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Improving Yield and Antioxidant Properties of Strawberries by Utilizing Microbes and Natural Products

Mahfuz Rahman, Mosaddiqur Rahman and Tofazzal Islam

Abstract

Consumption of strawberry has gone up worldwide due to its proven health benefits. Strawberry growers are using synthetic fertilizers and pest management products to maximize yield. This situation posed a risk by affecting sustainability of strawberry production and tainting reputation of a healthy fruit by placing it in the list of dirty dozen due to pesticide residues on fruit. Alternative approaches for increasing yield and pest management of strawberry to minimize environmental and health hazards are possible. Recent studies on alternative natural products (e.g., chitosan) and beneficial microbes (e.g., *Bacillus*, *Paraburkholderia*, etc.) indicated that growth, yield, and fruit quality enhancement are supported by these products and may help in sustainable strawberry production. This chapter reviews and updates our knowledge on the health benefit of strawberry and research findings on the use of natural products and probiotic bacteria for yield and quality improvement in strawberry.

Keywords: probiotic, antioxidants, sustainability, disease control, strawberry yield, microbial biostimulant

1. Introduction

Strawberries are a popular fruit in the US and worldwide. In the US, per capita consumption of strawberries has increased from 2 pounds/person/year to approximately 8 pounds/person/year in recent years [1]. This trend is also apparent in other developed and developing countries of the world. As a result, strawberry growers are using synthetic fertilizers and pest management products to maximize yield. As increased use of synthetic chemicals (fertilizers and pesticides) in crop production and protection has posed a threat to both environment and human health [2], an alternative approach for plant growth promotion, pest management, and sustainable agriculture is being explored all over the world. Strawberry and other fruits and vegetables that are mostly consumed fresh are getting special research attention to innovate production techniques excluding synthetic chemicals [3]. Strawberry growers are specifically eager to find new agro-techniques with special emphasis on the use of both plant growth promotion and nutritional quality improvement in a move toward a more sustainable and environment-friendly approach. In addition,

researchers have been testing novel, sustainable approaches to improve the quality and antioxidant properties of strawberries to increase health benefits. One of the reasons for strawberry demand and consumption has been going up as this fruit is an excellent source of natural antioxidants, such as carotenoids, phenolics, vitamins, anthocyanins, and flavonoids with remarkably high capacity of scavenging free radicals [4]. Improving fruit quality and yield sustainability without synthetic inputs is a research priority for this nutritious fruit. Beneficial microorganisms that are used as bio-fertilizers or bio-stimulants possess the ability to colonize the rhizosphere, plant roots, or both when applied to seeds or plant organs that are used for vegetative propagation (strawberry tips). Some of these microbes have shown potential to promote strawberry plant growth by the release of metabolites into the rhizosphere that may inhibit various pathogens as biocontrol agents [5–8]. However, Tomic et al. [9] found that the response to bacterial inoculation is cultivar-related in strawberries. These microbes were reported to improve plant nutrition and support plant development under natural or stressed conditions as well as increase yield and quality of many important crops and thus may play a crucial role in sustainable crop production in the future [10–12]. A small but significant body of literature also suggests that these microbes can increase strawberry fruit quality in terms of taste and nutritional value and thereby have a positive impact on human health with associated reduction of healthcare costs [13, 14]. The objective of this review is to update our knowledge on the research conducted on improving yield and quality of strawberry by using natural products and beneficial microbes around the globe. Major focus of the review is to relate bio-fortified strawberry fruit with human health benefit. Some novel eco-friendly approaches and potential mechanisms involved with yield and quality improvement in strawberry are also discussed.

2. Nutritional and health benefit profile of strawberry

Strawberries are an excellent source of essential and health benefitting nutrients (**Table 1**) and low in total calories with a 100 g serving providing only 32 kcal. Their sweet flavor makes them a delicious alternative to processed foods. Dietary fiber present in strawberries may contribute to regulating blood sugar levels by slowing digestion. Fiber content may also control calorie intake by its satiating effect. Strawberries contain fat-soluble vitamins (i.e., vitamin A and tocopherol) and carotenoids (i.e., lutein and zeaxanthin), but one of the aspects of major nutritional relevance is the extremely high content of vitamin C, even higher than citrus fruits. Together with vitamin C, folate plays a crucial role in the nutritional quality of strawberry as it is one of the richest natural sources of this essential micronutrient, and folate is an important factor in health promotion and disease prevention [15, 16]. Strawberry is a source of several other vitamins such as thiamin, vitamin B6, vitamin K, vitamin A, and vitamin E although to a lesser extent (**Table 1**). It is also an excellent source of manganese providing more than 20% of the daily adequate intake (AI) for this mineral per serving. The same amount of strawberries can provide about 5% of the AI for potassium and is known as a good source of iodine, magnesium, copper, iron, and phosphorus (**Table 1**).

2.1 Role of strawberry as a source of dietary antioxidants compared with similar sources

Strawberry consumption can help to prevent inflammation, oxidative stress, cardiovascular disease (CVD), certain types of cancers, type 2 diabetes, and obesity. The addition of berries to the diet can positively influence risk factors for CVD by

Type	Nutrient	Per 100 g
Minerals	Calcium (mg)	16
	Iron (mg)	0.41
	Magnesium (mg)	13
	Phosphorus (mg)	24
	Manganese (mg)	0.386
Vitamins	Vitamin C (mg)	58.8
	Folate (μg)	24
	Thiamin (mg)	0.024
	Lutein + zeaxanthin (μg)	26
	Vitamin E, α -tocopherol (mg)	0.29
	Vitamin K (μg)	2.2
Proximates	Vitamin B6 (mg)	0.047
	Dietary fiber (g)	2.0
	Fructose (g)	2.44

Adapted from [17].

Table 1.
Nutrient composition of fresh strawberries.

inhibiting inflammation, improving plasma lipid profiles, scavenging free radicals, and increasing LDL resistance to oxidation [18]. The mechanisms by which strawberries exert these positive effects are not completely understood. Among many potential mechanisms, its role as an antioxidant is the most relevant as strawberry supplementation significantly decreases oxidative stress, protecting mononuclear blood cells against DNA damage [19, 20]. Several studies have shown that strawberry generally possesses a high level of antioxidant activity, which is linked to the levels of phenolic compounds in the fruit rather than vitamin C [1, 21–23]. Wang and Jiao [24] showed that strawberry juice extracts exhibited a high level of antioxidant capacity against free radical species. Strawberry extracts also seem to modulate cell signaling in cancer cells by inhibiting proliferation of several types of cancer cells inducing cell cycle arrest and apoptosis and suppressing tumor angiogenesis [25]. An unavoidable result of aerobic metabolism in humans and other organisms is the production of reactive oxygen species (ROS). ROS include free radicals such as the superoxide anion ($\text{O}_2^{\bullet-}$) and hydroxyl radical ($\bullet\text{OH}$), as well as nonradical molecules like hydrogen peroxide (H_2O_2), singlet oxygen ($^1\text{O}_2$), etc. All ROS can be damaging to organisms at a concentration where its level exceeds the defense mechanism. These excess ROS can put cells in oxidative stress that eventually pose a threat to cells by causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids, and enzyme inhibition. The enhanced production of ROS during physiological stresses can also activate a programmed cell death (PCD) pathway that may lead to cell death [26–33]. Under normal conditions, ROS molecules are unable to cause any damage as they are constantly being scavenged by a range of antioxidative mechanisms [34]. But, the delicate equilibrium between the ROS production and their scavenging by antioxidants is disturbed by multiple stress factors. An efficient antioxidative system that includes nonenzymatic as well as enzymatic antioxidants in a cell can usually scavenge or detoxify excess ROS [35]. The human antioxidant defense system includes endogenous (enzymatic and nonenzymatic) antioxidants and exogenous antioxidants such as vitamin C, vitamin E, anthocyanidins,

carotenoids, flavonols, and polyphenols, with the diet being the main source [36–39]. Exogenous antioxidants play a key role in this delicate equilibrium between oxidation and antioxidation in living systems [36, 37, 40, 41]. Under physiological conditions, the human antioxidative defense system allows the elimination of excess ROS. However, our endogenous antioxidant defense systems are incomplete without exogenous reducing compounds such as vitamin C, vitamin E, carotenoids, and polyphenols. Therefore, there is a continuous demand for exogenous antioxidants to prevent oxidative stress.

Strawberry polyphenolic phytochemicals perform nonessential functions in plants but have large impacts on humans. Of the polyphenolic compounds, anthocyanins in strawberries are the best-known and quantitatively the most important. Studies have determined total anthocyanin content as 150–600 mg/kg of fresh weight. [17]. Strawberries also contain small amounts of other phenolic compounds as shown in **Table 2**. Evidence from in vitro studies shows that strawberry phenolics may have anti-inflammatory effects and suppress mutagenesis through antioxidative and genoprotective properties. Additionally, the content and composition of flavonols have been studied [42], and these compounds are identified as derivatives of quercetin and kaempferol, with quercetin derivatives being the most abundant [43]. The contents of the flavonoid groups, flavonols, and anthocyanins in strawberry extracts have been associated indirectly and directly, respectively, with the total antioxidant capacity for low-density lipoproteins [21]. Flavonoids in strawberries exhibit antioxidant [44, 45] and anticancer properties as well [46]. Elevated levels of these secondary metabolites should provide better health benefits to the consumers of strawberry.

Among numerous studies conducted on antioxidant contents in fruits and vegetables, results have shown that strawberry possessed a high level of antioxidant activity compared with others in the same group, and the activity was directly linked to the levels of phenolic compounds in the fruit [1, 21, 22]. A comparative study on the antioxidant activity of strawberry extract with other fruits based on the oxygen radical absorbance capacity assay indicated that its antioxidant capacity was higher than extracts from plum, orange, red grape, kiwifruit, pink grapefruit, white grape, banana, apple, tomato, pear, and honeydew melon [47]. However, Sun et al. [22]

Class	Group	Compound
Flavonoids	Anthocyanins	Cyanidin-3-glucoside
		Cyanidin-3-rutinoside
		Pelargonidin-3-glucoside
		Pelargonidin-3-rutinoside
		Pelargonidin-3-malylglucoside
	Flavonols	Quercetin-3-glucuronide
		Quercetin-glucoside
		Kaempferol-3-glucoside
	Flavanols	(+)-catechin
		Proanthocyanidin B1 (EC-4,8-C)
Proanthocyanidin B3 (C-4,8-C)		
Phenolic acids	Hydroxycinnamic acids	p-coumaroyl hexose

Adapted from [17].

Table 2.
Polyphenol composition reported in strawberries.

ranked fruit differently for antioxidant contents based on total antioxidant oxyradical scavenging assay. These results put strawberry behind cranberry, apple, and grape but before peach, lemon, banana, pear, orange, grapefruit, and pineapple in terms of antioxidant activity of fruit extracts. Total antioxidant activity of strawberry can also relate to the contents of anthocyanins, which are typically present at high levels in this fruit [47]. Great interest has developed in strawberries due to the extremely high content of vitamin C, which makes them an important source of this vitamin for human nutrition. Relatively high content of ellagic acid is also a reason of interest for strawberries to consumers. Ellagic acid is an antioxidant that has been proposed to exert antimutagenic and anticarcinogenic effects [48, 49]. Nutritional quality of strawberry is reflected in its high levels of vitamin C, folate, and phenolic constituents [17], most of which show relevant antioxidant capacities *in vitro* and *in vivo* [50]. Moreover, strawberries are economically feasible and commercially important, and are widely consumed as fresh or in processed forms such as jam, juice, and jelly. Due to the high nutritional quality, taste, and health benefits, strawberries are among the most studied berries from the aspects of horticultural, genomic, and sustainable production practices.

3. Enhancement of yield and antioxidant contents in strawberry by various natural products including chitosan

To overcome the challenge of increasing strawberry production with a significant reduction of agrochemical use and environmental pollution (especially from synthetic chemicals), a great deal of interest and research has been devoted to natural products and beneficial microbes in recent days. Many growers and researchers are actively looking for ways to create a more sustainable production system through use of natural inputs while simultaneously improving yield and antioxidant properties. A large body of literature suggested that integration of these products with conventional management tools could significantly reduce chemical use and make strawberry production more sustainable. Various natural products have been tested in strawberry production to improve yield and quality by preventing disease and stimulating growth and development. Among the natural products, chitosan is the most tested that has shown growth and yield stimulating effect together with efficacy against diseases in strawberries and other crops [51]. Chitosan is a polysaccharide derived from chitin outer skeletons of shell fish and crustaceans such as crab, crayfish, lobster, and shrimp. As chitin is deacetylated by sodium hydroxide to obtain chitosan, it is slightly basic and is soluble in dilute aqueous acidic solution ($\text{pH} < 6.5$). Once dissolved, it can be further diluted with water to apply on plants at all different growth stages. In general, it is nontoxic to humans and considered safe for agricultural uses due to its quick degradation in the environment. Once chitosan or its derivatives come in contact with plants, they bind with the cell plasma membrane and elicit defense responses through expression of pathogenesis-related (PR) genes, accumulation of phytoalexins, callose, oxidative burst, and formation of reactive oxygen species. Expression of these PR genes and accumulation of antimicrobial phytoalexins are believed to play a major role in controlling pre- and postharvest pathogenic diseases. A large body of published reports supports antimicrobial activities of chitosan against a wide range of phytopathogens [52]. Similar studies also found that the biostimulant chitosan promoted plant growth and development and provided enhanced disease suppression capability to plants through multiple mechanisms including induced systemic resistance [51, 53]. Chitosan has been widely used as fruit coatings to enhance storability and preserve anthocyanin and other antioxidants in strawberry [51], and various other fruit mainly for protection

from postharvest losses due to microbial infections [51, 54]. In addition, many investigators reported that chitosan use as a foliar spray increased vegetative growth, yield, and biochemical contents in plants [55–58]. Improvement of yield and functional properties of strawberry fruit through application of chitosan should be considered a sustainable option. A recent study by Rahman et al. [58] showed that multiple application of low concentrations (ppm level) of chitosan on the canopy of field grown strawberry plants at the prebloom stage significantly improved growth and yield. Authors also reported concurrent increase in various antioxidant contents and total antioxidant activities in treated fruit compared to nontreated control. This is an interesting and significant finding as total antioxidants and pigments such as anthocyanins are determinants of health benefits of strawberry fruit. Rahman et al. [58] also determined the effect of different doses of chitosan biopolymer on growth, fruit yield, and human health benefiting antioxidant properties of strawberry and found that both yield and contents of antioxidants are increased in a dose-dependent manner to some extent compared to untreated control. These findings indicate that the biostimulant chitosan can be an attractive agent for production of high quality and human health benefiting strawberry [58]. Results also indicated that foliar application of varying doses of chitosan on strawberry canopy stimulated all aspects of vegetative growth (plant height and root length) that may have influenced fruit yield and fruit quality compared with untreated control (**Table 3**). These findings were also interesting as all doses of chitosan improved growth of strawberry plants to some extent and may be experimented in similar crops being grown in soils with varying physical, chemical, and biological characteristics. This study was one of the few of its kind that determined the effects of natural products such as chitosan application on field-grown strawberry plants influencing yield and contents of multiple antioxidants in fruit. Experimental protocol for this study can be found in Rahman et al. [58].

Among a few different chitosan concentrations tested in the study, 500 ppm provided the highest fruit yield (42% higher than untreated control) in “Strawberry Festival” compared with untreated control (**Table 3**). Similar to yield response and a few other antioxidants, chitosan spray application on the canopy of strawberry also significantly increased fruit anthocyanin contents in a dose-dependent manner that plateaued at 500 ppm with 184.3 mg cyanidin-3-*O*-glucoside/100 g fruit. This increase of anthocyanin contents was equivalent to 2.3-fold higher compared

Treatment	Plant height (cm)	Root length (cm)	Total fruit weight/plant (g)	Total anthocyanin content	Total phenolic content	Total antioxidant activity
Control	19.5 ± 1.0b	19.25 ± 0.4c	246.6 ± 0.4d	81.11 ± 0.9d	310.4 ± 0.7c	250.9 ± 0.9c
Ch 125	20.41 ± 0.9b	21.16 ± 0.2bc	317.5 ± 0.7c	83.1 ± 1.0cd	356.5 ± 1.0b	252.6 ± 1.0c
Ch 250	21.75 ± 0.8b	22.66 ± 0.7ab	325.7 ± 0.5c	94.6 ± 0.5c	317.8 ± 0.5c	358.6 ± 1.0b
Ch 500	25.1 ± 1.0a	24.33 ± 0.2a	351.25 ± 0.5a	184.3 ± 1.9a	363.2 ± 0.4ab	374.42 ± 1.0b
Ch 1000	24.91 ± 1.5a	24.16 ± 0.6a	337.7 ± 0.4b	163.9 ± 0.6b	370.9 ± 0.4a	415.6 ± 0.5a

Five different concentrations, 0, 125, 250, 500, and 1000 ppm, of chitosan solution were prepared by dissolving the required amount in 0.1 N HCl and diluting with distilled water with pH adjusted at 6.5 by NaOH. Freshly prepared chitosan solutions were applied onto strawberry plants in each experimental unit prior to flowering and at 10% flowering stage by spraying up to run off at five different times with 10-d intervals. Cumulative fruit harvest from each plot was recorded. The required amounts of fruit tissues from first harvest were subjected to analyses for phenolics and other antioxidants mentioned in the table. Values are means ± standard errors of three independent replications (n = 3). Different superscripted letters within the column indicate statistically significant differences among the treatments according to Fisher's protected LSD (least significance difference) test at $p \leq 0.05$, adapted from [58].

Table 3.
Effect of chitosan application on yield and content of antioxidants in strawberry fruit.

with untreated control. The fruit produced by the plants treated with 1000 ppm chitosan solution in the study by Rahman et al. [58] had the highest total phenolic content (370.9 µg gallic acid/g fruit) indicating that chitosan concentration should be adjusted depending on the intended quality improvement in strawberry fruit. Total antioxidant activities of strawberry fruit obtained from both varied rates of chitosan treated and untreated control plants were assayed by utilizing the DPPH method, and the results were expressed as butylated hydroxytoluene (BHT) equivalents per gram of strawberry fruit. The highest total antioxidant activity was quantified in strawberry fruit obtained from 1000 ppm chitosan (415.6 µg BHT/g fruit) treated plants. These results reveal that application of chitosan on the canopy of strawberry could increase antioxidant activity in fruit up to 1.7-fold compared to untreated control (**Table 3**) [58].

A few other examples of natural products that have been investigated on strawberry with varying results are derived either from seaweed or compost. Seaweed products are used as nutrient supplements, biostimulants, and biofertilizers to augment plant growth and yield in agriculture. A study by Masny [59] found no effect on disease suppression of *Botrytis cinerea* on strawberry by applying seaweed products. However, application of these products had a significant influence on yield with an increase in the range of 17–42%. Compost or tea extracts were also used for plant disease control and for plant nutrition and growth promotion. Welke [60] assessed the effect of compost extract application on strawberry. Aerobically prepared extracts were effective in both disease suppression (*B. cinerea*) and increasing yield compared to the control.

4. Strawberry growth, yield, and quality improvement by probiotic bacteria

Beneficial microorganisms especially bacteria that are associated with host plants either as rhizoplane, phylloplane, or endophyte and enhance growth of the host plants including yield are popularly known as plant probiotic bacteria (PPB). These PPB can also suppress plant diseases by various modes of action when applied proactively in adequate amounts [61]. PPB that are used as biofertilizers or biostimulants possess the ability to colonize the rhizosphere, plant roots, or both when applied to seeds or crops. Some of these microbes have shown potential to promote strawberry plant growth by the release of metabolites into the rhizosphere that may inhibit various pathogens as biocontrol agents [5–8]. However, Tomic et al. [9] found that the response to bacterial inoculation is cultivar-related in strawberries, which indicates that a specific microbial strain should be tested for efficacy against a specific strawberry variety before large scale use. Microbes belonging to this group are also known as plant growth promoting rhizobacteria (PGPR) and were reported to improve availability of plant nutrient and support plant development under natural or stressed conditions as well as increase yield and quality. Although beneficial microbes have not been widely researched or used for improving yield and quality of strawberry, a large body of evidence indicates that many available beneficial microbes were found to provide growth and yield enhancement to diverse crop commodities [10–12], which can be tested for similar efficacy on strawberry and thus may play a crucial role in sustainable strawberry production in the future. A significant body of literature suggests that these microbes can increase strawberry fruit quality in terms of taste and nutritional value and thereby have a positive impact on human health with associated reduction of healthcare costs [13, 14]. A few relevant examples of positive effects of antagonistic microbes on multiple crops include protection against *Verticillium dahliae* [62] and protection of tomato

against *Alternaria solani* [63]. Some of these microbes were used in vitro and should be evaluated in vivo or in field conditions. For example, in vitro-beneficially-bacterized plantlets of grapevine not only grew faster than non-bacterized controls but also were sturdier, with a better developed root system and significantly greater capacity for withstanding gray mold fungus [64]. Similarly, banana plantlets treated with endophytes *Pseudomonas* and *Bacillus* species showed improved vegetative growth, physiological attributes, and strong defense against bunchy top diseases in the field [65, 66]. Seed treatment or augmenting beneficial microbial population in soil was also found to reduce seedling mortality from soil-borne diseases [67]. Biological agents such as *Trichoderma*, *Serratia*, and *Pseudomonas* and different plant extracts are some of the alternative strategies that have been explored to reduce the number of microsclerotia or wilt symptoms in multiple crops [65, 68–72]. A few studies also showed that application of beneficial bacteria significantly improved seed germination, seedling vigor, growth, yield, and early blight disease protection in tomato through multiple mechanisms including production of growth regulators and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase [63]. Although many different strains of microbes belonging to multiple genera and species have been identified and tested for their efficacy, major genera of PPBs include *Bacillus*, *Paraburkholderia*, *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, and *Serratia*. Major modes of action by which PPB provide beneficial effects to host plants include production of growth promoting hormones, antibiotics, and lytic enzymes that affect harmful microbes, nitrogen fixation from the atmosphere, nutrient solubilization from soil minerals for plant availability, and systemic resistance induction in the host or treated plants. Two PPB, *Bacillus amyloliquefaciens* and *Paraburkholderia fungorum* applied on strawberry by Rahman et al. [73] not only increased yield but also significantly improved contents of several antioxidants and total antioxidant activities of fruits. Treatments of strawberry plants with bacterial strains *B. amyloliquefaciens* and *P. fungorum* consistently produced higher antioxidants, carotenoids, flavonoids, phenolics, and total anthocyanins compared to nontreated control [73]. Flores-Félix [14] reported that application of a strain of genus *Phyllobacterium* on strawberry showed significant increase in vitamin C contents in fruits.

A recent study [73] explored an environment-friendly option for boosting strawberry plant growth, fruit yield, and functional properties of fruits through the application of two plant growth promoting probiotic bacteria and compared the results with that of nontreated control. Results showed significant improvement in plant growth, yield, various antioxidant contents, and total antioxidant activities of strawberry fruits by the application of both *B. amyloliquefaciens* BCh1 and *P. fungorum* BRRh-4 treatment compared to nontreated control. Inoculation of strawberry plants separately with two bacterial isolates significantly increased vegetative growth (plant height and root length) of the strawberry plants (**Table 4**). Generally, plant growth promoting rhizobacteria facilitate plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus, and essential minerals) or modulating plant hormone levels, or indirectly by inhibiting various pathogens as biocontrol agents [11]. Early colonization of root system has the potential to preclude pathogen colonization and infection in addition to induction of disease resistance or a range of beneficial secondary metabolites. Plant height and root length also were positively influenced and varied significantly due to the plant probiotic bacterial applications. The highest plant height (20.50 cm) was observed in BRRh-4 treated plants (**Table 4**). Similar to plant height, root length also significantly ($p < 0.05$) varied among the treatments and was reflected by plant vigor (**Figure 1**). A hypothetical pathway of strawberry growth, yield, and fruit quality improvement is shown in **Figure 2**. Results from this study indicated that vegetative

Treatment	Plant height (cm)	Root length (cm)	Total fruit weight/plant (g)	Total anthocyanin content	Total phenolic content	Total antioxidant activity
Control	18.6 ± 1.01a	19.3 ± 0.43b	316.6 ± 10.06b	81.1 ± 0.5b	317.1 ± 7.3b	250.9 ± 3.1b
BChi1*	119.3 ± 0.86a	22.7 ± 0.33a	453.0 ± 2.2a	187.5 ± 16.9a	377.8 ± 1.7a	382.0 ± 1.4a
BRRh-4	20.5 ± 0.26a	23.5 ± 1.15a	467.8 ± 2.2a	223.0 ± 3.6a	380.5 ± 5.1a	385.5 ± 3.4a

Cumulative fruit harvest from each plot was recorded. The required amounts of fruit tissues from first harvest were subjected to analyses for phenolics and other antioxidants mentioned in the table. Values are means ± standard errors of three independent replications (n = 3). Different superscripted letters within the column indicate statistically significant differences among the treatments according to Fisher's protected LSD (least significance difference) test at $p \leq 0.05$, adapted from [73].

Table 4.
 Effect of plant probiotic bacteria on yield and antioxidant content in strawberry fruit.

growth enhancement by probiotic bacteria may have also enhanced fruit yield and quality, enhancing secondary metabolites such as anthocyanins, phenolics, and total antioxidant activity (**Table 4**). Strawberry fruit from *Paraburkholderia fungorum* BRRh-4 and *Bacillus amyloliquefaciens* BChi1 treated plants had total phenolic content 380.5 and 377.72 µg gallic acid/g fruit, respectively compared with 317.08 µg gallic acid/g fruit in untreated control plants. Detailed experimental protocol can be found in a study by Rahman et al. [73].

One of the interesting findings of this study is that both plant probiotic bacteria significantly improved growth and yield of strawberry almost at the same level with some minor differences although they belong to different bacterial genera. Probiotic bacterium, BRRh-4 provided the highest fruit yield increase (48%) in plants of “Strawberry Festival” compared to nontreated control (**Table 4**). Treatments of strawberry plants with bacterial strains BRRh-4 and BChi1 consistently produced higher antioxidants, carotenoids, flavonoids, phenolics, and total anthocyanins compared to nontreated control [73]. A previous study showed that the members of the genus *Phyllobacterium* were good plant probiotics with the capacity of increasing fruit yield as well as quality [14]. Application of plant probiotic bacteria significantly increased total anthocyanin content in strawberry



Figure 1.
 Effect of different doses of chitosan and probiotic bacteria on vegetative and reproductive growth of cv. Strawberry Festival. Adapted from [58, 73].

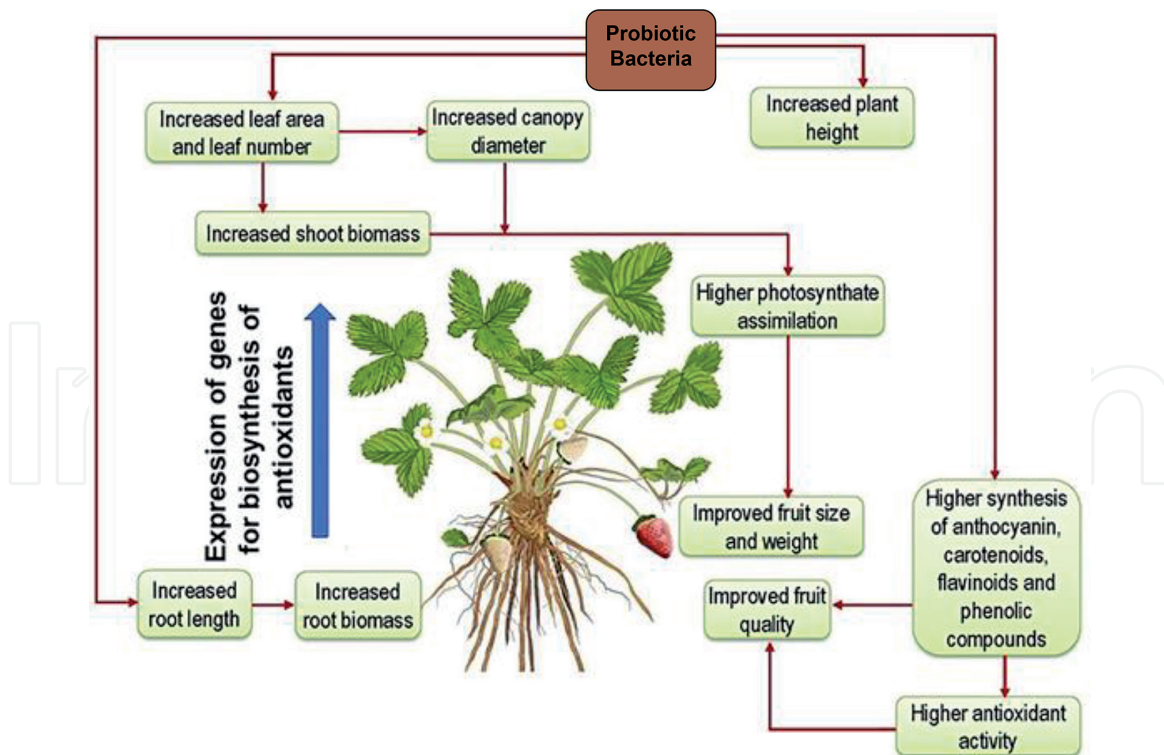


Figure 2.
A hypothetical pathway of stimulation of fruit yield and accumulation of antioxidants in strawberry fruit due to the root colonization by probiotic bacteria.

fruits compared to nontreated control. The highest anthocyanin content (222.0 mg cyanidin-3-*O*-glucoside/100 g fruit) in strawberry fruits was recorded in plants treated with BRRh-4 followed by BChi1 (187.47 mg cyanidin-3-*O*-glucoside/100 g fruit). To evaluate whether plant probiotic bacteria had any effect on antioxidant activities of strawberry fruits obtained from both probiotic bacteria and nontreated control plants, we estimated total antioxidant activities of fresh strawberry fruits by DPPH assay. The results of the DPPH assay for total antioxidant activity were expressed as butylated hydroxytoluene (BHT) equivalents per gram of strawberry fruit. As expected, the total antioxidant activity of fresh strawberry fruits was the highest in BRRh-4 (385.47 µg BHT/g fruit) followed by BChi1 treatment (382.00 µg BHT/g fruit) (Table 4) [73].

4.1 Bio-rational/natural product-based approach for strawberry root disease management for boosting yield

The strawberry black root rot complex (BRRC) and crown rot are increasing problems in perennial strawberry plantings worldwide and have been identified as limiting factors of sustainable strawberry production [74, 75]. Yield loss from black root rot alone can range from 20 to 50% [76], which can dramatically increase if crown rot occurs concurrently. Because several factors are involved in BRRC of strawberry, including a range of infectious agents (nematodes and root infecting fungi) and various abiotic factors such as poor soil characteristics [77], the disease control is complicated, and no general control measure is completely effective. On the other hand, crown rot disease of strawberry caused primarily by the fungal species *Colletotrichum gloeosporioides* and *Phytophthora cactorum* [78] can sometimes also incur significant yield loss in strawberry production in the US and other strawberry growing countries [78]. Although inoculum sources for crown rot in fruiting fields may be diverse, infected planting stock is the

most important source of *C. gloeosporioides* [79–83] whereas *P. cactorum* is mostly soil-borne and builds up in a strawberry field over time. Occurrence of crown rot caused by *Fusarium oxysporum* f.sp. *fragariae* is also on the rise. Mass [84] observed that in many cases where crop rotation was not an option, fumigation of soil was necessary to control soil-borne diseases. Methyl bromide (MeBr) was previously used as a preplant broad-spectrum soil fumigant to control soil-borne diseases, nematodes, insects, and weeds in high value crop such as strawberry [85]. However, with the disappearance of this highly effective soil fumigant MeBr, and restrictions on the allowed use of other alternative synthetic fumigants, the interest in the development of safe, sustainable, and economically viable fumigation strategies have increased to manage soil-borne fungi and nematodes [85]. More importantly, the demands from organic growers and small growers who cannot use synthetic fumigants have increased tremendously [86]. Alternative strategies are also required especially for strawberries as disease-resistant cultivars are unavailable [87]. Among multiple alternatives of soil fumigation with synthetic chemicals, glucosinolate-containing Brassica spp. is known to release volatile isothiocyanates (ITCs), which are toxic to different pathogens [88]. The chemistry involved in the biofumigation can be attributed to the action of myrosinase enzyme on the glucosinolates (GLS) to release ITCs, thiocyanates, nitriles, oxazolidine, dimethyl sulfide, and methanethiol, among other compounds [88, 89]. Several lines of evidence suggest that biofumigation with ITC-producing plants have shown promising results against soil-borne fungal pathogens, for example, *Rhizoctonia*, *Verticillium*, *Fusarium*, *Pythium*, and *Phytophthora* spp. [90–92]. However, the concentration of ITCs produced is influenced by mustard variety [93], soil texture, moisture, temperature, microbial community, and pH [94, 95], resulting in variable soil-borne disease control efficacy. From a NE-SARE funded project in the U.S., Balzano [93] found the highest glucosinolate content and biomass in “Caliente-199.” While these observations indicate a need for selecting the right variety, site-specific testing, optimization of the method such as selection of the best growth stage (highest content of glucosinolate), optimum tissue disruption, and quick soil incorporation may also play a significant role. This is crucial for the success of this approach as laboratory experiments indicated that the efficacy of the conversion to ITCs was only 5% of the potential when using tissue disruption methods (cutting and chopping) similar to those frequently used under field conditions [95, 96]. Matthiessen et al. [97] were able to increase soil ITC levels by 20-fold (100 nmol per g soil) using a tractor-drawn tissue pulverizing implement compared to when using a cutting and chopping implement. In addition, they showed that adding excess water to the pulverized tissue was necessary for maximum ITC release.

Another promising nonchemical soil-borne disease control alternative is anaerobic soil disinfestation (ASD), which was adapted from the previously described methods of biological soil disinfestation (BSD) and soil reductive sterilization [98, 99] to create a treatment suitable for strawberry [100]. A wide range of soil-borne plant pathogens and plant parasitic nematodes have been controlled in a variety of crops using ASD [100]. Implementation of ASD is done in three different steps. First, a labile carbon source is added to the soil followed by the generation of anaerobic conditions through application of water to fill soil pore space. In the third step, the soil is covered with plastic mulch to prevent oxygen exchange. The exact mechanisms that lead to disease suppression with ASD are not clearly understood but may involve production of organic acids and other biologically active volatiles [101] and amplification of specific microbes with biocontrol activity [102].

5. Future perspectives

Findings from many important and relevant studies indicated that natural products and plant probiotic bacteria especially the ones isolated from the native environment could be used as natural agents for sustainable production of high quality strawberry with no or little additional use of expensive synthetic inputs. However, researchers found that the effects are more pronounced in nutrient poor growing conditions. The additive effect of utilization of these products in growing environment with balanced nutrition has not been sufficiently researched. In addition, with the disappearance of soil fumigant methyl bromide that played an essential role in managing soil-borne diseases in high value crop like strawberry, more research should be directed to finding natural alternatives for sustainable management of both foliar and soil-borne diseases. Resistance development in plant pathogens against synthetic products is also a huge concern that dictates the need for developing sustainable options. Numerous natural products and microbial strains have been screened for antimicrobial properties with positive outcomes enabling plants to resist important phytopathogens and provide plant growth-promoting effects. However, only a few of these products are commercially available to growers. Large-scale use of these products did not occur due to the variability and inconsistency of results in field conditions. Lowering the variability and increasing the consistency of results from these products are among a few challenges that will have to be addressed. The scientific community must determine the factors that interfere with the reproducibility of results from one location to another or controlled condition to field condition over time. Integration of these products with other management options should help in reducing the variability of results and produce additive effects. The continuing need for natural products supporting sustainable strawberry production will make discovery and commercialization of natural and beneficial microbe-based products as an attractive and profitable pursuit in the coming days.

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