

GROWTH PROMOTION OF RASPBERRY AND STRAWBERRY PLANTS BY BACTERIAL INOCULANTS

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ABSTRACT

Study on potential mechanisms influencing the growth of raspberry and strawberry plants showed that the most active was *Bacillus* sp. strain AF75BC producing IAA and siderophores, and having the ability to release phosphorus. The latter feature was also present in the strains Sp115AD (*B. subtilis*) and SP116AC (*Paenibacillus polymyxa*). Two of the tested strains: SP116AC and JaFGU (*Lysobacter* sp.) showed the ability to fix atmospheric nitrogen, while the AF75AB2 (*Bacillus* sp.) produced siderophores and IAA. All strains showed an antagonism toward the most important pathogens of strawberry and raspberry, i.e. *Verticillium dahliae*, *Botrytis cinerea*, *Phytophthora cactorum* and *Colletotrichum acutatum*, limiting their growth to a different extent on the PDA medium. Inoculation of raspberry roots with the tested bacteria resulted in an increase of some growth parameters of their above-ground part in cv. Poemat. In the case of cv. Polana, a significant increase was found only in the chlorophyll content in the leaves. All the inoculants caused an increase in dry mass of roots in cv. Polana, and in cv. Poemat similar effect was observed after applying Inoculants 1 and 3. The treatments of strawberry roots with any of the inoculants resulted in a significant increase in the total leaf surface area in cv. Rumba, but they had no effect on the chlorophyll content in the leaves of either cultivar. All the inoculants significantly increased the total length of roots and their total surface area in cv. Rumba. This parameter also increased in cv. Elsanta, and the number of root tips also significantly increased in this cultivar. Our study showed that the tested inocula is a promising alternative as a bio-fertilizer for small fruit production in sustainable and organic agricultural systems.

Key words: growth mechanisms, antagonism, pathogens, growth parameters, microbial inoculants

INTRODUCTION

The decline in natural resources and the negative impact of conventional farming practices on the environment indicate the need to develop new, safe and effective technologies. To this end, integrated, holistic multidisciplinary research is being conducted in many countries. It meets the current EU strategy aimed at supporting the development of environmentally friendly methods promoting the growth and yielding of

plants and intended for the protection of plants against pests [Backer et al. 2018, Pylak et al. 2019, Rouphael and Colla 2020]. The directions set out in the Common Agricultural Policy document and other strategic documents, e.g. Green Deal EU and UN Sustainable Development Goals (SDG), are being promoted.

In order to preserve the biodiversity of agroecosystems and minimize the harmful effects of chemical-

ization, alternatives to chemical fertilizers and pesticides are seen as having good prospects for the future [Le Mire et al. 2016]. Included among them are biostimulants, i.e. “any substance or microorganism, in the form in which it is supplied to the user, applied to plants, seeds or the root environment with the intention to stimulate natural processes of plants benefiting nutrient use efficiency and/or tolerance to abiotic stress, regardless of its nutrient content, or any combination of such substances and/or microorganisms intended for this use” [Traon et al. 2014, du Jardin 2015].

The forthcoming European Union (EU) Fertilizing Products Regulation proposes a claim-based definition of plant biostimulants, stipulating that “plant biostimulant” means a product stimulating plant nutrition processes independently of the product’s nutrient content, with the aim of improving one or more of the following characteristics of the plant: nutrient use efficiency, tolerance to abiotic stress, crop quality traits or availability of confined nutrients in the soil and rhizosphere [Ricci et al. 2019]. Apart from bacteria and fungi, substances of natural origin, such as humic and fulvic acids, seaweed extracts, protein hydrolysates and amino acids, can thus be also regarded as biostimulants [Calvo et al. 2014].

Bacterial biostimulants can be based on one bacterial strain or a mixture of strains, i.e. bacterial consortia [Le Mire et al. 2016, Santos et al. 2019]. The positive influence of bacteria on plant growth and yielding occurs through direct and/or indirect mechanisms. The first of them include the ability to fix nitrogen (symbiotic and non-symbiotic bacteria, e.g. of the genera *Rhizobium*, *Azoarcus*, *Beijerinckia*, or the species *Pantoea agglomerans* and *Klebsiella pneumoniae*), to solubilize phosphates and potassium compounds (e.g. *Arthrobacter* spp., *Bacillus* sp., *Pseudomonas* spp., *Serratia* spp.), to produce siderophores (*Pseudomonas* spp.), exopolysaccharides (e.g. *Rhizobium* spp., *Azotobacter* spp., *Bacillus* sp., *Xanthomonas* spp.). The solubilization of P in the rhizosphere is the most common mode of action implicated in PGPR that increase nutrient availability to host plants [Richardson 2001]. The most thoroughly studied and used in practice is the symbiosis of nitrogen fixation between rhizobias and legumes. Inoculants based on these bacteria are the earliest example of commercial microbial products in agriculture and still represent the most wide-

ly used agricultural inoculants [Bashan 1998]. The phytohormone producing ability is widely distributed among bacteria associated with soil and plants. Studies have demonstrated that the PGPR can stimulate plant growth through the production of auxins (indole acetic acid) [Spaepen et al. 2008], gibberellins [Bottini et al. 2004] and cytokinins [Timmusk et al. 1999]. Many bacterial strains increase the availability of Fe through the production of organic acids or siderophores [Ahmed and Holmstrom 2014]. Iron is an essential growth element for all living organisms. The scarcity of bioavailable iron in soil habitats and on plant surfaces foments a furious competition [Loper and Henkels 1997]. Under iron-limiting conditions PGPR produce low-molecular-weight compounds called siderophores to competitively acquire ferric ion [Whipps 2001].

Indirect mechanisms, on the other hand, determine the ability of bacteria to prevent the harmful effects of factors of biotic origin, e.g. plant pathogens, by producing substances that have a toxic effect on them and/or increase the natural resistance of plants to infection. They include hydrolytic enzymes (e.g. chitinases, cellulases, proteases), various antibiotics, and siderophores [Gouda et al. 2018].

In Europe, the largest amounts of biostimulants are produced in France, Italy and Spain. By 2025, the global market for these products is predicted to reach over 4 billion dollars [Grand View Research 2018].

In recent years, there has been a growing interest in the use of beneficial bacteria in fruit crops to replace mineral fertilizers and maintain sustainability. Effective application of plant growth promoting rhizobacteria (PGPR) in strawberry organic production has been reported in studies of Esitken et al. [2010], Sas-Paszt et al. [2019a, 2019b, 2020a, 2020b]. Karlidag et al. [2013] proved that such bacteria showed the ability to reduce the harmful effect of salt stress on strawberry plants. According to Kurokura et al. [2017] application of PGPR could be a method to improve the content of valuable compounds for human health in strawberry fruits. The obtained results indicate in fact that PGPRs have the potential to increase yield while maintaining fruit quality, but the effect is cultivar dependent. It was also found that root and foliar application of various strains of *Bacillus licheniformis*, *B. subtilis*, and *Bacillus* sp. gave the best results with respect to growth,

yield and fruit quality of strawberry as compared to other application methods, including treatment of only the roots or only the leaves [Seema et al. 2018]. In this context noteworthy is work of Gutiérrez-Mañero et al. [2001], the results of which indicate that *B. pumilus* and *B. licheniformis* isolated from rhizosphere of alder promoted of alder stem elongation. Robledo-Buriticá et al. [2018] believe that bacteria *B. subtilis* and *B. pumilus* act as biofertilizers resulting in the long-term growth of blackberry (*Rubus glaucus*) effect similar to mineral fertilization. Mixtures of those bacteria can act synergistically. The first study showing that PGPR (*Bacillus* sp.) can increase yield, growth and nutrient content in raspberry plants was conducted by Orhan et al. [2006]. The influence of introducing *Pseudomonas fluorescens* (Pf5) bacteria into the soil under highbush blueberry has also been investigated, resulting in a significant increase in leaf surface area and diameter of the main shoot [de Silva et al. 2000]. Positive effects of PGPR have been also reported in many other horticultural crops such as: apple [Altaf et al. 2019], apricot [Esitken et al. 2003], cherry [Esitken et al. 2006], grape [Sabir 2013].

The aim of our study was to determine the influence of three inoculants, based on selected bacterial strains, on the growth of strawberry and raspberry plants in greenhouse conditions. Potential mechanisms of action of these bacteria related to growth stimulation and their effect on the most important pathogens of strawberry and raspberry were also determined.

MATERIAL AND METHODS

Bacterial strains used in inoculants

Five strains of rhizosphere bacteria were selected for the experiments. They were included in three inoculants: Inoculant 1 – Sp116AC (*Paenibacillus polymyxa*) and Sp115AD (*Bacillus subtilis*); Inoculant 2 – AF75AB2 (*Bacillus* sp.), Sp115AD (*B. subtilis*) and AF75BC (*Bacillus* sp.); Inoculant 3 – JaFGU (*Lyso-bacter* sp.). The composition of the inoculants were chosen according to the plant growth-promoting properties of the selected strains and their antagonistic activity toward each other. Strain Sp116AC was antagonistic towards AF75AB2, AF75BC, and JaFGU. JaFGU produced secondary metabolites toxic to the other

strains used in this experiment. The bacteria were identified based on the sequence of the gene encoding the 16S rRNA subunit. The GeneMatrix Bacterial & Yeast Genomic DNA Purification Kit (EURx) was used for DNA isolation. Amplification of the 16S rRNA gene was performed using the primer pair 27F/1492R [Lane 1991]. The obtained DNA sequences were compared with NCBI data using the BLAST tool (National Center for Biotechnology Information, https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch). All the strains used in this work are preserved in a sterile solution consisting of glycerol (30%), peptone (0.5%), yeast extract (0.3%) and distilled water (69.2%), and stored at –80°C in SYMBIO-BANK – a microorganisms collection (The National Institute of Horticultural Research, Department of Microbiology and Rhizosphere, Skierniewice, Poland).

Determination of potential mechanisms responsible for stimulation of vegetative growth of plants

Phosphate solubilization. The test was performed on Pikovskaya agar medium [Nautiyal 1999]. The agar plates were inoculated with the tested isolates and then incubated at 26°C for 14 days. The strains able to dissolve calcium phosphate from the medium produced a clear zone around the colony.

Siderophore production. The test was performed on CAS agar medium [Alexander and Zuberer 1991]. The agar plates were inoculated with the tested isolates and then incubated at 26°C for 2–3 days. The strains able to synthesize siderophores produced an orange zone around the colony.

Nitrogen fixation. The test was performed on semi-solid NFb medium [Ribeiro and Cardoso 2012]. The tubes with the medium were inoculated with 72–96-hour-old bacterial culture, sealed with cotton wool and incubated at 26°C for 7 days. The strains able to fix nitrogen produced biomass.

Production of indole acetic acid. The test was performed on lysogen broth with the addition of 5 mM l-tryptophan [Ribeiro and Cardoso 2012]. The tubes with the broth were inoculated with the tested strains and incubated at 26°C. After 24 h of incubation, the biomass was separated from the liquid medium by centrifugation at 20 598 g. Next, 250 µl of Salkovski reagent was added to 500 µl of each supernatant. After 30 min of incubation, the change in colour was assessed.

A red-rose colour of the supernatant in the presence of Salkovski reagent indicated that the tested bacteria produced IAA from l-tryptophan.

Biotic relationships between bacterial strains used in inoculants and pathogens of strawberry and raspberry on agar media

To estimate bacterial antibiosis towards pathogens, two types of agar media were used: nutrient-rich potato-dextrose agar (PDA) and nutrient-poor soil agar consisting of 5 g of air-dry soil, 15 g of bacteriological agar and 1000 g of distilled water. The plates with PDA or soil agar were inoculated with the pathogens *Verticillium dahliae*, *Botrytis cinerea*, *Colletotrichum acutatum* or *Phytophthora fragariae*. *V. dahliae* was spread on the whole surface of agar plates with the use of a cotton swab with the biomass scraped from a 7-day-old *V. dahliae* culture growing on PDA medium. Agar plugs with *B. cinerea*, *C. acutatum* or *Ph. fragariae* were placed in the centre of the agar plates. Then the plates with the pathogens were point-inoculated with the tested bacteria using a sterile wooden toothpick, and incubated at 26°C. The inoculated plates were examined every week for 21 days. Colonies of the isolates able to produce metabolites toxic to *V. dahliae* were surrounded by a inhibition zone of fungal growth. The isolates able to synthesize metabolites toxic to *B. cinerea*, *C. acutatum* or *Ph. fragariae* inhibited or slowed down the growth of the these pathogens.

Preparation of inoculants

The bacteria were grown on Plate Count Agar (PCA) with the following composition: glucose 1 g, yeast extract 2.5 g, peptone K 5 g, agar 15 g, H₂O 1000 g, pH 7.2 ±0.2. After 72 h of incubation at 26°C, the bacteria were transferred to 100 ml of 50% Tryptone Soya Broth (composition: casein peptone 8.5 g, soy peptone 1.5 g, glucose monohydrate 1.25 g, NaCl 2.5 g, K₂HPO₄ 1.25 g, H₂O 1000 g) in 250 ml Erlenmayer flasks. The final pH of the medium was 7.3 ±0.2. The inoculated medium was incubated at 30°C in a shaking water bath at 100 strokes per minute. After 72 h, 50 ml portions of culture fluid were each transferred to 400 ml of 50% TBS broth in 1000 ml Erlenmayer flasks. After another 72 h of incubation at 30°C in the shaking water bath at 100 strokes per minute, the

bacterial concentration was standardized to 1.0–5.0 × 10⁸ CFU ml⁻¹ with a spectrophotometer (BiowaveII, manufacturer of Biochrom WPA) at a wavelength of 590 nm and by plating on the PCA medium. The inoculants prepared in this way were used to treat the roots of raspberry and strawberry plants.

Plant material, growth conditions and treatments

The experiments were carried out for three months in greenhouse conditions on seedlings of raspberry cvs. Polana and Poemat, and strawberry cvs. Rumba and Elsanta, planted in a substrate consisting of coir and peat with a grain size of 7–20 mm, in a volumetric ratio of 2 to 1, respectively, in 3 litre pots. Granulated manure was added to the substrate at 5 g per pot. Before planting, the roots of the plants were immersed in a suitable inoculum for 30 min. The control combination consisted of plants immersed in tap water for 30 min. The experimental design was a randomized complete block with 4 replications. Each replication consisted of 3 plants. During the experiment the plants were watered with tap water.

Analysis of growth parameters of plants above-ground part

At the end of the study, leaf chlorophyll content was determined with a SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc.). It was measured on three fully expanded leaves from each plant. The results obtained for each plant were averaged for later processing [Neufeld et al. 2006].

Next, the above-ground part of the plants was cut off, placed on a tray and scanned with an EPSON EXPRESSION 10000 XL scanner. After the scan was completed, the total surface area (cm²) was determined using the WinRhizo software (WinRHIZO Pro v.2009c). Additionally, in case of raspberry the fresh and dry weight (g) of the above-ground part of plants as well as height (cm) of plants were determined. Dry weight was measured after drying the plant material at 55°C for 3 days. Both measurements were made on a laboratory balance (RADWAG WLC 3/A2/C/2).

Analysis of growth parameters of the root system

After having been cut off, the roots were placed on a sieve and gently rinsed with water to remove soil particles. Next the total length (cm), diameter (mm),

volume (cm³), fresh weight (g) and number of root tips were determined. Total area (cm²) was determined with the EPSON EXPRESSION 10000 XL scanner. Their dry weight (g) was determined after drying at 55°C for 3 days. Both fresh and dry weights of roots were determined with a laboratory balance (RADWAG WLC 3/A2/C/2). All root growth parameters were determined with the WinRhizo software. In case strawberry the weight of fresh and dry weight of roots was not determined.

Data analysis

The experimental design was a randomized complete block with 4 replications. Each replication consisted of 3 plants. The results were statistically analyzed by one-way analysis of variance using the Newman-Keuls test in the statistical program Statistica 13.1.

RESULTS AND DISCUSSION

Among the tested mechanisms of bacterial action that may affect the growth of raspberry and strawberry plants, the greatest activity was shown by the AF75BC (*Bacillus* sp.) strain producing IAA and siderophores, and having the ability to release phosphorus. The latter feature was also present in the strains Sp115AD (*B. subtilis*) and SP116AC (*Paenibacillus polymyxa*).

Two of the tested strains: SP116AC and JaFGU (*Lysobacter* sp.) showed the ability to fix atmospheric nitrogen, while the AF75AB2 (*Bacillus* sp.) produced siderophores and IAA (Tab. 1). There is a lot of information in the literature that bacteria occurring in the soil act on plants through a variety of mechanisms including nitrogen fixation, P-solubilization, siderophore production and IAA production [Bhattacharyya and Jha 2012, Calvo et al. 2014, Pii et al. 2015]. Phosphate solubilizing PGPR are included in the genera: *Arthrobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Microbacterium*, *Pseudomonas*, *Erwinia*, *Rhizobium*, *Mesorhizobium*, *Flavobacterium*, *Rhodococcus*, and *Serratia* and have attracted the attention of agriculturists as soil inoculate improve plant growth and yield [Gouda et al. 2018]. In recent years, there has been interest in developing commercial inoculants for free-living nitrogen-fixing bacteria, such as: *Azoarcus* sp., *Burkholderia* sp., *Gluconacetobacter* sp., *Diazotrophicus* sp., *Herbaspirillum* sp., *Azotobacter* sp., *Paenibacillus polymyxa*, and especially *Azospirillum* sp. [Vessey 2003]. These diazotrophs may provide N to a much wider range of crop plants than rhizobia. It is worth emphasizing study of Adesemoye and Kloepper [2009] which has been indicate that bacteria that do not fix N have been shown to increase N uptake in plants, thus increasing nitrogen use efficiency. Iwata et al. [2010] proved for the first time

Table 1. Production of siderophores and indole acetic acid (IAA), ability to phosphate solubilization and nitrogen fixation by bacterial strains used in inoculants

Strain	Siderophores	Phosphate solubilization (glucose)	Nitrogen fixation	IAA
SP116AC <i>Paenibacillus polymyxa</i>	–	+	+	+
Sp115AD <i>Bacillus subtilis</i>	+	++	–	–
AF75AB2 <i>Bacillus</i> sp.	++	–	–	++
AF75BC <i>Bacillus</i> sp.	+	+	–	++
JaFGU <i>Lysobacter</i> sp.	–	–	+	+

Reaction intensity: + weak, ++ medium, – no reaction or no growth on NFb medium

that bacteria of genus *Lysobacter* fixes nitrogen under free-living conditions, as shown by its ability to grow on nitrogen-free medium and accumulate relatively high amounts of ammonia in the culture broth. Also Satapute et al. [2012] determined that the soil isolate *Bacillus subtilis* strain AS-4 is salt tolerant, free living nitrogen fixing bacteria that could be exploited as soil inoculants and can be used for nitrogen fixation in soil with high concentration of salt, which is of long run, eco-friendly and cost ineffective. Noteworthy is the research of Singh et al. [2020], which showed that a substantial number of *Bacillus* isolates have N-fixation and biocontrol property against two sugarcane pathogens *Sporisorium scitamineum* and *Ceratocystis paradoxa*.

Swain et al. [2007] reported a positive effect of *Bacillus subtilis* IAA producing strains on the edible tubercle *Dioscorea rotundata* L. The application of a suspension of *B. subtilis* on the surface of the plants resulted in an increase in stem and root length, increase fresh weight of the stem and root, and the numbers of sprouts as compared with non-inoculated plants. Most commonly, IAA-producing PGPR are believed to increase root growth and root length, resulting in greater root surface area which enables the plant to access more nutrients from soil. Bacterial species-specific effects were observed in root hormone levels: indole-3-acetic acid concentration was elevated in roots inoculated with *P. polymyxa* L6 or Pw-2 [Bent et al. 2001]. Liu et al. [2017] documented that

siderophore-producing bacterial strains identified as *Paenibacillus illinoisensis* and *Bacillus* sp., enhanced root activity, chlorophyll and active iron content in leaves, total nitrogen, phosphorus and potassium accumulation of plants and increased the quality of peanut kernels and plant biomass over control. Valuable information was also provided by Zhang et al. [2020] research showing that *Paenibacillus triticisoli* BJ-18, a N₂-fixing bacterium, is able to promote plant growth due to siderophores and indolic acids production. On the other hand the siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on Fusarium wilt and promotes the growth of pepper [Yu et al. 2011].

Besides stimulating of plant growth by direct mechanisms the applied in our study bacterial strains can also indirectly improve growth of strawberry and raspberry plants by protecting them against soil-borne pathogens. However, the influence of tested phytopathogens on those plants were not the subject of our research. All our bacterial strains showed an antagonistic effect against the most important pathogens of strawberry and raspberry, i.e. *V. dahliae*, *B. cinerea*, *Ph. cactorum* and *C. acutatum*, limiting their growth to a different extent on the PDA medium (Tab. 2). On the soil agar, however, this effect was generally less pronounced, and some strains showed no ability to limit the growth of the tested pathogens at all. This indicates the importance of the composition of the media from which bacteria can synthesize substances

Table 2. The growth inhibition of strawberry and raspberry fungal pathogens by bacterial strains used in inoculants*

Strain	Pathogens (medium)							
	<i>Verticillium dahliae</i> (PDA)	<i>Verticillium dahliae</i> (soil agar)	<i>Botrytis cinerea</i> (PDA)	<i>Botrytis cinerea</i> (soil agar)	<i>Phytophthora cactorum</i> (PDA)	<i>Phytophthora cactorum</i> (soil agar)	<i>Colletotrichum acutatum</i> (PDA)	<i>Colletotrichum acutatum</i> (soil agar)
SP116AC	5 (+5)*	3	6	15	10	7	X	X
Sp115AD	0 (+4)	0	2	10	5	0	3	X
AF75AB2	0 (+4)	0	2	14	5	0	X	X
AF75BC	2 (+4)	0	5	6	2	4	3	X
JaFGU	6	4	4	13	10	8	5	7

* the values are given in mm after 21 days of incubation; values in brackets mean zone of disturbance of microsclerotia formation
X – ‘slowing down’; a slowdown in the growth of the pathogen, but after 21 days the bacterial colony was ‘overgrown’ by pathogen

that are toxic to other organisms living in the same biotope [Mikiciński et al. 2020]. Depending on the concentration of these substances, the degree of antagonism can vary from complete inhibition of growth to only slight limitation, or lack of this ability. Besides inhibition of growth of *Verticillium dahliae* almost all the tested strains revealed activity in disturbance of microsclerotia formation and decreased the growth rate of *Colletotrichum acutatum*, but after 21 days the bacterial colony was overgrown by pathogen (Tab. 2). It is worth emphasizing that although *in vitro* studies indicate the potential of bacteria to have a given ability, its manifestation in natural conditions will depend on many environmental factors. Research of Exposito et al. [2015] showed that the genus *Lysobacter* includes several species that produce a range of extracellular enzymes and other metabolites with activity against bacteria, fungi, oomycetes, and nematodes. Among strains of *B. subtilis* ZO4, in particular, was recorded very high growth inhibition against *V. dahliae*. These findings are consistent with several studies indicating that rhizosphere may be a common source for the selection of *Bacillus* species with important potentials that are useful for the biocontrol of both soil-borne and foliar pathogenic fungi [Govindasamy et al. 2010, Hinarejos et al. 2016].

Several authors have reported the large spectrum of antifungal activity of *Bacillus* sp. and have suggested that antibiosis could be the most common mode of antagonism observed among these species [Toral et al. 2018, Caulier et al. 2019]. Other studies have also reported that *Bacillus* sp. protect plants through a number of mechanisms, particularly through the

synthesis of different lipopeptides with inhibitory activity against phytopathogens [Falardeau et al. 2013]. Zhang et al. [2018] found that strain ShX301 of *Paenibacillus polymyxa* showed very high antagonistic activity against spore germination and mycelial growth of *V. dahliae*. It also appeared very effective in controlling *Verticillium* wilt of cotton and demonstrated a broad-spectrum of antifungal activity against other plant pathogens. Moreover, this strain significantly promoted the growth of cotton seedlings. Beneficial microorganisms have been extensively used to make plants more resistant to abiotic and biotic stress. Study of Zhang et al. [2019] showed that a consortium of three plant growth promoting rhizobacteria (PGPR) strains (*Bacillus cereus* AR156, *Bacillus subtilis* SM21, and *Serratia* sp. XY21) is a promising and environmentally friendly biocontrol agent against phytophthora blight of sweet pepper. Also Cheng et al. [2020] determined the inhibitory effects of two plant-growth-promoting rhizobacterial (PGPR) strains, namely *Bacillus tequilensis* C-9 and *Sphingobacterium* A1, against *V. dahliae* *in vitro* and in the field.

Inoculation of raspberry roots with the test bacteria resulted in an increase of some growth parameters of their above-ground part in cv. Poemat, as compared to untreated plants (Tab. 3). A significant increase in the height of plants of this cultivar was found, with the highest increase (by approx. 56%) after application of Inoculant 1. All the inoculants also caused a significant increase in the fresh weight of plants (from 81.6% to 89.9%) and in the total surface area of plants of this cultivar (approx. 30%). On the other hand, in the case of plants of cv. Polana, a significant

Table 3. Effects of treatment with bacterial inoculants on above ground part of raspberry plants (2019)

Treatment	Plants height (cm)		Fresh weight (g)		Dry weight (g)		Total plant area (cm ²)		Chlorophyll content in leaves (SPAD)	
	Polana	Poemat	Polana	Poemat	Polana	Poemat	Polana	Poemat	Polana	Poemat
Control	33.3 a	45.0 a	15.8 a	15.8 a	5.6 a	7.4 a	906.9 a	1193.8 a	21.4 a	23.4 a
Inoculant 1	39.8 a	70.1 b	21.1 a	28.7 b	6.7 a	12.1 a	1006.7 a	1546.9 b	25.4 b	23.5 a
Inoculant 2	39.0 a	60.3 ab	19.9 a	29.2 b	6.6 a	12.8 a	1088.4 a	1565.1 b	25.3 b	23.1 a
Inoculant 3	48.3 a	59.1 ab	29.4 a	30.0 b	10.1 a	12.2 a	1092.8 a	1534.4 b	30.5 c	23.5 a

Results of analyses verified with univariate analysis of variance using Statistica 13.1. Homogenous groups determined with Newman-Keuls test for $\alpha = 0.05$

increase was found only in the chlorophyll content in the leaves, the highest after applying Inoculant 3 (over 42%). Practically none of the inoculants affected any of the assessed parameters of raspberry roots, and in the case of their total length, they even caused a significant reduction in both cultivars (from 31 to over 43%)

(Tab. 4). This tendency was also observed in the number of root tips, but only in cv. Polana (by approx. 35%). All the inoculants caused an increase in dry mass of roots in cv. Polana, the highest after application of Inoculant 3 (by 67%). A similar effect on this parameter was observed after applying Inoculant 1 in cv.

Table 4. Effects of treatment with bacterial inoculants on roots of raspberry plants (2019)

Treatment	Total length (cm)		Total area (cm ²)		Diameter (mm)		Volume (cm ³)		Number of root tips (pcs)	
	Polana	Poemat	Polana	Poemat	Polana	Poemat	Polana	Poemat	Polana	Poemat
Control	1186.8 b	896.4 b	570.7 a	589.0 a	1.52 a	2.10 a	22.2 a	31.2 a	4698 b	3588 a
Inoculant 1	729.1 a	510.0 a	550.2 a	587.4 a	2.72 a	6.03 a	37.6 a	83.6 a	3058 a	3188 a
Inoculant 2	672.7 a	590.4 a	557.8 a	567.2 a	3.31 a	3.60 a	43.1 a	49.3 a	3020 a	3543 a
Inoculant 3	731.5 a	619.4 a	521.4 a	582.9 a	2.90 a	3.32 a	38.8 a	47.8 a	3108 a	3405 a

Results of analyses verified with univariate analysis of variance using Statistica 13.1. Homogenous groups determined with Newman-Keuls test for $\alpha = 0.05$

Tabela 5. Effects of treatment with bacterial inoculants on raspberry root fresh and dry weight of roots (2019)

Treatment	Fresh weight (g)		Dry weight (g)	
	Polana	Poemat	Polana	Poemat
Control	44.8 a	112.5 a	9.9 a	26.2 a
Inoculant 1	60.7 a	135.7 a	14.2 ab	37.8 b
Inoculant 2	51.6 a	133.4 a	12.3 ab	27.9 a
Inoculant 3	69.1 a	123.3 a	16.5 b	31.0 ab

Results of analyses verified with univariate analysis of variance using Statistica 13.1. Homogenous groups determined with Newman-Keuls test for $\alpha = 0.05$

Table 6. Effects of treatment with bacterial inoculants on leaf surface size and chlorophyll content of strawberry plants (2019)

Treatment	Total leaf surface area (cm ²)		Chlorophyll content in leaves (SPAD)	
	Rumba	Elsanta	Rumba	Elsanta
Control	413.0 a	884.0 a	28.0 a	33.9 a
Inoculant 1	560.4 b	855.8 a	30.7 a	32.7 a
Inoculant 2	535.4 b	886.1 a	29.2 a	30.5 a
Inoculant 3	476.0 ab	934.2 a	30.3 a	31.2 a

Results of analyses verified with univariate analysis of variance using Statistica 13.1. Homogenous groups determined with Newman-Keuls test for $\alpha = 0.05$

Poemat (increase by 44%) and Inoculant 3 (increase by over 18%) (Tab. 5). Studies on the use of bacteria in raspberry cultivation are noteworthy. Treatments of plant roots and rhizosphere with *Bacillus* sp. bacteria resulted in a significant increase in fruit yield (by 33.9% and 79.9%), an increase in shoot length (13.6% and 15%), as well as an increase in the amounts of nitrogen, phosphorus and calcium in the leaves, compared to untreated plants. These bacteria are believed to be particularly useful in crops grown by environmentally friendly means [Orhan et al. 2006].

The treatments of strawberry plant roots with any of the inoculants resulted in a significant increase in the total leaf surface area in cv. Rumba (from 15 to over 35%) (Tab. 6), but they had no effect on the chlorophyll content in the leaves of either cultivar. In contrast, Karlidag et al. [2013] recorded an increase in the chlorophyll content (SPAD readings) in strawberry plants treated with *Bacillus atrophaeus* bacteria (strain EY6) and cultivated under soil salinity conditions. They suggested that inoculations with PGPR could alleviate the deleterious effects of salinity conditions in the soil by increasing plant growth, chlorophyll content, and altering mineral uptake, therefore enhancing salt tolerance in strawberry plants. Another study of Karlidag et al. [2011] data suggest that root inoculation of strawberry plants with PGPR strains tested increased root weight, shoot weight, ionic composition of leaves of strawberry and yield. The results of the study show that PGPR application may increase organic manure use efficiency and have capacity to stimulate strawberry growth and yield. Kurokura et al. [2017] supposed that increase of strawberry yield can

be correlated with the increase of leaf area but not by the photosynthetic ability per unit area, as it was reflected in SPAD. All the inoculants significantly increased the total length of roots (by 28% to 35%) and their total surface area (by about 18%) in cv. Rumba. This parameter also increased in cv. Elsanta (by approx. 15%), and the number of root tips also significantly increased in this cultivar (by approx. 45.5%) (Tab. 7).

Beneficial effects with bacterial strains of the genera *Pseudomonas* and *Bacillus* have been obtained in the cultivation of strawberry plants, the roots of which were immersed in bacterial suspensions before planting, while during the growing season the plants were sprayed several times with bacterial inoculants. Applied strains have potential to increase the yield, growth and P, Fe, Cu and Zn content of the strawberry plant and to increase the soil P, Fe, Zn, K, and Mg availability. Depending on the combination, fruit yield increased by 10.5–33.2% [Esitken et al. 2010]. Other studies have also found that bacteria of the species *Bacillus simplex* (RC19) and *Paenibacillus polymyxa* (RC05) increased the fruit yield of strawberry cv. Fern by 1.98–20.85%, as well as soluble solids content by approx. 14% and vitamin C by over 26% [Erturk et al. 2012].

In conclusion, it is difficult to determine specifically which of the potential mechanisms identified in the bacterial strains used played a particular role in stimulating the growth of the strawberry and raspberry cultivars studied. According to Bhattacharyya and Jha [2012] the interaction between PGPR with plants depends on the type of bacterial strain, plant species and environmental factors. Our study showed that tested inocula is a promising alternative as a bio-fertilizer for

Table 7. Effects of treatment with bacterial inoculants on roots of strawberry plants (2019)

Treatment	Total length (cm)		Total surface area (cm ²)		Diameter (mm)		Volume (cm ³)		Number of root tips (pcs)	
	Rumba	Elsanta	Rumba	Elsanta	Rumba	Elsanta	Rumba	Elsanta	Rumba	Elsanta
Control	356.6 a	356.5 a	112.3 a	121.9 a	1.00 a	1.08 a	3.53 a	3.95 a	1728 a	631 a
Inoculant 1	456.1 b	396.9 a	132.2 b	133.0 ab	1.1 a	1.3 a	4.5 a	4.9 a	1620 a	918 b
Inoculant 2	480.6 b	412.6 a	137.1 b	140.0 b	1.2 a	1.3 a	4.3 a	5.0 a	1729 a	937 b
Inoculant 3	481.3 b	364.7 a	132.6 b	141.7 b	1.1 a	1.3 a	4.2 a	4.6 a	1776 a	914 b

Results of analyses verified with univariate analysis of variance using Statistica 13.1. Homogenous groups determined with Newman-Keuls test for $\alpha = 0.05$

small fruit production in sustainable and organic agricultural systems.

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