termed as nodules [25, 26]. The capability of rhizobia to fix nitrogen on legume plants was the first described and characterized bacterial mechanism of plant growth promotion, with the first reports about this symbiosis dating from the beginning of the XIX Century [35]. From that time, many studies have reported yield increments in different legume crops after the application of rhizobial inoculants, reducing or even replacing chemical fertilization [36–38]; thus, the application of rhizobia-based biofertilizers is an efficient agronomic practice to fertilize legume crops in a friendly and sustainable manner, avoiding the environmental contamination and the human health risks derived from chemical fertilization. In these studies, the commonly analyzed parameters are shoot and root weight, nodule number and weight, grain yields, and nutrients content [39].

Moreover, during the last decade, several studies have focused on the analysis of changes in the bioactive substances content of legumes after inoculation with rhizobial strains. Legumes are historically known to contain bioactive compounds such as isoflavones, phenolic acids, and procyanidins, which constitute the major phenolic compounds present in their grains and have been proved to protect against different diseases, such as cancer, obesity, and other metabolic diseases, as well can reduce menopausal symptoms [30, 40, 41]. The molecular structure of isoflavones is similar to that of 17-β-estradiol molecules, so isoflavones can induce similar effects to those of estrogens, but they lack the risks associated with these drugs treatments [42]. Related to the isoflavones content in legumes, several recent studies have found a positive effect of soybean intake in women for the prevention of cancer diseases related with estrogenic levels, such as endometrial and breast cancers [43, 44]. Soy-based foods consumption has also been correlated with a lower risk of prostate and colorectal cancers [42, 45, 46]. Proanthocyanidins are other phenolic compounds with antioxidant potential present in legume seeds [47–51] and have been related to the prevention of cancer, diabetes, and cardiovascular diseases [52–54]. Tocopherols, compounds with vitamin E activity, are present in important amounts in legume grains [55–58] helping in the prevention of several diseases related with vitamin E deficiency, such as neuromuscular problems [59]. Legume seeds also contain different carotenoids, such as lutein, zeaxanthin, and β-carotene [55], which have been described as potent antioxidants [56] and are precursors of vitamin A, which plays an important role in the prevention of macular degeneration and other eye diseases [60]. Finally, some legume seeds, such as soybeans, peanuts, chickpeas, lentils, beans, and lupins, constitute an important source of unsaturated fatty acids, such as oleic and linoleic [55–57, 61], which have an effect in the lowering of cholesterol levels [62], helping in the prevention of coronary diseases [63, 64].

Most of the research studies on the effects of the inoculation with rhizobial bacteria on the contents of bioactive compounds in legumes have been performed in soybean (*Glycine max*). Silva and collaborators [61] indicated that the inoculation of soybeans with *Bradyrhizobium japonicum* sv *glycinearum* induced an increase in some volatile compounds and organic acids in seeds, confirming the results previously obtained by Couto and collaborators [65], who showed that soybean plants inoculated with *B. japonicum* presented a higher increase in the content of phenolic compounds and organic acids. Besides, grains from inoculated plants presented a higher total fatty acids content, with an increase in both monounsaturated and polyunsaturated fatty acids [61]. On the other hand, the inoculation of faba bean (*Vicia faba*) induced a considerable augmentation in the antioxidant constituents and the total content of flavonoids, phenols, tannins, and proteins [66]. And moreover, a study performed using a *Mesorhizobium* strain to inoculate chickpea (*Cicer arietinum*) showed an increase of flavonoids content in the inoculated plants compared to those ones of the negative control [67].

Finally, some studies have been performed on medicinal legume plants, such as *Psoralea corylifolia* L. The seeds of this plant, known as "Buguzhi," are used in the traditional Chinese medicine for various disorders treatments, particularly vitiligo [68]. *Psoralea corylifolia* contents psoralen [69], a tricyclic furocoumarin, are used for the treatment of hypo-pigmented lesions of the skin for its potent photosensitizing capability [70]. The inoculation of *P. corylifolia* with *Ensifer meliloti* and *Rhizobium leguminosarum* strains has shown an increase in the psoralen content in the seeds of this legume [71].

Considering all these studies and the fact that legumes are regarded as functional foodstuff and recommended as nutraceuticals, we can assume the importance of deepening in the studies of how plant growth-promoting bacteria affect their content in bioactive molecules, not just the mentioned in the already performed studies, but also other not yet considered, which may greatly influence consumers' health.

3 Improvement of Beneficial Substances in Berries by Bacterial Inoculation of Theirs Crops

Berries-type fruits are a heterogeneous group of fruits widely consumed because of their nutraceuticals qualities. These fruits present low energy contents and high antioxidant activity due to high concentrations of bioactive compounds such as vitamins or phenolic compounds [3]. There are several species integrated in the group of the so-called berries-type fruits, i.e., bilberries (*Vaccinuim myrtillus*), lingonberries (*Vaccinium vitis-idaea*), blueberries (*Vaccinium corymbosum),* cranberries (*Vaccinuim macrocarpon* or *Vaccinuim oxycoccos*) strawberries (*Fragaria ananassa),* red raspberries (*Rubus idaeus),* cloudberries (*Rubus chamaemorus*), arctic brambles (*Rubus articus*), loganberies (*Rubus loganobaccus*), honeyberries (*Lonicera caeulea*), rowan berries (*Sorbus* spp.), or crowberries (*Empetrum nigrum*, *E. hermaphroditum*), which present different bioactive compounds profiles, for example, blue and black colored berries show the highest antioxidant activity, which is related to the highest content in anthocyanins [72]. Moreover, berry fruits show good nutritional characteristics, present low amounts of fat contents, high fiber concentration, and an excellent mineral profile [73].

Berries production is usually carried out using conventional agricultural systems, but consumers have started to demand new quality standards of production [74]. In this point, plant growth promotion rhizobacteria (PGPR) could be a key tool to improve their nutraceutical characteristics. However, compared to other type of crops, the number of yield experiences with PGPR is poor yet, but they show good expectations.

Probably, strawberry is the berry which more studies have received due to the relevance of this crop in agriculture. Alvarez-Suarez et al. [75] showed that the consumption of strawberries could improve cardiovascular health and induce an increase on immunological fitness [76]. For this reason, several PGPR studies have been focused on the biofertilizer influence in vitamin contents in strawberries. Different bacteria have been employed to improve nutraceutical qualities of strawberry as *Pseudomonas*, *Bacillus*, *Paenibacillus*, or *Phyllobacterium* [77–79]. The inoculation of *Pseudomonas* BA-8, *Bacillus* OSU-142, and *Bacillus* M-3 produces an increase of vitamin C concentration in strawberry when they are applied by root, foliar, or combined way [77]. Strawberry plants inoculated with *Paenibacillus polymyxa* RC05 produce fruits with high levels of vitamin C concentration [78]. Similar results have been obtained with *Phyllobacterium endophyticum* PEPV15 inoculation under greenhouse conditions. This strain can increase in a 79% the vitamin C concentration comparing with the uninoculated control [79]. Esikten et al. [80] showed that the application of *Pseudomonas* and *Bacillus* strains can increase plant biomass and nutrient content through the production of organic acids from bacteria.

Another important way reported to improve strawberry production is the co-inoculation of PGPR bacteria with mycorrhizal fungi. According to Bona and collaborators, the co-inoculation of *Pseudomonas* strains and an arbuscular mycorrhizal fungi (AMF) produces an increase in vitamin C with respect to the uninoculated control. The authors also show that the vitamin B9 content only presents in a significant high level in double co-inoculation with AMF and PGPB treatment [81].

Other significant compounds in berries with nutraceutical qualities are flavonoids, which activity is directly related to their concentration [82]. Anthocyanins are the main flavonoids involved in antioxidant activity in berries as strawberry, blackberry or raspberry [83]. Reported results from experiments in strawberry when plants are inoculated with a mixture of plant growth promoting bacterial strains (*Pseudomonas fluorescens* Pf4 and 5Vm1K) and a mycorrhizal fungus (*Glomus* sp.) show how the co-inoculation produces an increment in anthocyanin contents [84]. Orham et al. [85] showed than *Bacillus* OSU-142 and *Bacillus* M3 are also efficient biofertilizers with the ability to improve raspberry production and nutrient quality, including their content in flavonoids.

Basu and Maier [86] showed that all different flavonoids presented in berries crops have antioxidant activity with potential to improve several aspects of human health. Raspberry, blackberry, and other dark berries have a relevant role as flavonoid sources [72]. The inoculation of blackberry plants with the strain *Pseudomonas fluorescens* N21.4 produces an increase in flavonoid content of 22% compared to the uninoculated treatment. These results are especially significant during summer and autumn months [87]. This strain of *Pseudomonas fluorescens* is also able to increase or stabilize total flavonoid content in blackberry fruits under adverse environmental conditions [88], and further experiments suggested that these results are based on differential expression of genes involved in flavonoid synthesis which suffer stimulation when blackberry plants are inoculated with *Pseudomonas fluorescens* N21.4 [89].

4 Increase of Nutrients and Bioactive Compounds in Horticultural Vegetables by PGPR Inoculants

Horticultural crops provide humans with a big variety of essential vitamins and indispensable elements [90]. According to Ramsay et al. [91], the consumption of a wide range of horticultural fruits is linked to overall diet quality.

In addition to be required for many physiological functions, vitamins prevent deficiency syndromes which can affect humans when there is an irregularity of their contents [92]. Scientists are developing different ways and methods to increase the total content of vitamins in horticultural crops. Here, we present some of the studies in which bacterial plant probiotic inoculation has been used to produce an improvement in food quality.

Bona et al. [93] reported that the inoculation of tomato plant with *Pseudomonas* sp. 19Fv1T enhances crop yield and improves vitamin C content in tomatoes, compared to the control treatment. Gül et al. [94] also reported that the highest levels of vitamins content in tomato fruits were obtained after the bacterial inoculation of two *Bacillus amyloliquefaciens* strains (FZB2 and FZB42). Additionally, Shen et al. [95] showed that a mixture of *Bacillus amyloliquefaciens*, *Bacillus megaterium*, and vermicompost also increased tomato yields and vitamin C fruit content. Although Tomato is one of the most horticultural cultivated crops in the world [96], this fruit is also regarded as an excellent source of antioxidants compounds [97]. Ochoa-Velasco et al. [98] have described an important improvement in vitamin C and total phenols contents with a reduction of nitrogen doses after the inoculation with *Bacillus licheniformis*.

An increase in lycopene antioxidants levels have also been reported in tomato fruits after bacterial inoculation. According to Ordookhani et al. [99], the mix of *Pseudomonas putida*, *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Glomus lipoferum, Glomus mossea*, and *Glomus etunicatum* not only increase lycopene antioxidants levels but also is related to an improvement in potassium fruit contents. The nutrient contents of nitrogen, calcium, magnesium, potassium, and phosphorus were significantly improved in tomato fruits after the inoculation with five combinations of plant growth promotion rhizobacteria such as *Pseudomonas*, *Azotobacter*, and *Azospirillum* [100].

Basil crop (*Ocimum basilicum* L.) is an interesting medicinal plant used worldwide for cooking. Its antioxidant activity has been improved with plant probiotics bacteria. Compared to the uninoculated treatment, the highest antioxidants levels were achieved after the inoculation with a mixture of *Pseudomonas putida*, *Azotobacter chroococcum*, and *Azospirillum lipoferum* strains [99]. Under water stress and after the inoculation with *Pseudomonas* sp., *Bacillus lentus*, and *Azospirillum brasilense*, the antioxidant activity in basil crops also improved [101].

5 Conclusion

Expansion and improvement of a more sustainable and at the same time proficient agriculture, which will guarantee food resources to feed the growing world populations, fighting the hunger in developing countries, with limited land and energy resources while at the same time protecting the environment, is one of the principal challenges of the nowadays human society. There is a plethora of research studies proving that certain bacteria improve yields in agricultural crops, by aiding in nutrients supply, producing growth-stimulating phytohormones, preventing pathogen-induced plant diseases, and inducing plant resistance to biotic or abiotic stresses, so the application of these bacteria as biofertilizers is one of the strategies that could help to achieve the goal.

In parallel, there is an increasing worry about food quality and heathy diets, and many people in developed countries and wealthy families in developing countries demand quality functional foods which improve human health and aid in the prevention of diseases. Consumers' demand of organic foodstuff is increasing, and at the same time most countries are developing several policies to limit or banish the application of chemical fertilizers in crop production.

As shown in this chapter, contrasting chemical fertilizers, the application of plant probiotic bacteria as crops biofertilizers increases not only yields, but also the nutritional quality of seeds, fruits, and horticultural vegetables. Research in this field showed how several bacterial inoculants interact with different plant species, and as a result there is an increase of some plant compounds, which are beneficial to human health. Thus, the application of probiotic bacteria to reduce chemical fertilizers is an excellent alternative, not just to sustain crop production while limiting chemical fertilizers, but also to improve food quality.

The still scarce number of studies orientated to the application of plant probiotic bacteria to improve the quality of fruits, grains, and horticultural crops has showed an increase in the levels of vitamins, antioxidants, and flavonoids, among other values. Still, there are many other bioactive compounds which could also be enriched by the application of bacterial inoculants. Therefore, new research approaches such as metabolomics, comparing plant foodstuffs produced with and without the application of plant probiotic bacteria, may reveal additional bioactive compounds whose quantity may be enlarged by the applications of bacterial inoculants. Also, more studies on the effects of different bacterial strains on the food quality increase in different plant species are necessary; this would allow for a better selection of plant probiotic bacteria with the potential to increase not just crops yields, but also their quality and health benefiting properties.

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