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THE IMPACT OF THREE BACTERIA ISOLATED FROM CONTAMINATED PLANT CULTURES ON *IN VITRO* MULTIPLICATION AND ROOTING OF MICROSHOOTS OF FOUR ORNAMENTAL PLANTS

Research note

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ABSTRACT

The strains of bacteria *Paenibacillus glucanolyticus*, *Curtobacterium pusillum* and *Methylobacterium extorquens* were isolated as non-deleterious contaminations from hosta or raspberry tissue cultures. Microshoots of chrysanthemum, gerbera, hosta and rose were inoculated with these bacteria and their influence on shoot multiplication and rooting was evaluated. None of the investigated bacteria caused symptoms of hypersensitivity or vitropathy on the shoot explants at rooting and shoots multiplication. *C. pusillum* stimulated axillary shoot formation in all studied plant genotypes. Length and number of rose roots and their length were higher but number of roots and their length in chrysanthemum were lower in inoculated than in controls. The number and the length of shoots and roots in gerbera and hosta and the number of shoots in chrysanthemum inoculated with *M. extorquens* were higher but shoot length of chrysanthemum and rose, and root length of rose were lower as compared with controls. *P. glucanolyticus* influenced higher number and length of chrysanthemum shoots and root length of chrysanthemum and gerbera than non-inoculated control but the number of gerbera and hosta roots was lower and root length of rose was as low as 0.2 cm. All assessed bacteria were able to assimilate atmospheric nitrogen and *M. extorquens* and *P. glucanolyticus* were able to produce IAA.

Key words: beneficial bacteria, contamination, inoculation, rooting, shoot multiplication

INTRODUCTION

According to contemporary knowledge, it is difficult to imagine plant life without any bacteria (Holland & Polacco 1994; Beattie 2006; Ryan et al. 2008). Bacteria populate surfaces of leaves, stems, fruits and roots (epiphytes), and the interior of the plant body, where they inhabit the vascular bundles, intercellular spaces and cell insides (endophytes). Some of them can be observed in an electron microscope as stable endosymbionts belonging to different bacterial groups (Almeida et al. 2009). Only strains of about 100 species of bacteria have been described as plant pathogens. Other bacteria species do not have a detrimental impact on plants and some were defined as beneficial for plants they colonize. Beneficial effects in stimulating growth and biomass increase resulted from the facilitation of nutrients absorption and production and provision of a variety of plant secondary metabolites, such as plant growth regulators (Ortiz-Castro et al. 2009), chitynolytic enzymes protecting against pathogenic organisms (Compant et al. 2005) and osmoprotectants that enable the overcoming of abiotic stresses (Sziderics et al. 2007). Endophytic and epiphytic bacteria take part in the expression of functional traits of plants (Friesen et al. 2011). The level of the beneficial impact of bacteria on plants depends on

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the bacterial traits, type of bacterial interactions with plant genotypes and biotic and abiotic factors that influence the interactions (Beattie 2006; Hardoim et al. 2008).

In vitro cultures are usually not microorganism free, as microorganisms can survive the surface decontamination within a plant body. Bacteria may appear soon after the initiation of cultures but often only after several passages (Cassels 1997, 2011).

Our survey of contaminated *in vitro* plant cultures obtained from different laboratories yielded 104 isolates of bacteria. Based on 16S rDNA sequencing and BIOLOG tests, they were assigned to 29 taxa (data not published). Some bacteria, although they often multiplied abundantly during the time of passage, which was visible as bacterial growth around microshoots or leakages into the medium, did not have a clear negative effect on the host explants. Using three bacterial strains that seemed to have a beneficial influence on contaminated cultures, the experiments were conducted to answer the question of whether they can modify the multiplication and rooting of microshoots of chrysanthemum, gerbera, hosta and rose.

MATERIAL AND METHODS

The chosen isolates were classified to the genus by sequencing nucleotides of 16S rDNA obtained using rd1/fd1 primers (Weisburg et al. 1991) and then to the species using BIOLOG tests, as *Curtobacterium pusillum* (N), *Methylobacterium extorquens* (67) and *Paenibacillus glucanolyticus* (I). The first two were isolated from raspberry shoot cultures and the third from hosta shoot cultures. The ability of strains to produce auxins (according to Pilet & Chollet, after Glickmann & Dessaux 1995), to fix atmospheric nitrogen (according to Ribeiro & Cardozo 2012) and to dissolve inorganic phosphate (according to Panhwar et al. 2009) was assessed. As a control, strain *Burkholderia phytofirmans* PsJN™ (kindly gifted for research by Prof. Angela Sessitsch from the Austrian Institute of Technology) was used.

Experiments included microshoots of *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam 'Ludo', *Hosta* Tratt. 'Paradigm', *Gerbera jamesonii* Bolus ex Hook 'Kormoran' and *Rosa* L. 'White Gem'. The shoots for experiment were harvested from the stock cultures "free of cultivable bacteria". These stocks were founded from microshoots indexed in two subsequent subcultures for the bacterial contamination by placing fragments of the lowest parts of shoots on two bacteriological media: Nutrient Agar (NA) and 523 medium (Viss et al. 1991). The shoots, from which bacteria did not grow, were considered as free of cultivable bacteria and micropropagated for the purpose of the experiment. The gerbera, hosta and rose were propagated by axillary shoots and chrysanthemum by single shoot elongation from nodal segments. Media used contained macro- and microelements according to Murashige and Skoog (1962), 100 $mg \cdot l^{-1}$ of inositol, WPM (woody plant medium) (Lloyd & McCown 1981) vitamins, 30 $g \cdot l^{-1}$ of sucrose and 6 $g \cdot l^{-1}$ of agar Plant (Duchefa) for gerbera, rose and chrysanthemum or 3 $g \cdot l^{-1}$ of Gelrite (Duchefa) for hosta. The medium for chrysanthemum contained 0.1 $mg \cdot l^{-1}$ kinetin (KIN) and 2 $mg \cdot l^{-1}$ gibberellic acid (GA_3), medium for hosta 5 $mg \cdot l^{-1}$ 6-benzylaminopurine (BAP) and 0.1 $mg \cdot l^{-1}$ 1-naphthalene acetic acid (NAA); for gerbera 2 $mg \cdot l^{-1}$ KIN and 0.1 $mg \cdot l^{-1}$ indole-3-acetic acid (IAA), and for rose 2 mg l^{-1}BAP, 1 $mg \cdot l^{-1}$ GA_3 and 0.1 $mg \cdot l^{-1}$ indole-3-butyric acid (IBA). The pH of the media was adjusted to 5.7 before autoclaving. The temperature in the growth room was 23 ± 2 °C. An illumination of 16 h was provided by 40 W fluorescent lamps, and the intensity of irradiation was between 40 and 50 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. Culture media were sterilized in an autoclave for 17 min at 121 °C and pressure of 0.1 Mpa. The shoot explants were grown in glass jars of 350 ml in volume, containing 35 ml of a medium. There were five shoots in a jar and six jars per treatment.

In the initial experiment, different methods of microshoots inoculation with bacteria (bacterization) were compared. The most effective one was used in the experiment described here. The excised shoots were transferred to jars containing perlite saturated with liquid rooting medium and 100 µl of 24 h bacterium inoculum of strains *Curtobacterium* and *Paenibacillus* and 48 h of *Methylobacterium* strain was added immediately in the centre of a jar of each plant species. Rooting media contained salts

of MS (½ MS for rose), WPM vitamins, inositol 100 $mg \cdot l^{-1}$, 30 $g \cdot l^{-1}$ sucrose with the addition of auxin (for chrysanthemum 0.5 $mg \cdot l^{-1}$ IAA; for hosta 0.1 $mg \cdot l^{-1}$ NAA, for gerbera 5 $mg \cdot l^{-1}$ IAA, and for rose 1 $mg \cdot l^{-1}$ IBA). For the initial 7 days, the cultures were incubated in the dark and then in the light at the photoperiod of 16/8 and intensity of about 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

After 4 weeks, the rooting was assessed by recording the length and the number of roots. Then, the lower parts of the shoots with roots were cut off and placed on the above-mentioned bacteriological media- NA and 523 for checking the presence of bacteria. The shoot tips were transferred to the shoot multiplication media to determine the effect of bacteria on the ability to form axillary shoots. The composition of the multiplication media was the same as above, with the exception that shoot tips of chrysanthemum were cultured on the medium with the kinetin content increased to 0.5 $mg \cdot l^{-1}$. During multiplication, the shoot cultures were visually inspected for the bacterial presence and symptomatic bacterial leakages from the shoot proximity observed in the medium were streaked on the two above-mentioned bacterial media to check the presence and morphological identity of bacteria.

The results of shoot multiplication were assessed after 4 weeks, when the number and length of axillary shoots were recorded.

The microshoots from the above-mentioned step were used for the second experiment on rooting. The media were similar as in the first experiment, but solidified with agar. Rooting was assessed after 5 weeks, when the number and length of roots, as well as length of shoots and number of leaves, were scored. In this trial, only bacteria/plant combinations, which were beneficial for rooting in the first experiment, were studied.

The experiments were arranged as 1-factorial (bacteria inoculation), completely randomized with 30 microshoots in combination. The analysis of variance (ANOVA) was performed for the number of axillary shoots, number of roots, length of shoots and roots, and number of leaves according to the Freeman-Tukey's function. The significance of differences between means was tested using the Duncan's Multiple Range Tests at $p = 0.05$.

The results are presented in chronological order.

RESULTS

Characteristics of bacteria

None of the assessed bacteria was able to solubilize phosphates. All were able to assimilate atmospheric nitrogen. Two bacteria – *M. extorquens* (67) and *P. glucanolyticus* (I) – were able to synthesize IAA. *P. glucanolyticus* had a high potential in IAA production, comparable to *B. phytofirmas*, whereas *M. extorquens* produced twice less IAA than the two above-mentioned strains (Table 1).

Table 1. Characteristics of strains used for bacterization and *Burkholderia phytofirmans* PsJN™

Strain	16S rDNA identification	BioLog identification	Auxin production in $\mu g \cdot ml^{-1}$		Air nitrogen assimilation	Phosphate solubilization
			after 24 h	after 48 h		
N	*Curtobacterium* spp.	*Curtobacterium pusillum*	-	-	+	-
67	*Methylobacterium* spp.	*Methylobacterium extorquens*	0.181	0.476	+	-
I	*Bacillus* spp.	*Paenibacillus glucanolyticus*	0.643	0.900	+	-
PsJN™	*Burkholderia phytofirmans*	Not tested	0.318	0.919	+	-

Indexing of bacteria in explants

From all the bottom parts of the shoots rooted in the medium inoculated with bacteria, bacteria of the morphology similar to the ones used for bacterization were re-isolated on NA and 523 media. The shoots were transplanted into the shoot multiplication media solidified with agar and examined visually for the presence of bacteria (clouding below or transparent halo around explant). Symptoms of bacterial presence appeared on nearly all explants within the first 1-3 weeks after transferring of the microshoots. *M. extorquens* strain growth turned pink after 2-3 weeks. Only explants in which the presence of bacteria was confirmed were accounted to as experimental objects. None of the investigated bacteria caused any hypersensitivity reaction or other vitropathy symptoms on the shoot explants during the rooting or the shoot multiplication.

Rooting of shoots directly after inoculation with bacteria

In this trial *C. pusillum* stimulated rooting - the number and the length of roots of rose (4.3 *versus* 2.3 roots, 1.6 *versus* 1.3 cm), and the root length of hosta (3.2 *versus* 1.0 cm) in comparison to the non-inoculated control. This bacterium decreased the number of roots by 1.7 and their length by 1.0 cm in chrysanthemum as compared to the uninoculated control. It had no influence on the number of the roots of gerbera and hosta (Table 2). *M. extorquens* strain increased twice the number and the root length of gerbera in comparison with non-inoculated control. The rooting of hosta was influenced even stronger as number of roots was 4.8 *versus* 1.8 and length 5.9 *versus* 1.0 in comparison with control (Table 2). *P. glucanolyticus* stimulated only the root length of chrysanthemum and gerbera but diminished the number of roots of hosta and reduced root length of rose up to 0.2 cm (Table 2).

Multiplication of shoots derived from shoots inoculated with bacteria (4 weeks after inoculation)

C. pusillum strain stimulated the number of axillary shoots of gerbera and hosta and doubled the shoot number of rose in comparison to uninoculated control. It also slightly stimulated shoot elongation in rose. *M. extorquens* had stimulatory effect on the number of shoots of chrysanthemum, gerbera and hosta and the length of shoots of gerbera and hosta. It doubled the shoot number of gerbera and hosta in comparison with the control. It also diminished the shoot length of chrysanthemum and rose. *P. glucanolyticus* influenced only shoot multiplication in chrysanthemum and caused decrease in the shoot length of hosta (Table 3).

Rooting of shoots derived from the multiplication passage (9 weeks after inoculation with bacteria)

In the second rooting trial, only combinations, where a positive interaction between host and bacteria were observed, were studied. A positive influence on rooting was confirmed for all bacteria/host combination with the exception of *M. extorquens*/*Hosta* where bacteria decreased root length (Table 4). Rooted shoots of chrysanthemum derived from cultures inoculated 9 weeks before experiment were higher (6.1 *versus* 4.6 cm) with more leaves (18.4 *versus* 10.0) and longer roots (11.4 *versus* 7.5 cm) in comparison with non-inoculated control. Gerbera shoots inoculated with *M. extorquens* had more leaves (8.8 *versus* 7.1) and longer roots (5.9 *versus* 3.8 cm) in comparison with the control. Only in combination *C. pusillum*/*Rose* all parameters of the rooted shoots were higher than in non-inoculated control (Table 4). Shoots were 35% heigher and leaves 38% more numerous, 93% more roots was formed and they were 23% longer.

Table 2. Rooting of microshoots directly after inoculation with bacteria. Shaded cells show means, which differ negatively from non-inoculated control. Bolded means are significantly different from control in a positive sense

Plant genotype	Control		*Curtobacterium pusillum* (N)		*Methylobacterium extorquens* (67)		*Paenibacillus glucanolyticus* (I)	
	Root No.	Root length (cm)	Root No.	Root length (cm)	Root No.	Root length (cm)	Root No.	Root length (cm)
Chrysanthemum × *grandiflorum* 'Ludo'	4.9b	1.7b	3.2a	0.7a	4.8b	1.9b	5.2b	**3.5c**
Gerbera jamesonii 'Kormoran'	1.8b	0.6a	2.4b	0.5a	**3.6c**	**1.2c**	1.0a	**0.8b**
Hosta 'Paradigm'	1.8b	1.0a	2.4b	**3.2b**	**4.8c**	**5.9c**	1.0a	1.1a
Rose 'White Gem'	2.3a	1.3b	**4.3b**	**1.6c**	2.2a	0.9a	2.5a	0.2a

Table 3. Multiplication of microshoots derived from microshoots inoculated before 4 weeks. Shaded cells show means, which differ negatively from non-inoculated control. Bolded means are significantly different from control in a positive sense

Plant genotype	Control		*Curtobacterium pusillum* (N)		*Methylobacterium extorquens* (67)		*Paenibacillus glucanolyticus* (I)	
	Shoot No.	Shoot length (cm)	Shoot No.	Shoot length (cm)	Shoot No.	Shoot length (cm)	Shoot No.	Shoot length (cm)
Chrysanthemum × *grandiflorum* 'Ludo'	2.0a	5.8b	2.2a	5.6b	**2.5b**	5.3a	**3.2c**	**6.5c**
Gerbera jamesonii 'Kormoran'	1.9a	2.4a	**2.5b**	2.4a	**3.8c**	**4.1b**	2.2ab	2.2a
Hosta 'Paradigm'	1.9a	1.8b	**2.7b**	1.5b	**3.9c**	**2.7c**	2.0a	1.0a
Rose 'White Gem'	2.2a	2.1b	**4.7b**	**2.6c**	2.7a	1.7a	3.3a	2.0b

Table 4. Rooting of second vegetative generation microshoots derived from cultures inoculated before 9 weeks. Shaded cells show means, which differ negatively from non-inoculated control. Bolded means are significantly different from control in a positive sense

Plant genotype/bacteria	Shoot height (cm)		Leaves No.		Root No.		Root length (cm)	
	Bacteria absent or present							
	-	+	-	+	-	+	-	+
Chrysanthemum × *grandiflorum* 'Ludo'/ *Paenibacillus glucanolyticus* (I)	4.6a	**6.1b**	10.0a	**18.4b**	5.2b	4.0a	7.5a	**11.4b**
Gerbera jamesonii 'Kormoran'/ *Methylobacterium extorquens* (67)	1.3a	1.6a	7.1a	**8.8b**	1.8a	2.2a	3.8a	**5.9b**
Hosta 'Paradigm'/ *Methylobacterium extorquens* (67)	1.2a	1.0a	7.5a	8.0a	6.5a	7.0a	12.0b	7.5a
Rose 'White Gem'/ *Curtobacterium pusillum* (N)	1.4a	**1.9b**	6.4a	**8.8b**	3.0a	**5.8b**	1.2a	**1.6b**

DISCUSSION

Since the 1970s, it has been known that bacteria that survive the surface decontamination within the plant body and reproduce at a moderate rate, often being covert, and not causing a clear negative impact on the explants (Leifert et al. 1991; Cassels 1997, 2011), can inhabit the in vitro plant cultures. As early as in 1994, Holland and Polacco suggested that covert contaminants could be responsible for modifications of plant explants' behavior *in vitro*. The observation on beneficial effects of different bacteria strains on the explants *in vitro* raised the idea of bacterization (biotization) of plant cultures with useful bacteria (Nowak 1998; Nowak et al. 1998). The best-known candidate for biotization is the *Burkholderia phytofirman*s PsJN^{TM} strain, formerly named *Pseudomonas* spp. PsJN, which does not grow on the plant medium in the absence of plant explants (Sessitsch et al. 2005). It colonizes both the surface and the internal tissues (Frommel et al. 1991; Nowak 1998), and stimulates the growth of microshoots and microroots, makes the use of water more efficient and increases the plant resistance to pathogens (Sharma & Nowak 1998; Theocharis et al. 2012) and coldness (Fernandez et al. 2012). Another bacteria, *Pseudomonas* spp., F strain was reported to produce polysaccharides, which can inhibit an excessive hydration of oregano (Shetty et al. 1995), raspberry (Ueno et al. 1998) and anise (Bela et al. 1998) cultures. *Methylobacterium* sp. D10 and *Methylophilus glucoseoxidans* stimulated the production of morphogenetic callus from wheat embryos (Kalyaeva et al. 2003). The strain of *Bacillus circulans* allowed the induction of somatic embryogenesis from callus derived from geranium hypocotyls (Murthy et al. 1999) and *Curtobacterium citreum* stimulated outgrowth of axillary shoots in geranium cultures (Panicker et al. 2007). *Azotobacter chroococcum* strain increased the number of shoots in wheat (Andressen et al. 2009), *Bacillus* spp. stimulated root growth, and *Sphingomonas* spp. facilitated acclimatization of micropropagated strawberries in the greenhouse (Diaz et al. 2009) and *Azospirillum barasilense* 243 enhanced acclimatization of micropropagated fruit rootstocks (Vettori et al. 2010). The interaction between *Paenibacillus* P22 that is able to assimilate atmospheric nitrogen with poplar shoot explants resulted in essential changes in plant metabolism (Scherling et al. 2009). These observations are often consistent with the results of experiments on the interaction of bacteria with plants grown in the *in vivo* conditions (Russo et al. 2012).

Curtobacterium pusillum belonging to Microbacteriaceae was not only found in oil-brine fields (Ijah & Ukpe 1992) but was also isolated from human clinical specimens (Funke et al. 2005). Its strain was one of endophytic bacteria isolated from leaves of rice plants (Mano et al. 2007) and leaves of soybean and corn (Dundleavy 1989). Strains of this bacterium were often isolated from air samples taken in food processing industries (Góra et al. 2009). El-Mehalawy (2007) selected *C. pusillum* strain producing inhibitory compounds against plant pathogen *Rhizoctonia solani*. Moraes et al. (2012) isolated an endophytic symbiont, the strain of *C. pusillum*, which was classified as plant growth promoting bacteria (PGPB) due to the production of IAA. *C. citreum* could replace cytokinin in the medium for propagation of chrysanthemum (Panicker et al. 2007).

Methylobacteria are microorganisms commonly found in soil and on plant surfaces, but they also exist as endophytes. Many beneficial interactions of this group of bacteria with plants were detected (Madhaiyan et al. 2011). *M. extorquens*, which produces a pink pigment, belongs to one of the most characterized and studied methylotrophs (Christoserdova et al. 2003). Pirtilä et al. (2000) obtained the strain of this bacterium from meristematic cells of *Pinus silvestris*. When this strain was inoculated on the callus of that plant, it influenced the growth and regeneration by diverse mining (Pirtilä et al. 2008). The cell-free supernatant of the bacterial culture of one of *M. extorquens* strains isolated from strawberry promoted the growth of various seedlings (Abanda-Nkpwatt et al. 2006).

Paenibacillus genus differs in nutritional requirements and growth conditions. Many strains were isolated from soil, water, rhizosphere, plant body and food (Lorentz et al. 2006), and from *in vitro* cultures (Ulrich et al. 2008). Bacteria of this

systematic group are known to secrete several enzymes and metabolites to the environment, including growth regulators (Timmusk et al. 1999). *P. glucanolyticus* was isolated from soil, but in our research bacteria isolated from hosta propagated *in vitro* was used. Sangeeth et al. (2012) found that the strain *P. glucanolyticus* isolated from the root zone of black pepper is capable of solubilizing potash, which may be important in fertilization of plants with potassium. In addition, the antimicrobial activity of several *Paenibacillus* strains belonging to different species was reported (Lorentz et al. 2006).

All strains of bacteria studied here were able to stably colonize *in vitro* explants of all four plant species but their impact on plant hosts differed. The results showed the significant influences of bacterization of microshoots of chrysanthemum, gerbera, rose and hosta using bacterial strains – *C. pusillum*, *M. extorquens* and *P. glucanolyticus* – isolated from the *in vitro* cultures. The character of influence depended on the host and type of organogenesis.

C. pusillum stimulated axillary shoot growth of all species and was especially effective for rose. Chrysanthemum was the only plant species which rooted worse with this bacterium. *M. extorquens* was especially helpful for the rooting and shoot multiplication of gerbera and hosta. It increased the number of shoots of chrysanthemum, but decreased shoot length of chrysanthemum and rose. *P. glucanolyticus* positively stimulated chrysanthemum for both shoot multiplication and rooting. It decreased rooting and shooting of gerbera and hosta and retarded root elongation of rose. The stimulation of shoot proliferation of all four-plant species by *C. pusillum* strain may be related to its modification of the balance of growth regulators. This strain does not produce IAA and production of any other growth regulator was not evaluated. All studied bacteria were able to assimilate atmospheric nitrogen but this fact cannot explain their specialization for different hosts.

The observed effects were not a breakthrough in micropropagation of the investigated genotypes because they are relatively easy to micropropagate without bacterial stimulation. With our experiments, we can confirm that the strains isolated from one species could colonize and be beneficial for others, hopefully also for recalcitrant genotypes, but it needs to be proved experimentally.

According to Penrose and Glick (2003), the impact of endophytes on plants is small or insignificant when they grow under optimal conditions and in fertile soil. Bacterization of plants cultivated in poor soils results in a greater stimulation of growth (Glick et al. 1998). Consequently, the influence of the bacteria on *in vitro* explants may not be important because in most cases cultures are grown under optimal conditions in terms of nutrition, temperature and water abundance. On the other hand, it is known that bacteria can produce all plant hormones and their facilitation can harmonize and optimize hormonal balance of plant explants (Friesen et al. 2011). Beneficial effects of bacteria may appear clearly under stress, which in micropropagation culminates during the acclimatization in the greenhouse when microplants have to develop mature covering and conducting tissues to protect plants from losing water, microbial attack, ensure the absorption and conduction of water and nutrients, and to develop the autotrophy (Chandra et al. 2010). Improving acclimatization is probably the most promising application of beneficial bacteria in the *in vitro* propagation industry. Digat et al. (1987), who inoculated the synthetic substrate MILCAP with strains of *P. putida* and *P. fluorescens*, suggested for the first time a need of bacterization of in vitro cultures at microshoots rooting. In their experiment, the inoculation was beneficial to some extent for *Primula obconica* but not for *Rosa* and *Hydrangea quercifolia*. The fully beneficial effect of bacterization of *Robinia pseudoacacia* with *Rhizobium* strains was observed by Balla et al. (1998), and *Photinia fraseri* with *Azospirillum brasiliense* by Larraburu et al. (2007). Zakharchenko et al. (2010) reported better growth of potato and strawberry at acclimatization, when microshoots were inoculated before rooting with strain of *Pseudomonas aureofaciens*. A strain of *Azospirillum brasilense* enhanced rooting and acclimatization of *Prunus cerasifera* (Russo et al. 2008). According to Thomas et al. (2010), bacterization of explants *in vitro* can be unsuccessful due to lack of colonization or culture overgrowth. In many cases, better results in plantlet survival and

growth can be obtained when bioinoculants are used to amend the soil or during an *in vitro* rooting in the perlite, as in our experiment. Moreover, our experiments indicate a kind of bacteria/host specialization. Only *C. pusillum* affected all four species in the same way, but *M. extorquens* had a positive effect only on hosta and gerbera, and *P. glucanolyticus* only on chrysanthemum. In comparison, *Burkholderia phytophirmans* PsJN™ seems to have an exceptional genotype, because of its wide range of host plants (Sessitsch et al. 2005).

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