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Use of *Bacillus* spp. as growth promoter in carrot crop

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Growth promoting rhizobacteria may increase the yield of some crops. Several microbial products that stimulate plant growth have been marketed. Therefore, the objective of this study was to evaluate the effect of bacteria from Bacillus genera on the production of commercial carrot roots (*Daucus carota* L.) in field conditions. The following isolates: SF 264 (*Bacillus* spp.), SF 268 (*Bacillus* spp.) and commercial formulations based on SF 202 (Rizos®, *B. subtilis*), SF 266 (Quartz®, *B. methylotrophicus*) and SF 267 (Onix®, *B. methylotrophicus*) were evaluated in four experiments conducted in commercial fields of carrot production in the municipality of Rio Paranaíba, Minas Gerais - Brazil. Each plot was 6 m long and 1.75 m wide (10.5 m^2) including four double rows. An additional treatment containing only water was the control treatment. The experiment was designed as randomized blocks with five replications. The products Rizos®, Quartz® and Onix® containing *Bacillus* spp. increased the production of commercial carrots roots of all cultivars and sites. The SF 268 and SF 264 isolates were efficient only in two and three experiments, respectively.

Key words: *Daucus carota*, rhizobacteria, plant growth, *Bacillus.*

INTRODUCTION

In Brazil, carrot is among the five main garden crops grown and 80 % of the total production supplies Brazilian domestic market. The Southeast, Northeast, and South regions are the largest producers of this root crop. The State of Minas Gerais stands out in the production of this vegetable crop and the Alto Paranaiba region is one of the main producers. Yield potential of the crop is from 100 to 120 t ha^{-1}; however, the Brazilian average is much lower around 33 tons ha^{-1} (Embrapa, 2010).

Free-living bacteria are found in the rhizosphere of plants and parts of them are known as Plant Growth Promoting Rhizobacteria - PGPR (Alves, 2007). *Pseudomonas, Bacillus, Azospirillum, Agrobacterium* and *Azotobacte* have been reported as PGPR, being *Pseudomonas* and *Bacillus* the PGPR widely reported.

The mechanisms that PGPR, including *Bacillus* spp., increase the plant growth may vary from production or changes on phytohormones concentration, ethylene synthesis inhibition, siderophore and antibiotics production, nitrogen fixation, phosphate solubilization and systemic resistance induction to pathogens (Singh et al., 2011; Vafadar et al., 2014). The beneficial effect of

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rhizobacteria in commercially cultivated crops is world-wide known in onion (Harthmann et al., 2010), potato (Sottero et al., 2006) and tomato Mena-Violante and Olalde-Portugal, 2007). However, this potential is not yet known in several crops, such as carrot (Konusny-Andreani et al., 2014).

Carrot marketing depends on the roots pattern that considers the length, diameter and the defects absence (Ceasaminas, 2015). Thus, the application of PGPR may increase the production of commercial roots, resulting in lower waste and consequently higher profitability to farmers. *Bacillus* spp. in special is easily grown in liquid culture media with low cost, which make easier its mass production in industrial fermenters. Furthermore, they produce resistant endospores, which increase the shelf life of products and integrated use with chemical products (Lanna Filho, 2010).

Therefore, the aim of this study was to evaluate the efficiency of five *Bacillus* spp. isolates in increasing the production of commercial roots of carrot under field conditions.

MATERIALS AND METHODS

PGPR Isolates

The following isolates: SF 264 (*Bacillus* spp.), SF 268 (*Bacillus* spp.), SF 202 (*Bacillus subtilis* - commercial product Rizos®), SF 266 (*B. methylotrophicus* - commercial product Quartz®) and SF 267 (*Bacillus methylotrophicus* - commercial product Onix®). They were tested in 4 fields with commercial carrot production in the municipality of Rio Paranaíba - MG (site 1 – S.: 19°19´09´´; W.: 46°14´04.2''; site 2 – S.: 19°14´14.7´´; W.: 46°08´47.5.2''; site 3 – S.: 19°25´45.8´´; W.: 46°14´10.2''; site 4 – S.: 19°13´04´´; W.: 46°14´20''). Isolates and commercial products belong to Farroupilha`s group from Patos de Minas, Minas Gerais. The experiments were carried out during February and March 2012.

Carrot cultivars

The cultivar Brasilia, developed by EMBRAPA, was used in two sites. In the two other sites the cultivars were Suprema Max® (Isla Sementes Ltda, Porto Alegre, Brasil) and Juliana® (Seminis Vegetable Seeds, St. Louis, EUA), respectively.

Soil and sites characterization

The chemical and physical characteristics of the soils are presented in the Table 1.

All isolates were prepared in liquid culture media and formulated as a protocol used in the company. The concentration of the isolates SF 264, SF 266, SF 267 and SF 268 was 1 x 10^9 colony forming units (cfu) per mL, while the concentration of SF 202 was 5 x 109 cfu mL^{-1}.

At 15 days after planting, the isolate suspensions were applied on the plants using a CO_2 -pressurized backpack sprayer furnished with three 110-02 nozzles working in constant pressure of 2.2 bar, spaced at 0.5 m. The dose and volume of the spray solution were 4 L ha^{-1} and 200 L ha^{-1}, respectively. Immediately after the application of the bacterial isolates, each experimental area was irrigated at 5 mm water depth in order to facilitate the bacteria propagules percolation in the soil profile. The sprays were done after 3 pm.

Each plot was 6 m long and 1.75 m wide (10.5 m^2), including four double rows. An additional treatment containing only water composed the control treatment. The experiment was designed as randomized blocks. Crop management was carried out following the pattern adopted by the growers, including irrigation, topdressing fertilizations, pest management and rough-hewing.

Yield evaluation

The harvest was carried out at 105 days after planting. The fresh weight of commercial roots of carrot was evaluated in 1 m^2, located at the center of each plot (useful plot). All roots in the useful plots were classified according to the carrot standard classification of a Brazilian program for commercial standards and horticultural packaging improving (Ceasaminas, 2015). It was considered only the roots with a length between 14 and 26 cm without defects. Later the selected roots were weighed using a digital scale.

Statistical analysis

The data were submitted to variance analysis (F test, P = 0.05) and the means compared by Scott-Knott test (P = 0.05) using the software SISVAR 5.1 Build 72 (Ferreira, 2007).

RESULTS AND DISCUSSION

The bacteria isolates increased the production of commercial roots of the cultivar Juliana in both sites (Table 2). For the cultivar Suprema Max, the SF 267, SF 266, SF 264 and SF 202, even in different groups, increased the carrot production. For the cultivar Juliana, yields with SF 202, SF 266 and SF 267 application were 10.40; 12.48 and 23.32 higher than the control.

The SF 267, SF 266 and SF 202 isolates of *B. methylotrophicus* are soil nitrifying bacteria, which means, they may convert ammonia to nitrite and later nitrite to nitrate which is a readily assimilable nitrogen form by plant roots (Zhang et al., 2012). This characteristic may explain the outstanding performance observed for SF 266 and SF 267 isolates to increase the production of commercial roots of carrot. Furthermore, Yan et al. (2011) and Dev Sharma et al. (2013) reported in several studies that *B. methylotrophicus* isolates produced antagonistic metabolites against several pathogenic bacteria and fungi.

Andreani et al. (2014) evaluated the effect of rhizobacteria isolates from *Crotalaria spectabilis* on the development of carrot (cultivar Nantes), in greenhouse conditions, through seed microbiolization with bacterial suspensions. In this study the bacterial isolates could not be identified. They were merely coded as UCCBj-CE's. The UCCBj-CE 04, UCCBj-CE 11, UCCBj-CE 17 and UCCBj-CE 18 isolates induced higher productivity in carrot crop.

According to Lanna Filho (2010), the growth-promotion induced by *B. subtilis* may be a consequence of increasing nitrogen fixation, nutrients solubilization, hormone synthesis and soil conditions improvement,

Table 1. Chemical and physical characterization of commercial fields with carrot cultivation.

Chemical and physical analysis	Cultivar			
	Brasilia - Site 1	Brasilia - Site 2	Suprema Max - Site 4	Juliana - Site 5
pH water	6.27	6.43	5.97	6.73
P-rem (mgL^{-1})	11.13	10.27	12.25	7.06
O. M. (dag kg^{-1})	3.48	3.21	3.70	3.14
P (mg dm^{-3})	47.77	43.87	45.69	11.76
K (mgdm^{-3})	123.00	176.00	81.00	86.00
Ca (cmoldm^{-3})	4.49	3.83	4.01	3.87
Mg (cmoldm^{-3})	0.83	0.95	0.87	0.88
Al (cmol dm^{-3})	0.04	0.04	0.04	0.04
H+Al (cmol dm^{-3})	3.10	2.71	4.04	2.08
BS (cmol dm^{-3})	5.63	5.23	5.09	4.97
CEC (t) (cmoldm^{-3})	5.67	5.27	5.13	5.01
CEC (T) (cmol dm^{-3})	8.32	7.94	9.13	7.05
m (%)	0.70	0.76	0.78	0.80
V (%)	64.54	65.87	55.72	70.54
B (mg dm^{-3})	0.31	0.22	0.13	0.18
Cu (mg dm^{-3})	1.40	7.70	4.30	1.10
Fe (mg dm^{-3})	28.50	32.40	28.30	20.90
Mn (mg dm^{-3})	8.10	13.40	8.80	6.10
Zn (mgdm^{-3})	11.10	25.70	22.40	8.50
S (mg dm^{-3})	13.97	5.85	3.77	5.66
Clay (g kg^{-1})	416.00	446.00	424.00	418.00
Silt (gkg^{-1})	160.00	174.00	172.00	176.00
Sand (gkg^{-1})	424.00	380.00	404.00	406.00
TOC (dagkg^{-1})	2.02	1.86	2.15	1.82

P, K, Fe, Zn, Mn, Cu: Mehlich I extractor. Ca, Mg, Al: KCl 1 mol L^{-1} extractor. CEC (t): Effective cation exchange capacity. CEC (T): Cation exchange capacity at pH 7.0. Texture: Pipette Method. V: Base saturation. m: Aluminum saturation. H + Al: SMP extractor. B: Hot water extractor. P-rem: Remaining phosphorus, concentration of P of the equilibrium solution after stirring the air-dry soil during 1 h with $CaCl_2$ solution at 10 mmol L^{-1}, containing 60 mg L^{-1} of P (1:10). S: Monocalcium phosphate in acetic acid. Organic matter: Oxidation - $Na_2Cr_2O_7$ 4N + H_2SO_4 10N. TOC - Total Organic Carbon: Oxidation with $Na_2Cr_2O_7$ 4N + H_2SO_4 10 mol L^{-1}.

Table 2. Fresh weight of commercial carrot roots (kg m^{-2}) after different applications of *Bacillus* spp. isolates.

Treatment	Cultivar			
	Brasilia - Site 1	Brasilia - Site 2	Suprema Max - site 3	Juliana - site 4
SF 202 (Rizos®)	5.8550^{a}	9.0880^{a}	6.5030^{b}	6.5038^{a}
SF 264(*Bacillus* spp.)	6.1770^{a}	8.5900^{a}	6.6260^{b}	6.1788^{b}
SF 266 (Quartz®)	6.0760^{a}	8.5740^{a}	6.1780^{b}	6.6262^{a}
SF 267 (Onix®)	6.4090^{a}	8.5070^{a}	7.2650^{a}	7.2650^{a}
SF 268(*Bacillus* spp.)	6.2130^{a}	9.0990^{a}	5.6580^{c}	5.6588^{b}
Control(water)	5.2758^{b}	6.9890^{b}	5.8910^{c}	5.8916^{b}
CV(%)	8.79	9.54	8.88	7.39

Means followed by the same letter, in the columns, are not different at 5% probability by Scott-Knott test. CV: coefficient of variation.

besides indirect benefits by environmental suppression against pathogens. Additionally, the beneficial association provides an increase in physiological metabolites that unleash the root system sensitivity to external conditions, which may improve the nutrient uptake (Manjula and Podile, 2005).

Isolates of *B. subtilis* also has the ability to induce hormonal regulation in plants, as reported by Tsavkelova et al. (2006), Persello-Cartieaux et al. (2003) and Lanna Filho (2010) controlling the root growth by auxin,

gibberellin and cytokinin synthesis. It may also explain the responses of *B. subtilis* isolates in all experiments. Besides the genetic differences among isolates and their possible influence on root colonization, the interaction between isolates and carrots cultivar may also influenced the plant growth (Romeiro et al., 2005; Choudhary et al., 2007).

For the cultivar Brasilia, all isolates increased the carrot production. The SF 264 isolate in the cultivar Juliana and the SF 268 in the cultivar Suprema Max and Juliana did not have the same beneficial effect. It is possible that the production and root exudates composition among cultivars are different; therefore, the SF 268 and SF 264 isolates have not been able to colonize efficiently the rhizosphere of Suprema Max® and Juliana® cultivars, and consequently act as growth promoter.

When a microorganism is introduced into a new environment, it must overcome the competition with other microorganisms (microbiostase), in order to develop and perform its ecological functions (Tsavkelova et al., 2006).There are reports that show the high specificity of growth - promoting bacteria to colonize specific hosts. This specificity was not observed in the present study. Raasch et al. (2013) stated that eucalyptus production with Rizolyptus® (*B. subtilis*) varied according to the tested clones, suggesting a high specificity of root colonization regarding the host.

Mello et al. (2002) reported the lack of specificity of the C210, ENF10, ENF16 and RAB9 isolates, obtained from cabbage, beans and radish, respectively, regarding growth promotion in seedlings of pineapple micro-propagation. In another study, isolates of C116 (*Bacillus pumilus*) and C25 (*Bacillus thuringiensis* subvar. *kenyae*), from cabbage were effective in promoting the growth of lettuce seedlings (Gomes et al., 2003).

Besides the specificity of bacterial isolates to the host, stands out the differences in the soil of rooting for rhizobacteria colonization, once abiotic factors (pH, nutrient availability, moisture retention, aeration etc.) and biotic (qualitative and quantitative composition of the microbiota and others) may favor or not the colonization, survival and beneficial activity of rhizobacteria (Mafia et al., 2009).

Such variables may explain the difference in the production of commercial carrot when using a same bacterial isolate and in different experiments, i.e. under different environmental conditions. This was observed for the cultivar Brasilia - site 1, where the SF 267 isolate (*B. methylotrophicus*, Onix®) was used, the fresh weight was 6.41 kg while in the site 2 it was 8.51 kg.

In the cultivation fields of the cultivar Suprema Max and Juliana, the potassium content in the soil was around 40% lower than the other fields as highlighted in the Table 1. There may be some relationship between potassium and phosphorus contents in the soil and the capacity of the SF 268 isolate colonize the rhizosphere of carrot plants and promote the plant growth. Moreover, the SF 267, SF 266 and SF 202 isolates do not seem to depend on the presence of high amounts of these nutrients in the soil, considering that some PGPR solubilize phosphates and/or provide potassium to the plants (Gomes et al., 2003; Viruel et al., 2014). Further studies are recommended to investigate whether the beneficial effect of the SF 267, SF 266 and SF 202 isolates in increasing the production of commercial carrot roots is due to their action as nutrients solubilizers in the soil.

Conclusions

The application of *Bacillus* spp., present in the products Rizos®, Quartz® and Onix®, enhanced the yield of commercial carrot roots.

Conflict of Interests

The authors have not declared any conflict of interest.

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REFERENCES

Alves E (2007). Mecanismos estruturais na resistência de plantas a patógenos. Summa Phytopathol. 33:154-156.

Andreani DRK, Agiado JC, Junior RA (2014). Efeito de bactérias rizosféricas sobre o desenvolvimento da cenoura. Rev. da Universidade Vale do Rio Verde 12: 211-220.

CEASAMINAS. Centros de abastecimento de Minas Gerias. Informações de mercado: Cenoura. (2015). Available at: http://www.ceasaminas.com.br/informacoesdemercado/contador_procedenciaprodutos.asp (accessed in June 2016).

Choudhary DK, Prakash A, Johri BN (2007). Induced systemic resistance (ISR) in plants:mechanism of action. Indian J. Microbiol 47:289-297.

Dev Sharma SC, Shovon MS, Jahan MGS, Asaduzzaman AKM, Rahaman MA, Biswas KK, Abe NR (2013). Antibacterial and cytotoxic activity of *Bacillus methylotrophicus*-scs2012 isolated from soil. J. Microb. Biotech. Food Sci. 2:2293-2307.

Empresa Brasileira de Pesquisa Agropecuária – Embrapa (2010). Sistemas de Produção: Cenoura [internet]. Brasília, DF; 2010 [accessed on Jul. 25, 2016]. Available at: http://www.cnph.embrapa.br

Ferreira DF (2007). SISVAR: Sistema de análise de variância para dados balanceados, versão 5.1 Build 72. Lavras: DEX/UFLA.

Gomes AMA, Mariano LRR, Silveira EB, Mesquita JCP (2003). Isolamento, seleção de bactérias e efeito de *Bacillus* spp. na produção de mudas orgânicas de alface. Hort. Bras. 21:699-703.

Harthmann OEL, Mógor AF, Wordell FJA, Luz WC (2010). Rizobactérias no crescimento e produtividade de cebola. Cienc. Rural 40:462-465.

Konusny-Andreani DI, Agiado JC, Andreani JR (2014). Efeito de

bactérias rizosféricas sobre o desenvolvimento da cenoura. Rev. da Universidade Vale do Rio Verde12:211-220.
Lanna FR, Ferro HM, Pinho RSC (2010). Controle biológico mediado por *Bacillus subtilis*. Rev. Trópica – Ciênc. Agrárias e Biol. 4:12-20.
Mafia RG, Alfenas AC, Ferreira EM, Binoti DHB, Mafia GMV, Mounteer AH (2009). Root colonization and interaction among growth promoting *rhizobacteria* isolates and eucalypts species. Rev. Árvore 33:1-9.
Manjula K, Podile AR (2005). Increase in seedling emergence and dry weight of pigeon pea in the field with chitin-supplemented formulations of *Bacillus subtilis* AF 1. World J. Microb.Biot. 21:1057-1062.
Mello MRF, Mariano RLR, Menezes M, Câmara TR, Assis SMP (2002). Seleção de bactérias e métodos de bacterização para promoção de crescimento em mudas de abacaxizeiro micropropagadas. Summa Phytopathol. 28:222-228.
Mena-Violante HG, Olalde-Portugal V (2007). Alteration of tomato fruit quality by root inoculation with plant growth-promoting *rhizobacteria* (PGPR): *Bacillus subtilis*BEB-13bs. Sci. Hort.113:103-106.
Persello-Cartieaux F, Nussaume L, Robaglia C (2003).Tales from the underground: Molecular plant-rhizobacteria interactions. Plant Cell Environ. 26:186-199.
Raasch LD, Bonaldo SM, Oliveira AAF (2013). *Bacillus subtilis*: enraizamento e crescimento de mini estacas de eucalipto em Sinop, Norte de Mato Grosso, Brasil. Biosci. J. 29:1446-1457.
Romeiro RS, Lanna-Filho R, Vieira JR, Silva HSA, Baracat-Pereira MC, Carvalho MG (2005). Macromolecules released by a plant growth-promoting *rhizobacterium* as elicitors of systemic resistance in tomato to bacterial and fungal pathogens. J. Phytopathol. 153:120-123.
Singh JS, Pandey VC, Singh DP (2011). Efficient soil microorganisms: a new dimension for sustainable agriculture an environmental development. Agric. Ecosys. Environ. 140:339-353.
Sottero NA, Freitas SS, Melo AMT, Trani PE (2006). Rizobactérias e alface: colonização rizosférica, promoção de crescimento e controle biológico. Rev. Bras. Ci. Solo 30:225-234.
Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI (2006). Microbial Producers of Plant Growth Stimulators and Their Practical Use: A Rev. Appl. Biochem Micro 42:117-126.
Vafadar F, Amooaghaie R, Otroshy M (2014). Effects of plant-growth-promoting rhizobacteria and arbuscularmycorrhizal fungus on plant growth, stevioside, NPK, and chlorophyll content of Stevia rebaudiana. J. Plant Interactions 9:128-136.
Viruel E, Erazzú LE, Calsina LM, Farrero MA, Lucca ME, Siñeriz F (2014). Inoculation of maize with phosphate solubilizing bacteria: effect on plant growth and yield. J. Soil Sci. Plant Nut. 14:819-831.
Yan X, He L, Song G, Wang R (2011). Antagonistic bioactivity of endophytic strains isolated from *Salvia miltiorrhiza*. Afr. J. Biotechnol. 10:15117-15122.
Zhang QL, Liu Y, Ai GM, Miao L, Zheng HY, Liu ZP (2012). The characteristics of a novel heterotrophic nitrification–aerobic denitrification bacterium, *Bacillus methylotrophicus* strain L7. Bioresour. Technol. 108:35-44.