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ORIGINAL PAPER

Phenotypic and genotypic characterization of endophytic bacteria associated with transgenic and non-transgenic soybean plants

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Abstract

Endophytic bacteria isolated from non-transgenic and transgenic Roundup Ready® glyphosate-resistant (GR) soybean plants were investigated to analyze the correspondence between phenotypic and genotypic characteristics and to determine whether or not the strains could be grouped based on the source of isolation in transgenic or non-transgenic plants, respectively. Most of the strains recovered from GR plants have shown the ability for plant growth promotion (PGP) by means of IAA production and inorganic phosphate solubilization, and 100% of the strains showed great motility (swarm or swim); in addition, 90% of the strains were able to metabolize the majority of carbon sources tested. GR soybean fields showed higher endophytes abundance than non-transgenic; however, analyzing the phylogenetic trees constructed using the partial 16SrRNA gene sequences, higher diversity was observed in non-transgenic soybean fields. Overall the majority of isolated endophytes could utilize multiple patterns of carbon sources and express resistance to antibiotics, while isolates varied widely in the PGP ability. The greater pattern and frequency of utilization of carbon sources and frequency and intensity of antibiotic resistance compared with PGP ability within the soybean endophytes community suggest that carbon sources metabolism and antibiotic resistance confer a greater relative fitness benefit than PGP ability. In conclusion, cluster analysis of the phenotypes and 16SrRNA gene sequences reveals lack of correspondence between the pattern of bacterial isolates and the transgenic character of plants, and the heterogeneity of clustering suggested that various adaptive processes, such as stress response, could have contributed to generate phenotypic variability to enhance endophytes overall fitness.

Keywords Transgenic soybean · Endophytic bacteria · 16SrRNA · Antibiotics · Carbon sources metabolism · Plant growth promotion

Introduction

The composition and diversity of the bacterial community associated with a plant are influenced by a variety of factors such as plant species, cultivar or environment (Zinniel et al. 2002; Kuklinsky-Sobral et al. 2004, 2005; Andreote et al. 2010). Bacteria that can colonize internal tissues and intercellular spaces of plants without determining any negative influence on their host are considered as endophytes (Schulz and Boyle 2006; Hardoim et al. 2008; Etesami and Maheshwari 2018; Vigani et al. 2018). According to the literature, many endophytic bacteria can promote plant growth because they have specific characteristics such as hormone production and ability to solubilize inorganic phosphates, or even to antagonize phytopathogens (Kang et al. 2007; Goes et al. 2012). There are several factors that may affect the endophytic bacterial community within plants, including plant genotype and agronomic management such as the use of agrochemicals. The agrochemical effects on the soil microbiota as well as endophytic bacterial community have been hardly investigated (Kuklinsky-Sobral et al. 2005; Andreote et al. 2008; Barriuso et al. 2010; de Almeida Lopes et al. 2016). Glyphosate [*N*-(phosphomethyl) glycine] is a

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broad-spectrum, non-selective herbicide that controls both broad-leaf and grass weeds. It has a short mean active life in the soil environment and is marketed as Roundup® by Monsanto Company, St. Louis, MO (Franz et al. 1997). Glyphosate blocks the synthesis of phenylalanine, tyrosine and tryptophan, the aromatic amino acids, by blocking the shikimic acid pathway. This pathway is present in plants and microorganisms but not in animals, and this is the reason for its low mammalian toxicity (Jaworski 1972; Fisher et al. 1986). *Agrobacterium* strain CP4 is insensitive to glyphosate due to the insensitivity to the herbicide of its 5-enolpiruvylshikimic acid-3-phosphate synthase (EPSPS) gene. Insertion of this gene in soybean confers resistance to glyphosate allowing the functional expression of the shikimic acid pathway in its presence (Padgette et al. 1995; Zablotowicz and Reddy 2004). Therefore, there are some bacteria that are able to use glyphosate as a source of energy and nutrients, whereas it can be toxic to others (Busse et al. 2001; Kuklinsky-Sobral et al. 2005). Thus, some studies suggest that the application of pre-planting glyphosate could influence bacterial communities (Kuklinsky-Sobral et al. 2005; de Almeida Lopes et al. 2016).

The release of genetically engineered soybean cultivars has raised the question of whether the bacterial community can be also affected by the genotype or not. The use of herbicides may affect the benefits of bacterial plant growth promotion directly through effects on the bacteria or indirectly through effects on plant host and also in the symbiosis. Therefore, it is important that the biotechnological advances that have enabled the generation of genetically modified soybeans are also accompanied by rigorous studies of environmental safety. This is necessary not only to ensure the occurrence of minimal impacts on the environment, but also to secure that gains already obtained from research are not negatively affected.

The effect of the plant genetic modification in bacterial communities has been demonstrated in different plant species. Andreote et al. (2008) compared the bacterial communities associated with the rhizosphere of transgenic tobacco lines with those found in the wild type and showed that the rhizosphere microbial communities can be affected by the cultivation of transgenic plants. However, according to the authors, the original bacterial diversity was able to restore to the original condition, so the shift was not permanent. Similarly, Assumpção (2008), assessing cultivable endophytic bacteria isolated from non-transgenic and transgenic soybean seeds, found that 63% of the isolates were present in non-transgenic seeds, while 37% were from transgenic seeds, but the diversity of the bacteria isolated from transgenic seeds was higher. Hence, an efficient characterization of the endophytic community in both conditions is of great importance to make a reliable comparison between them, even with and without glyphosate, both in genetically modified and non-modified plants. However, it is often difficult to know which characteristics should be evaluated, in addition to the low correspondence between certain phenotypic characteristics with the genotype (Torsvik et al. 1990; Vela et al. 2000; Baines et al. 2007; Freitas et al. 2008).

Torsvik et al. (1990), comparing the phenotypic and genotypic diversity of bacterial strains isolated from soil, found that the major part of DNA of this bacterial fraction of soil is very heterogeneous and generates a good estimate of the bacterial diversity and agreed with different phenotypic diversity measures. Nevertheless, Baines et al. (2007), assessing the correspondence between phenotypic and genetic parameters of *Streptomyces* isolated from a prairie soil, observed that there was no correspondence between 16SrRNA gene sequence groups and antibiotic phenotypes.

Therefore, in this study endophytic bacteria isolated from glyphosate-resistant transgenic and non-transgenic soybean grown in field sites in Brazil were analyzed phenotypically, with regard to plant growth promotion (PGP) characteristics like antibiotic resistance and carbon sources metabolism, and also genotypically, with the aim to investigate whether or not endophytic bacterial communities isolated from such soybean could be grouped based on the source of isolation, whether plants were transgenic or not.

Materials and methods

Plant material

Soybean samples for isolation of endophytes were taken from three experimental fields site of the Brazilian Agricultural Research Corporation (Embrapa Soybean) as previously reported (de Almeida Lopes et al. 2016). Field sites were in Ponta Grossa (PR), Guarapuava (PR) and Campos Novos (SC), Brazil. The soil in Ponta Grossa (PR) (25º05′42″S, 50º 09′43″W) is characterized for having Red Latossol soil and Cfb climate; Guarapuava (PR) (25º23′43″S, 51º 27′29″W) has Purple Latossol Aluminic soil and Cfb climate—mesothermal humid subtropical; and Campos Novos (SC) (27º24′06″S, 51º13′30″W) has Purple Latossol Aluminic soil and Cfa climate—bland super humid subtropical mesothermal, according to Köppen classification (Embrapa 2006).

BRS 245RR and BRS 133 were the related genotypes used as sources for the isolation of endophytes. The cultivar BRS 245 RR has been genetically engineered to tolerate the herbicide glyphosate, e.g., Roundup Ready®, was developed by Brazilian Agricultural Research Corporation (Embrapa Soybean). BRS 245 RR Roundup Ready® and BRS 133 have been used to allow comparison between related transgenic and non-transgenic genotypes because BRS 245 RR Roundup Ready® is essentially the derived transgenic

version from the protected conventional cultivar BRS 133. The other cultivars included in this study were BRQ09-11694 (C) and the BMX Energia (GR) grown in Guarapuava (PR) and Campos Novos (SC) as previously reported by de Almeida Lopes et al. (2016). The breeding line BR Q09-11694 (C) was also developed by Embrapa Soybean. Seed companies, Brasmax Genetic, Tropical Breeding and Genetic, and Syngenta LTDA, developed cultivars BMX Energia, TMG 801 and NK 7059, respectively.

Soybean seeds were sown during the period 10-20 November, 2012. An individual replicate was a composite of three plants. Plants were harvested at the full seed beginning (reproductive stage, when the pods were green (R5) at the period from April 4 to 11, 2013. The soil management adopted was non-tillage, and 350 kg ha^{-1} of commercial fertilizer formula 0-20-20 of NPK was used in each location. The herbicide glyphosate was used in pre-sowing in all experimental field sites. One post-sowing application of glyphosate was used for the transgenic cultivars. The post-sowing chemical weed control in the non-transgenic cultivars trials was made with one application of the herbicide Fluazifop-P-Butil + Fomesafen.

The experimental design of the assay with the soybean cultivars was factorial, completely at random and each cultivar, at each site, had three replicates. Endophytic bacteria were isolated from roots, stems and leaves of soybean cultivars. Soil management, sowing, glyphosate and other chemicals used to control weeds applications, sampling and harvesting criteria were those reported by de Almeida Lopes et al. (2016).

Isolation and counting of endophytic bacteria in roots, leaves and stems

Samples of leaves, roots and stems, collected in triplicate, were processed as previously described (de Almeida Lopes et al. 2016). Briefly, samples were subjected to serial washes with water, 75% alcohol, sodium hypochlorite (2%) and phosphate buffer solution (PBS—1.44 g of Na_2HPO_4, 0.24 g of KH_2PO_4, 0.20 g of KCI, 8.00 g of NaCl, pH 7.4). The samples were weighed and macerated in 1 ml PBS buffer. Resulting suspensions were serially diluted in PBS up to 10^{-3} (v v^{-1}) according to Döbereiner et al. (1995) and used for both isolation and counting, after culturing onto solid media in three replicas. Samples were also placed onto Petri dishes and the absence of growth of both fungi and bacteria monitored to control the efficiency of tissues disinfection. The culture media used were Nutrient Agar (NA) plus 20% glycerol, and Trypticase Soy Agar (TSA), according to Kado and Heskett (1970) and Döbereiner et al. (1995), respectively. The inoculated media were incubated at 28 °C for 7–8 days.

Single colonies were individually streaked onto Petri dishes and grown at 30 °C for 2–3 days until pure cultures were obtained. Single isolates were cultivated in Nutrient Broth (NB) and Trypticase Soy Broth (TSB), respectively, for 18 h at 30 °C under constant agitation and stored in 15% sterile glycerol at − 80 °C until subsequent molecular and biochemical characterization.

Phenotypic characterization of isolated strains

Indoleacetic acid (IAA) production

IAA production was tested according to Loper and Schroth (1986). Briefly, bacterial cultures were inoculated in NB medium with tryptophan (1, 2, and 5 mg ml^{-1}) or without tryptophan and incubated at 28 ± 2°C for 1–2 weeks. Cultures were centrifuged at 360× for 30 min. Two milliliters of the supernatant were mixed with 2 drops of orthophosphoric acid and 4 ml of Salkowski's reagent (50 ml, 35% perchloric acid; 1 ml 0.5 $FeCl_3$). Absorbance at 530 nm was measured after 30 min at room temperature. A standard curve was used to calculate the concentration of IAA from each culture medium. The isolates were classified into: (−) no production of IAA; (+) low production, up to 10 µg ml^{-1} of IAA; (++) medium production, from 10 to 50 µg ml^{-1} of IAA; (+++) high production, more than 50 µg ml^{-1} of IAA.

Phosphate solubilizing activity

The ability of isolates to solubilize phosphates was determined according to the method described by Nautiyal (1999) and modified as follows. Bacterial strains were tested qualitatively by plate assay using NBRI-P medium (per liter: glucose, 10 g; Gafsa rock phosphate, 5 g; $MgCl_2.6H_2O$, 5 g; $MgSO_4.7H_2O$, 0.25 g; KCl, 0.2 g; $(NH_4)_2SO_4$, 0.1 g). Bacteria were inoculated onto Petri dishes using sterile loops and plates incubated at 30 °C for 7 days. The formation of a clear halo around the colonies indicated phosphate solubilizing activity; isolates were classified into: (−) no halo, or lack of activity; (+) small halo (up to 2 mm), low activity; (++) medium halo (2–4 mm), median activity; (+++) large halo (greater than 4 mm), high activity.

Production of exopolysaccharides (EPS)

The production of EPS was assayed by streaking single colonies in Yeast Extract Mannitol Medium (0.5 g of yeast extract $liter^{-1}$ and 4 g of mannitol $liter^{-1}$ supplemented with 15 g of agar $liter^{-1}$), as described previously (Zlosnik et al. 2008). The morphology of the colony and the viscous aspect were monitored, and isolates were classified accordingly into (−) no EPS production; (+) low production, halo up

to 2 mm; (++) average production, halo from 2 to 4 mm; (+++) high production, halo greater than 4 mm.

Siderophore production

Siderophores production was measured using the universal chemical assay on Chrome Azurol S (CAS) agar plates (Schwyn and Neilands 1987) adapted as follows. The isolates were streaked onto Petri dishes and incubated at 30 ± 2 °C for 2 days. The medium color change from blue to orange/yellow indicated the production of siderophores that, binding iron more tightly than the ferric complex of Chrome Azurol S, removed iron from the CAS agar medium. Siderophore production was estimated by the size of orange halos formed around the colonies; isolates were classified into (−) no production, no orange halo; (+) low production, orange halo up to 2 mm; (++) average production, orange halo from 2 to 4 mm; and (+++) high production, orange halo greater than 4 mm.

Secreted enzymatic activities

Production of proteolytic enzymes was assayed on Petri dishes containing skim milk (1%), yeast extract (0.01%), KH_2PO_4 (0.15%), $MgSO_4.7H_2O$ (0.05%), NaCl (0.5%) and agar (1.5%); the strains were inoculated onto Petri dishes and plates incubated at 30 °C overnight.

For detection of lipase activity, TBA plate assay was used (per liter: bacto-peptone 5 g, yeast extract 3 g, Tributyrin 10 g, agar 12 g), according to Kumar et al. (2012). Bacteria inoculated onto Petri dishes were incubated at 30 °C overnight.

Chitin hydrolysis was measured using Bacillus minimal medium (BMM) (100 ml solution: KH_2PO_4, 0.65 g; K_2HPO_4, 1.5 g; NaCl, 0.25 g; $(NH_4)_2SO_4$, 0.5 g; $MgSO_4$, 0.12 g; $MgCl_2$, $ZnSO_4$, $FeCl_3$ and $CaCl_2$ were added to give a final concentration of 10^{-6} mol l^{-1}) with the addition of colloidal chitin (1%). Colloidal chitin was prepared according to the method of Wen et al. (2002) using commercial chitin from shrimp shells. Degradation was assessed by measuring the clear halo around colonies and using the same classification criteria described above: (−) no enzymatic activity; (+) low activity, halo up to 2 mm; (++) average activity, halo from 2 to 4 mm; (+++) high activity, halo greater than 4 mm.

Swarming and swimming motility

Swarming motility assay was performed using M8 medium plates with the addition of 0.5% agar (300 ml H_2O, 1.5 g agar, 9.07 g Na_2HPO_4, 1.8 KH_2PO_4, 0.3 NaCl, 1.2 g glucose, 0.3 g glutamic acid, 1.2 ml 1 M $MgSO_4$, 50 µl 1 M $CaCl_2$) as previously described (Murray and Kazmierczak 2006). A 0.5 µl spot of overnight culture was placed at the center of the Petri dish and plates were incubated at 30 °C for 24–48 h.

Swimming motility assay was performed as described above for swarming, except the concentration of agar in M8 medium, 0.25% instead of 0.5%.

Classification of motility was as follows: (−) no motility; (+) low motility (diameter of the colony up to 1 cm); (++) medium motility (from 1 to 4 cm); (+++) high motility (more than 4 cm). All tests were repeated in triplicate with independent experiments and the average estimated.

Biochemical characterization

Carbon sources utilization

API 20 E system was used to test the carbon source utilization patterns of selected endophytes. The test was carried out according to the manufacturer's protocol (BioMérieux, Marcyl'Etoile, France). Cultures were grown during 24 h in YEM liquid medium and uniform suspensions were prepared by suspending the cells in the medium provided in the kit. Suspensions for each isolate were standardized to 0.5% McFarland solution. Then, 150 µl of suspension was inoculated into each well of the strip. The strips were incubated at 28 °C for 48 h under static conditions in aseptic environment of growth chamber.

Out of the total of 54 endophyte bacteria selected, 42 bacteria were examined for the carbon pattern in the 20 basic API 20E tests as following: nitrates reduction (NO_2) or nitrogen reduction (N_2), indole production (TRP), glucose assimilation (GLU), arginine dihydrolase (ADH), urease production (URE), hydrolysis from β-glucosidase (ESC), hydrolysis from protease (GEL), β-galactosidase production (PNG), and glucose fermentation (GLU1), fermentation/oxidation of arabinose (ARA), fermentation/oxidation of mannose (MNE), fermentation/oxidation of mannitol (MAN), *N*-acetyl-glucosamine (NAG), fermentation/oxidation of maltose (MAL), potassium gluconate (GNT), capric acid (CAP), adipic acid (ADI), malate (MLT), trisodium citrate (CIT) and phenylacetic acid (PAC) assimilation.

Antibiotic resistance

The antibiograms of bacterial isolates were determined by inoculating them onto Petri dishes containing NA medium with and without antibiotic. Plates were incubated at 30 °C overnight. The absence of growth indicated antibiotic sensitivity. The following antibiotics at three different concentrations were used: ampicillin (5, 100 and 200 µg ml^{-1}), gentamicin (10, 20 and 40 µg ml^{-1}), kanamycin (25, 50 and 100 µg ml^{-1}), streptomycin (50, 100 and 200 µg ml^{-1}) and tetracycline (10, 20 and 40 µg ml^{-1}).

Genotypic characterization

Extraction of genomic DNAs was carried out with PureLink® Genomic DNA Mini Kit (Life Technologies) and PCR amplification of the fragments was performed using primers 338F and 778R or 27F and 800R as described previously (Lane 1991; Anzai et al. 1997; Rösch and Bothe 2005; Xing et al. 2008). The resulting PCR products were purified with PureLink® Quick Gel Extraction Kit (Life Technologies) and sequenced by Macrogen (Macrogen Inc., Seoul, South Korea).

Data analysis

Phenotypic data included the ability to produce indole-3-acetic acid (IAA), phosphate solubilizing activity (Psolub), production of exopolysaccharides (EPS), secreted enzymatic activities such as protease (Prot), lipase (Lipase) and chitin hydrolysis (Chitin), swarming (Swarm) and swimming (Swim) motility and biochemical characterization including nitrates reduction (NO_2) or nitrogen reduction (N_2), indole production (TRP), glucose fermentation (GLU1), arginine dihydrolase (ADH) production, urease production (URE), hydrolysis from β-glucosidase (ESC), hydrolysis from protease (GEL), β-galactosidase production (PNG), glucose assimilation (GLU), fermentation/oxidation of arabinose (ARA), fermentation/oxidation of mannose (MNE), fermentation/oxidation of mannitol (MAN), *N*-acetyl-glucosamine (NAG), fermentation/oxidation of maltose (MAL), potassium gluconate (GNT), capric acid (CAP), adipic acid (ADI), malate (MLT), trisodium citrate (CIT) and phenylacetic acid (PAC) assimilation. In addition, the strains were characterized for the following antibiotics at several concentrations, as reported above: ampicillin (AMP), gentamicin (GN), kanamycin (KM), streptomycin (ST) and tetracycline (TC). All data were evaluated by means of construction of phenograms. Data regarding PGP characteristics (IAA, Psolub, EPS, Chitin, Swarm and Swim) were converted and transferred into a data matrix where 3 represented a great activity, 2 an average activity, 1 a low activity, and 0 a negative activity; for antibiotic resistance and carbon utilization patterns, data were converted into a binary data matrix where 1 represents positive result and 0 negative result against each carbon source. Pairwise comparisons of the isolates were used to generate similarity coefficients. The resulting similarity coefficients were further used to drive relationships among endophytic bacteria strains of each sample in the form of cluster analysis using an unweighted pair group method with arithmetic averages. Finally, the phenogram was constructed according to the cluster analysis by applying the unweighted pair group method with arithmetic mean (UPGMA) categorical analysis, using BioNumerics software version 7.6 (Applied Maths).

The phylogenetic analysis of the 16SrRNA gene fragments was performed by comparing sequences and analyzing homology with other sequences in the GenBank database using Basic Local Alignment Search Tool BLAST program (National Center for Biotechnology Information) available at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST). Software MEGA 6.0 (www.megasoftware.net/) was used for multiple alignments of sequences (Clustal W) and phylogenetic tree construction using neighbor-joining algorithm (Jukes–Cantor model). Neighbor-joining tree reliability was estimated by bootstrap analysis with 1000 pseudo-replicate data sets.

Results

Different transgenic and non-transgenic soybean cultivars were grown in different field sites and analyzed for density and composition of endophytic bacterial populations in tissues of different plant organs. Along the experiment with soybean, the following average minimal and maximal temperatures were observed in Ponta Grossa (PR), Guarapuava (PR), and Campos Novos (SC): 16.4 and 26.5 °C; 15.6 and 26.4 °C and 14.5 and 27.2 °C, respectively. The rainfall average was 903.4 mm in Ponta Grossa (PR), 1250.4 mm in Guarapuava (PR) and 732.6 mm in Campos Novos (SC). The recovered isolates in all field sites were selected based on their characteristics of plant growth promotion and antagonistic activity against soybean pathogens as already reported by de Almeida Lopes et al. (2016).

The comparisons between means observed between non-transgenic (C) and glyphosate-resistant transgenic (GR) soybean and among tissues of different organs were made by Dunn's test. A significant difference ($P < 0.05$) was observed in the number of $CFUg^{-1}$ fresh weight among cultivars, with the transgenic cultivars having the highest abundance of bacterial strains and most of the isolates were obtained from roots. The density of the strains expressed in $CFUg^{-1}$ fresh weight recovered from roots of glyphosate-resistant transgenic/non-transgenic cultivars was 2.79/2.70; 3.28/1.97 and 1.99/0.03 at Ponta Grossa, Guarapuava and Campos Novos, respectively. The analysis of the phenotypic characterization of the endophytic bacterial population associated with non-transgenic (Fig. 1) and glyphosate-resistant transgenic soybean (GR) (Fig. 2) including the PGP characteristics, frequency and quality of antibiotic resistance and carbon source metabolic patterns of the isolates was clustered at Euclidian distance of 10. The patterns of carbon source utilization of the isolates recovered from non-transgenic and glyphosate-resistant plants were expressed as percentages of positive or negative results for each strain and are shown in Figs. 1 and 2, respectively.

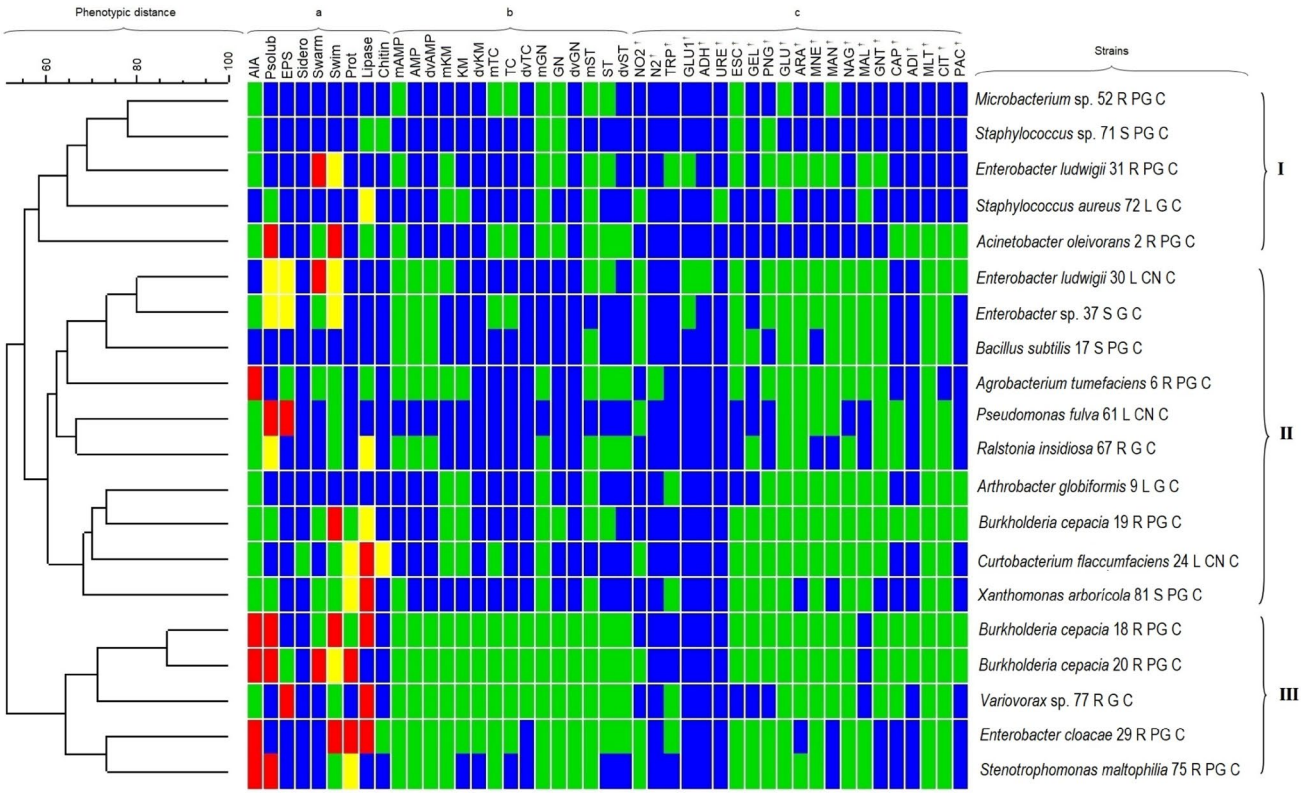

Fig. 1 Dendrogram constructed using the phenotypic data obtained from endophytic isolates from roots (R), stems (S) and leaves (L) of non-transgenic soybean grown in Ponta Grossa/PR (PG), Guarapuava/PR (G) and Campos Novos/SC (CN), Brazil. Cluster analysis was performed by applying UPGMA categorical analysis. The numbers of isolates phenotypes are given on the right. The % of sequence similarity of the 16S rRNA genes is given in brackets. †Evaluation according to the use of different carbon sources using Api kit 20 E (BioMérieux, Marcy l'Etoile, France). **a** Plant growth promotion characteristics—*IAA* indoleacetic acid production; *Psolub* phosphates solubilization, *EPS* exopolysaccharide production; *Sidero* siderophore production, *Swarm* swarming motility; Swim: swimming motility; Prot: protease production; Lipase: lipase production; Chitin: chitinase production. Yellow = high activity (+++); red = average activity (++); green = low activity (+); blue = no activity (−). **b** antibiotic resistance at half the dose (mAMP), normal dose (AMP) and double dose (dvAMP) of ampicillin, kanamycin (mKM, KM and dvKM), tetracycline (mTC, TC, dvTC), gentamicin (mGN, GN, dvGN) and streptomycin (mST, ST, dvST). Green = positive resistance (+); blue = negative resistance (−). **c** NO_2 reduction of nitrates to nitrites, *N2* reduction of nitrates to nitrogen, *TRP* indole production, *GLU1* fermentation, *ADH* arginine dihydrolase, *URE* urease, *ESC* hydrolysis from β-glucosidase, *GEL* hydrolysis from protease, *PNG* hydrolysis from β-galactosidase, *GLU* glucose assimilation, *ARA* arabinose assimilation, *MNE* mannose assimilation, *MAN* mannitol assimilation, *NAG N*-acetyl-glucosamine assimilation, *MAL* maltose assimilation, *GNT* potassium gluconate assimilation, *CAP* capric acid assimilation, *ADI* adipic acid assimilation, *MLT* malate assimilation, *CIT* trisodium citrate assimilation, *PAC* phenylacetic acid assimilation. Green = positive activity (+); blue = negative activity (−)

The endophytic bacteria isolated from non-transgenic soybean clustered into three groups at 57% similarity (Fig. 1). Group I joined isolates from roots, leaves and stems, mostly from Ponta Grossa and included bacteria with low (+) motility, IAA production and phosphate-solubilizing ability, except for *Enterobacter ludwigii,* which showed positive metabolism pattern for 50% of the 21 carbon sources tested, while the other strains metabolized less than 20% of the same carbon sources utilized. The largest group was cluster II with 50% of the selected strains, grouped from tissues of different plant organs with homogeneous carbon sources utilization pattern and able to metabolize most of the carbon sources tested (about 70%). Cluster III contained only isolates recovered from plant roots, mostly obtained from Ponta Grossa, and comprising mostly PGP bacteria with high IAA production (+++), significative motility (++), high EPS production and high capacity to solubilize inorganic phosphates. The strains from cluster III presented resistance to most of the antibiotics evaluated. In addition, the strains from cluster III were homogenous for carbon sources metabolism and were able to use most of the carbon sources used in the test (70%) (Fig. 1).

A broader range of carbon sources metabolism was observed in the strains recovered from non-transgenic plants compared to strains recovered from GR plants. Glutamine (GLU) and malate (MLT) were metabolized by 90% and 80% of the strains recovered from non-transgenic soybean, respectively, followed by ESC, PNG, ARA, MNE, MAN,

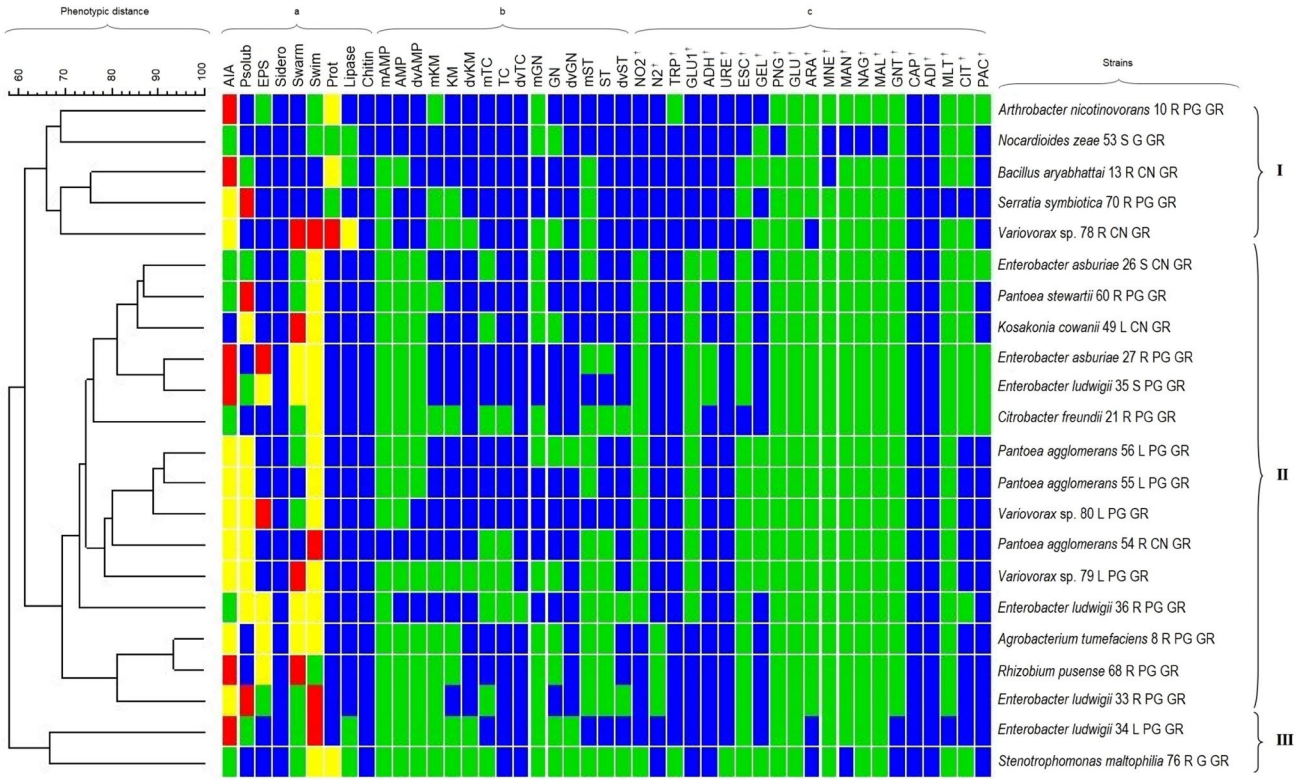

Fig. 2 Dendrogram constructed using the phenotypic data obtained from endophytic isolates from roots (R), stems (S) and leaves (L) of glyphosate-resistant transgenic soybean grown in Ponta Grossa/PR (PG), Guarapuava/PR (G) and Campos Novos/SC (CN), Brazil. Cluster analysis was performed by applying UPGMA categorical analysis. The numbers of isolates phenotypes are given on the right. The % of sequence similarity of the16S rRNA genes is given in brackets. †Evaluation according to the use of different carbon sources using Api kit 20 E (BioMérieux, Marcy l'Etoile, France). **a** Plant growth promotion characteristics—*IAA* indoleacetic acid production, *Psolub* phosphates solubilization, *EPS* exopolysaccharide production, *Sidero* siderophore production, *Swarm* swarming motility, *Swim* swimming motility, *Prot* protease production, *Lipase* lipase production, *Chitin* chitinase production. Yellow = high activity (+++); red = average activity (++); green = low activity (+); blue = no activity (−). **b** = antibiotic resistance at half the dose (mAMP), normal dose (AMP) and double dose (dvAMP) of ampicillin, kanamycin (mKM, KM and dvKM), tetracycline (mTC, TC, dvTC), gentamicin (mGN, GN, dvGN) and streptomycin (mST, ST, dvST). Green = positive resistance (+); blue = negative resistance (−). **c** NO_2 reduction of nitrates to nitrites, N_2 reduction of nitrates to nitrogen, *TRP* indole production, *GLU1* fermentation, *ADH* arginine dihydrolase, *URE* urease, *ESC* hydrolysis from β-glucosidase, *GEL* hydrolysis from protease, *PNG* hydrolysis from β-galactosidase, *GLU* glucose assimilation, *ARA* arabinose assimilation, *MNE* mannose assimilation, *MAN* mannitol assimilation, *NAG* *N*-acetyl-glucosamine assimilation, *MAL* maltose assimilation, *GNT* potassium gluconate assimilation, *CAP* capric acid assimilation, *ADI* adipic acid assimilation, *MLT* malate assimilation, *CIT* trisodium citrate assimilation, *PAC* phenylacetic acid assimilation. Green = positive activity (+); blue = negative activity (−)

NAG, GNT, MLT and CIT which were metabolized by 75% of the strains (Fig. 1). N_2, urea (URE), and ADH were the least utilized being metabolized by only 5% of the strains. The metabolism of three or less carbon sources was determinant for clustering strains 52, 71, 72 and 2 in Cluster I (Fig. 1).

Cluster analysis based on distance matrix for PGP characteristics, antibiotics resistance and carbon sources utilization pattern of endophytic bacteria recovered from glyphosate-resistant transgenic soybean is presented in Fig. 2. Cluster analysis divides 22 endophytic bacteria into three groups at distance of 62% of similarity. Cluster I joined five strains recovered from roots and stems of soybeans grown in Ponta Grossa, Guarapuava and Campos Novos. We observed that Cluster I included bacteria with limited PGPR ability, low motility (only two strains showed swim motility), no siderophore and chitinase production and low P solubilizing ability. The strains in cluster I were homogeneous for the lack of antibiotics resistance. Among the five strains of this cluster, only *Variovorax paradoxus* recovered from roots in Campos Novos showed low resistance to kanamycin, ampicillin and gentamicin. On the other hand, bacteria from Cluster I were homogeneous for the ability to metabolize carbon sources, with the majority using up to 60% of carbon sources tested. Cluster II was the largest group with 15 isolates (about 78% of the strains) recovered from transgenic plants, mostly from Ponta Grossa (80%) and from roots (50%). These strains showed high IAA production and P solubilization and all of them had motility. Although

they present variations regarding the pattern of antibiotic resistance, they were all resistant to at least two antibiotics. Lower diversity for carbon sources metabolism was found in strains recovered from GR transgenic plants (Fig. 2) compared with strains recovered from non-transgenic (Fig. 1). Glucose was the most used carbon source for strains recovered from transgenic plants (100%), followed by ESC, PNG, ARA, MNE, MAN, NAG, MAL, GNT and MLT which were metabolized by 80% of the strains (Fig. 2).

Isolates from transgenic soybean of Cluster II showed homogenous pattern for carbon source utilization, where 100% showed ability to metabolize 50% of carbon sources tested. Glucose was the most used carbon source, metabolized by 100% of the strains, followed by galactose (PNG), arabinose (ARA), mannose (MNE), mannitol (MAN), *N*-acetyl-glucosamine (NAG), maltose (MAL) and potassium gluconate (GNT). Cluster III comprised only two strains with PGP properties such as high IAA production and P solubilization, lipase and protease activity and high swimming motility; in addition, strains from Cluster III were resistant to all antibiotics tested and able to metabolize 80% of the carbon sources tested (Fig. 2).

The nearly complete sequences of the 16SrRNA gene from these isolates were also compared with those of other 16SrRNA sequences in the GenBank database. Among the 84 selected isolates 32 showed a 16SrRNA gene sequence with 99% similarity to the same sequence of the closest strain found in the GenBank database (Table 1). Considering that in our study tissues of different plant organs were analyzed, *Enterobacter* was the most abundant genus among all recovered strains (23 isolates) and it was present in all plant tissues (Table 1). The strains with 99% or more of identity were assigned to the genus *Enterobacter* and to the genera *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Burkholderia*, *Citrobater*, *Curtobacterium*, *Enterobacter*, *Kosakonia*, *Microbacterium*, *Pseudomonas*, *Ralstonia*, *Staphylococcus* and *Stenotrophomonas*. Partial sequences of 16SrRNA genes were aligned and the relationships between endophytic bacterial strains from non-transgenic soybean (Fig. 3), GR transgenic soybean (Fig. 4) and both non-transgenic and GR transgenic soybean (Fig. 5) were evaluated by neighbor-joining algorithm. Considering that trees are dynamic structures that change on the basis of the quality and availability of the data used for their calculation, only high-quality sequences were used for the phylogenetic analysis. Therefore, isolates with low sequence quality were not considered for construction of phylogenetic trees. The phylogenetic trees constructed from partial sequences of the 16SrRNA of isolates from both soybean management systems, non-transgenic and GR soybean, grouped the recovered strains into six groups of similarity at the genetic distance of 65.1. However, higher homogeneity was observed among bacteria recovered from GR soybeans, where 22 strains represented 11 genera, while in the non-transgenic soybean, the 20 recovered strains represented 14 genera.

Bacterial classification at species level was based on BLAST determined similarity of 16SrRNA amplicons with known 16SrRNA genes and was considered only when sequence similarity was higher than 95% if compared with the sequence of 16SrRNA gene of the type strains.

The genera *Burkolderia* sp., *Ralstonia* sp., *Acinetobacter* sp., *Pseudomonas* sp., *Xanthomonas* sp., *Curtobacterium* sp., *Microbacterium* sp., *Staphylococcus* sp. were found exclusively in non-transgenic soybean plants, while *Serratia* sp., *Pantoea* sp., *Kosakonia* sp., *Citrobacter* sp. and *Nocardioides* sp. were found exclusively in GR cultivars.

Significant differences were found in the abundance and diversity of bacterial communities associated with GR and conventional soybean. The GR soybean showed higher average population density when compared to the non-transgenic (2.36 Log CFU g^{-1} and 1.54 Log CFU g^{-1}, respectively, $P < 0.05$ Tukey's test) and most isolates were found in the roots. In Ponta Grossa, locality with the highest abundance of isolates, significative difference between cultivars was observed. The non-transgenic BRS 133 cultivar showed average population density of 1.85 Log CFU g^{-1}, while in its essentially derived GR BRS 245RR an average population density of 2.41 Log CFU g^{-1}, $P < 0.05$ Tukey's test, was observed.

Discussion

Endophytes are considered bacteria that can colonize intercellular spaces of tissues of different organs of plants without causing negative effects on their host (Hardoim et al. 2008; Schulz and Boyle 2006; Etesami and Maheshwari 2018; Vigani et al. 2018). They have been reported as being present in almost all plant species and have been isolated from all plant organs such as stem, root, seed and leaf, therefore representing a great reservoir of bacterial diversity. Many endophytic bacteria are able to play several useful roles for their host such as nitrogen fixation, production of growth regulators, participation in immune reactions, and protection from abiotic stresses such as salinity, drought, heavy metal toxicity, and nutritional imbalance in plants (Gaiero et al. 2013; Garipova 2014; Glick and Stearns 2011; Zhang et al. 2012 Miliute et al. 2015). These functions can have a remarkable biotechnological potential and the use of endophytic bacteria may lead to plant health improvements with an important role in reduction of the amount of chemicals and achievement of sustainable agricultural productivity.

Since endophytes are protected from adverse environmental conditions and are more closely related to the host, competition for nutrients with other microorganisms and

Table 1 Endophytic bacteria isolated from non-transgenic (C) and glyphosate-resistant transgenic (GR) soybean roots (R), stems (S) and leaves (L), grown in field trials in Ponta Grossa/PR (PG), Campos Novos/SC (CN) and Guarapuava/PR (G) in Brazil. NCBI accession numbers of the most identical 16SrRNA gene with the same gene of each isolate and the % of identity are also provided

Isolate number	Closest NCBI match	Cultivar	Tissue	Field site	NCBI accession number	% Identity
1	*Acinetobacter calcoaceticus* PUCM1031	C	R	CL	GQ469890.1	97
2	*Acinetobacter oleivorans* SSPR10	C	R	PG	MF521562.1	99
3	*Acinetobacter* sp. AAU-20B	C	R	CL	MG557790.1	96
4	*Acinetobacter* sp. UASWS1705	GR	R	CL	MG813733.1	89
5	*Acinetobacter* sp. 4081	GR	L	CL	KC236603.1	99
6	*Agrobacterium tumefaciens* NTW1	C	R	PG	HE995808.1	98
7	*Agrobacterium tumefaciens* C58	GR	S	CN	AE007870.2	99
8	*Agrobacterium tumefaciens* S33	GR	R	PG	CP014260.1	99
9	*Arthrobacter globiformis* FS-1	C	L	G	KY649415.1	98
10	*Arthrobacter nicotinovorans* S32115	GR	R	PG	AB648947.1	98
11	*Arthrobacter nicotinovorans* g52	GR	R	PG	KM019883.1	97
12	*Arthrobacter* sp. RC 1.1	GR	S	CN	KP267832.1	99
13	*Bacillus aryabhattai* PSB53	GR	R	CN	HQ242766.1	95
14	*Bacillus cereus* TYg2-1	C	R	CL	HQ290100.1	98
15	*Bacillus megaterium* 0100	C	L	CL	KP236235.1	98
16	*Bacillus megaterium* SR7	C	L	CL	CP022674.1	99
17	*Bacillus subtilis* M1	C	S	PG	MG977677.1	95
18	*Burkholderia cepacia* FC2980	C	R	PG	MG871245.1	99
19	*Burkholderia cepacia* EB01	C	R	PG	MF109897.1	99
20	*Burkholderia cepacia* JBK9	C	R	PG	CP013732.1	99
21	*Citrobacter freundii* CF9527	GR	R	PG	MF288078.1	98
22	*Citrobacter freundiiJCM2628*	GR	S	G	LC416049.1	99
23	*Cupriavidus taiwanensis* EJ2	GR	R	CL	KF646764.1	97
24	*Curtobacterium flaccumfaciens* P259/26	C	L	CN	AJ310414.1	99
25	*Curtobacterium sp.* MR_MD2014	GR	S	CL	KU740254.1	99
26	*Enterobacter asburiae* IHB B 1510	GR	S	CN	KF475835.1	99
27	*Enterobacter asburiae* L1	GR	R	PG	CP007546.1	95
28	*Enterobacter* sp. CVv	GR	R	PG	KF956722.1	97
29	*Enterobacter cloacae* L17	C	R	PG	GQ995670.1	85
30	*Enterobacter ludwigii* IF2SW-B4	C	L	CN	KF835823.1	95
31	*Enterobacter ludwigii* 1	C	R	PG	MH251720.1	99
32	*Enterobacter ludwigii* IF2SW-P3	GR	R	PG	KY218816.1	99
33	*Enterobacter ludwigii* K170	GR	R	PG	AB900620.1	90
34	*Enterobacter ludwigii* SJR3	GR	L	PG	KF383271.1	95
35	*Enterobacter ludwigii* IF2SW-P3	GR	S	PG	KY218816.1	99
36	*Enterobacter ludwigii* K9	GR	R	PG	EF175735.1	99
37	*Enterobacter* sp. MLB05	C	S	G	JQ765415.1	96
38	*Enterobacter* sp. L3R3-1	C	L	CL	KF010363.1	99
39	*Enterobacter* sp. Gamma-8	C	S	CL	MH703439.1	98
40	*Enterobacter* sp. GD106	C	R	CL	JX112354.1	100
41	*Enterobacter* sp. Ps08	C	R	CL	KR856303.1	99
42	*Enterobacter* sp. UIWRF1243	C	R	CL	KR189401.1	97
43	*Enterobacter* sp. SR1.1	C	R	CL	JQ912523.1	96
44	*Enterobacter* sp. BSRA1	C	R	CL	FJ868805.1	95
45	*Enterobacter* sp. GD106	GR	L	CL	JX112354.1	98
46	*Enterobacter* sp. Osibote30	C	S	CL	LC349902.1	99
47	*Enterobacter* sp. NOD6	C	R	CL	MH392321.1	99

Table 1 (continued)

Isolate number	Closest NCBI match	Cultivar	Tissue	Field site	NCBI accession number	% Identity
48	*Enterobacter* sp. TV1	C	R	CL	KX373988.1	93
49	*Kosakonia cowanii* R28	GR	L	CN	MH141462.1	99
50	*Kosakonia cowanii* Noori9	GR	R	G	MG586239.1	90
51	*Microbacterium binotii* JZ R-90	GR	R	PG	MH119727.1	99
52	*Microbacterium* sp. TRB26	C	R	PG	KX981237.1	98
53	*Nocardioides zeae* SCSIO 43764	GR	S	G	MH283848.1	98
54	*Pantoea agglomerans* SGS9	GR	R	CN	KJ529102.1	84
55	*Pantoea agglomerans* Y34	GR	L	PG	JX113242.1	97
56	*Pantoea agglomerans* SN 13	GR	L	PG	KR088615.1	93
57	*Pantoea agglomerans* FC2948	GR	L	CL	MH532498.1	93
58	*Pantoea agglomerans* TPJ1	GR	L	CL	KT766082.1	93
59	*Pantoea agglomerans* FC2948	GR	L	CL	MH532498.1	98
60	*Pantoea stewartii* LMR298	GR	R	PG	KU182804.1	89
61	*Pseudomonas fulva* S29	C	L	CN	AY741159.1	99
62	*Pseudomonas monteilii* 151	C	R	CL	MH910255.1	99
63	*Pseudomonas putida* MG-Y2	GR	R	CL	EU179737.1	99
64	*Pseudomonas* sp. EA-S-10	C	R	CL	KJ642335.1	96
65	*Pseudomonas* sp. H9zhy	C	S	CL	AM410625.1	99
66	*Pseudomonas* sp. EpD2	GR	R	CL	KX914639.1	90
67	*Ralstonia insidiosa* M11	C	R	G	LN890097.1	99
68	*Rhizobium pusense* A1143	GR	R	PG	JX266311.1	94
69	*Rhizobium sp./Agrobacterium sp.*	GR	S	CL	KC488176.1	97
70	*Serratia symbiotica* 28238	GR	R	PG	KP866578.1	85
71	*Staphylococcus* sp. C0021-01F	C	S	PG	MH447012.1	98
72	*Staphylococcus aureus* O17	C	L	G	CP032051.1	99
73	*Staphylococcus capitis* Y47	GR	L	CL	JX094954.1	99
74	*Staphylococcus pasteuri* HN-35	C	S	PG	KT003275.1	98
75	*Stenotrophomonas maltophilia* T215	C	R	PG	KC764984.1	95
76	*Stenotrophomonas maltophilia* ISMMS2	GR	R	G	CP011305.1	99
77	*Variovorax* sp. AMF1302	C	R	G	JQ316399.1	98
78	*Variovorax* sp. AL104	GR	R	CN	MG819595.1	90
79	*Variovorax* sp. 287	GR	L	PG	KY682047.1	89
80	*Variovorax* sp. SaNR1	GR	L	PG	HQ730968.1	88
81	*Xanthomonas arborícola* 17-331	C	S	PG	LC388645.1	95
82	*Xanthomonas axonopodis* UF-CrpMy	C	R	PG	KF926682.1	96
83	*Xanthomonas campestris* FJ-X1	GR	L	PG	MF285891.1	95
84	*Xanthomonas translucens* JS1023	GR	R	PG	KX507147.1	97

their effectiveness are significantly different if compared to the bacteria isolated from the soil and rhizosphere (Sturz et al. 2000; Siciliano et al. 2001; Compant et al. 2010; Etesami et al. 2014; Garipova 2014). Therefore, considering the several useful roles played by endophytic bacteria in plants and the favorable environmental conditions inside the host, the use of these bacteria could help reducing the adverse effects on crops of environmental stresses. Some endophytic bacteria are also able to completely degrade organic compounds such as organochlorine herbicides several times more frequently than in rhizobacteria. In their study, Germaine et al. (2006) reported that pea plants inoculated with *Pseudomonas*, an endophytic bacterium that naturally possesses the ability to degrade 2,4-dichlorophenoxyacetic acid, showed a higher capacity for 2,4-dichlorophenoxyacetic acid removal from soil and showed no 2,4-dichlorophenoxyacetic acid accumulation in their aerial tissues. This demonstrates the usefulness of bacterial endophytes not only to enhance the phytoremediation of herbicide-contaminated substrates but also to reduce levels of toxic herbicide residues in crop

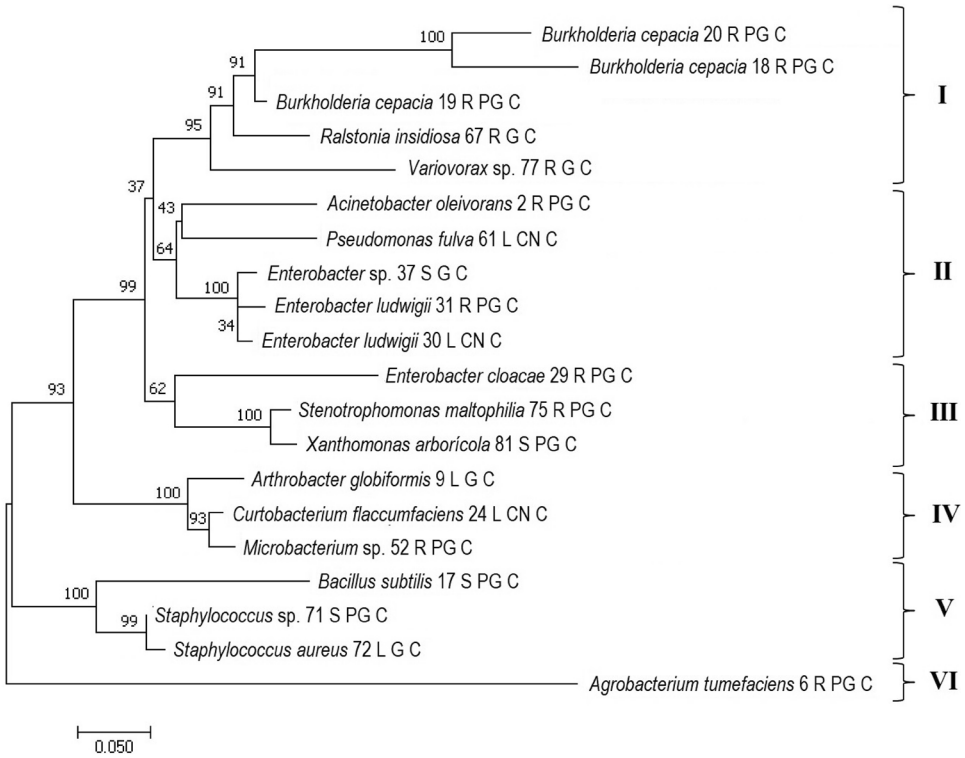

Fig. 3 Neighbor-joining phylogenetic tree of 16S rRNA constructed using the partial nucleotide sequence from the endophytic isolates obtained from roots (R), steams (S) and leaves (L) of non-transgenic soybean grown in Ponta Grossa/PR (PG), Guarapuava/PR (G) and Campos Novos/SC (CN), Brazil. The reliability of the neighbor-joining tree using MEGA 6.05 software package was estimated by bootstrap analysis with 1000 pseudoreplicate data sets. Confidence levels (%) above each node were generated from bootstrap. The numbers of isolates phenotypes are given on the right. The scale bar indicates 0.05 substitutions per nucleotide position

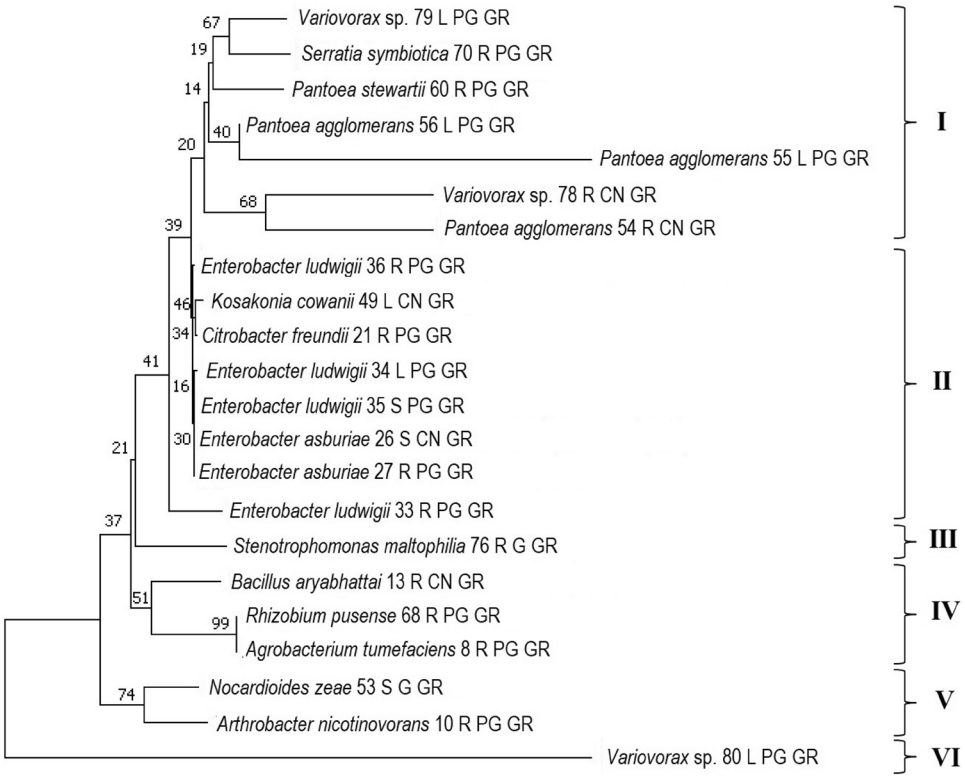

Fig. 4 Neighbor-joining phylogenetic tree of 16S rRNA constructed using the partial nucleotide sequence from the endophytic isolates obtained from roots (R), steams (S) and leaves (L) of glyphosate-resistant transgenic soybean grown in Ponta Grossa/PR (PG), Guarapuava/PR (G) and Campos Novos/SC (CN), Brazil. The reliability of the neighbor-joining tree using MEGA 6.05 software package was estimated by bootstrap analysis with 1000 pseudoreplicate data sets. Confidence levels (%) above each node were generated from bootstrap. The numbers of isolates phenotypes are given on the right. The scale bar indicates 0.10 substitutions per nucleotide position

plants. Differences in the composition of the root-associated microbial community between transgenic and non-transgenic plants have been described (Donegan et al. 1995; Siciliano and Germida 1999; Di Giovanni et al. 1999). This study indicated that endophytic bacteria isolated from transgenic and non-transgenic soybean field sites differ in their capacity

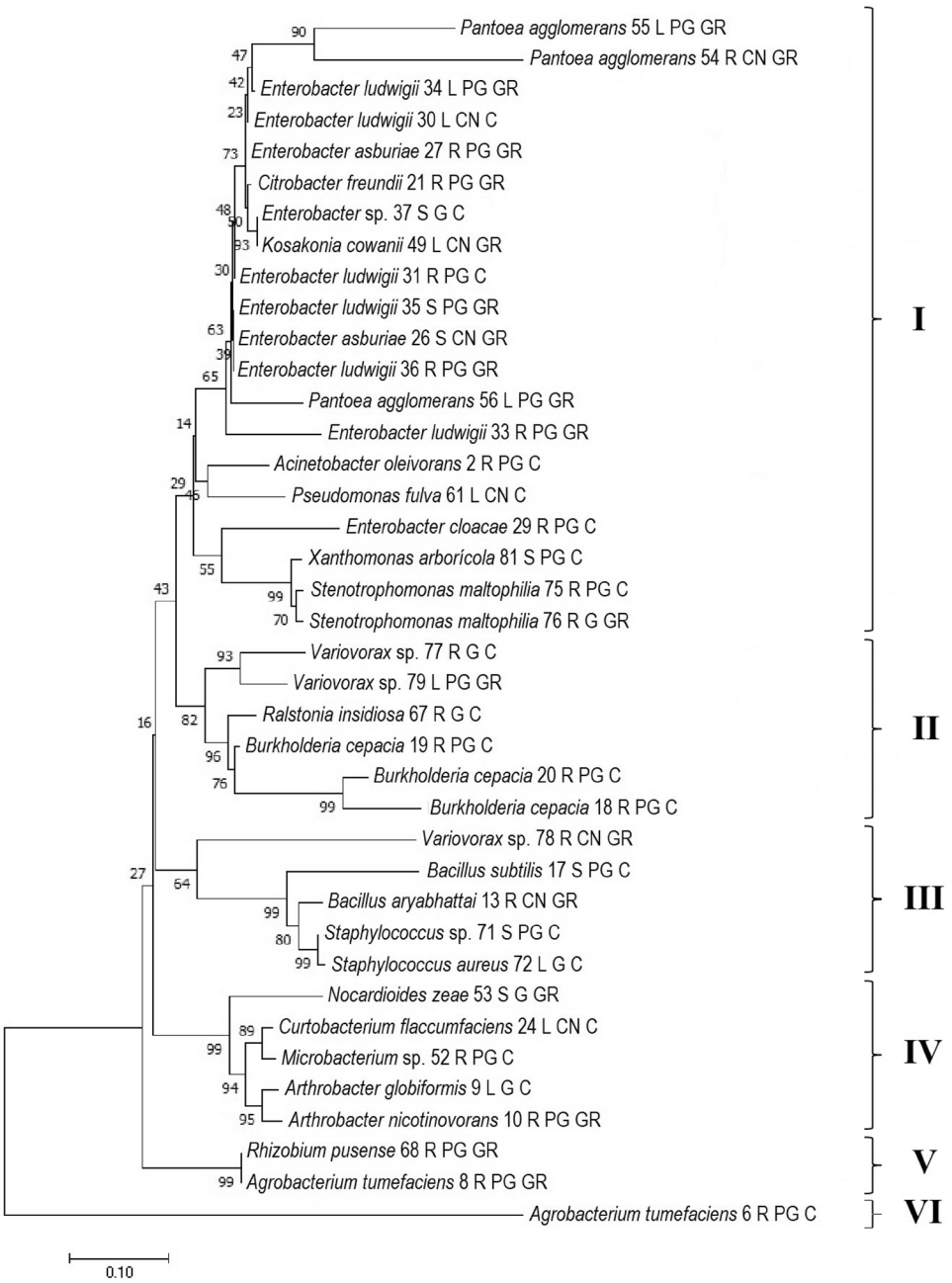

Fig. 5 Neighbor-joining phylogenetic tree of 16S rRNA constructed using the partial nucleotide sequence from the endophytic isolates obtained from roots (R), steams (S) and leaves (L) of non-transgenic (C) and glyphosate-resistant (GR) transgenic soybean grown in Ponta Grossa/PR (PG), Guarapuava/PR (G) and Campos Novos/SC (CN), Brazil. The reliability of the neighbor-joining tree using MEGA 6.05 software package was estimated by bootstrap analysis with 1000 pseudoreplicate data sets. Confidence levels (%) above each node were generated from bootstrap. The numbers of isolates phenotypes are given on the right. The scale bar indicates 0.10 substitutions per nucleotide position

to promote growth (PGPs). Overall, resistance to antibiotics and carbon sources metabolism were generally more common than PGP ability. The majority of isolated endophytes could utilize multiple patterns of carbon sources and express resistance to antibiotic, while isolates varied widely in the PGPs ability (Figs. 3 and 4). The greater frequency of carbon sources utilization as well as the frequency and intensity of antibiotic resistance compared with PGP ability within the soybean endophytes community suggests that carbon metabolism and antibiotic resistance confer a greater relative fitness benefit than PGP ability. Alternatively, carbon sources utilization pattern and antibiotic resistance may be less costly energetically to maintain in the absence of direct selection pressure. Further, carbon metabolism and antibiotic resistance may be more readily disseminated among individuals via horizontal gene transfer (Davelos et al. 2004; Liu et al. 2018). These data are consistent with the expectation that carbon sources utilization and antibiotic resistance are an absolute requirement for survival.

There was substantial diversity in our study when we analyzed tissues from different plant organs. The distinct differences among the frequency of antibiotic resistance in the phenotypes isolated from various tissues suggested that antibiotic interactions may vary in their significance to

fitness among plant tissues. The highest frequency of strains expressing antibiotic resistance was recovered in roots (R). In leaves (L) and stems (S), where the density of bacterial population was lower, the isolates had relatively little antibiotic resistance ability (Figs. 3, 4). These data suggest that antibiotic resistance-mediated competitive interactions within the community of bacterial endophytes could be less important where the population density is low. Similarly, a significant variation was observed among the soybean field sites. In Ponta Grossa/PR (PG), where soybean showed the highest density of isolates, strains with the highest frequency of antibiotic resistance were isolated, while in Campos Novos/SC (CN), field location with the lowest density, isolates had lower frequency of antibiotic resistance. These data support the hypothesis that antibiotic resistance is an important requirement for survival, even more important where the competition is higher.

The composition and density of the endophytic bacterial community depend on plant tissues and genotype, but also on temperature and soil type of the location where the plant is being grown. Our results showed that endophytic bacteria isolated from non-transgenic soybean grown in Campos Novos (SC) were significantly less abundant than those isolated from other localities. Although Campos Novos (SC), during the soybean season, did not show greater temperature difference comparing to the other sites evaluated, the annual temperature average in Campos Novos was lower than the other localities with additional significant higher temperature gradient during the year (data not shown). This could be a reason for differences in endophytes species abundance. The effect of temperature and soil type on plant growth-promoting bacteria associated with wheat was evaluated by Egamberdiyeva and Hoflich (2003). Wheat inoculated with *Mycobacterium* sp., *Pseudomonas fluorescens* and *P. agglomerans* isolated from semi-continental climate showed a significantly increased root and shoot growth at 16 °C compared with that at 26 °C and plant growth-promoting effect was different in loamy sand and sandy loam soils.

The structure of soil microbial community may be altered by culture management and several studies have demonstrated that the application of herbicides, such as glyphosate, could result in changes in the microbial community in plants (Busse et al. 2001; Kuklinsky-Sobral et al. 2005, Barriuso et al. 2010; de Almeida Lopes et al. 2016). Although there is conflicting literature on the effects of glyphosate on microbioma community, most of the literature regarding long-term studies showed that shifts in bacterial community structure could not be clearly associated with glyphosate dissipation, suggesting the presence of redundant bacteria populations of potential degraders. Sorption coefficient in soil, cation exchange capacity, pH, soil nutrients status, clay and organic matter influence the capacity of soil to adsorb the herbicide, suggesting that bioavailability was a key factor for the persistence of glyphosate. In this respect, it is important to consider the relationship between bacterial taxa exposed to the herbicide and the importance of soil properties as predictor of the possible rate of degradation and persistence of glyphosate in soil (Guijarro et al. 2018).

The full range of resistance or sensitivity to glyphosate within the soil microbial community is not fully known. In plants, glyphosate blocks the synthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan through the competitive inhibition of EPSPS, which also causes accumulation and excretion of shikimate-3-phosphate and hydroxybenzoic acids. In bacteria, the sensitivity of EPSPS to glyphosate varies widely in sensitive and relatively insensitive strains. Among the insensitive ones, *Agrobacterium* CP4 is the source of the GR-EPSPS transgene in most GR cultivars and the resistance to glyphosate results from variations in the amino acid sequence of EPSPS (Duke et al. 2012; Reddy and Duke, 2014). Three mechanisms of microbial growth inhibition by glyphosate were proposed by Fischer et al. (1986): (1) interruption of aromatic amino acid synthesis, (2) energy consumption and drainage due to accumulation shikimate-3-phosphate, and (3) toxicity resulting from the shikimic acid pathway intermediate products accumulation. Hungria et al. (2014) used three pairs of near isogenic cultivars to study the effect of the GR transgenic trait on N_2 fixation variables such as nodule mass, total biomass, N and ureide-N and found some negative effects, although soybean grain yields were not affected over the three-year study period. In addition, according to the results from the multivariate analyses, it was found that Biological Nitrogen Fixation (BNF) and yield parameters were more affected by cropping season, location and cultivar than by the transgene, weed management strategy or herbicides used. However, longer term effects on BNF and N accumulation should be monitored in spite of the lack of effects of the transgene on yield in the three-year period. The effects of glyphosate on soybean nodulation and N fixation were reported by Zablotowicz and Reddy (2004): glyphosate treatments determined reduced nodulation, as well as delayed N fixation, plant biomass accumulation and N fixation. However, several factors affected the severity of these effects such as the number of glyphosate applications, glyphosate formulation, and GR soybean cultivar. Cultivars responses to glyphosate, but also the influence on *Bradyrhizobium* growth and nodulation, were studied by Oliveira et al. (2008) in a field trial with 20 GR soybean cultivars. Glyphosate was applied at four rate and timing combinations and soybean without glyphosate application was the negative control. *Bradyrhizobium* nodulation was less affected by glyphosate in the BRS variety group (BRS 245 RR, BRS 242 RR, BRS 243 RR, BRS 244 RR, BRS 246 RR), compared to other genetic materials, showing no significant nodulation reduction. Glyphosate was also found to reduce growth of *B.*

japonicum in laboratory conditions, while *Rhizobium* spp. was able to degrade glyphosate (Liu et al. 2018). Therefore, these effects on nodulation and N fixation may be due in part to the inhibitory effects of glyphosate on *B. japonicum*, but may also be related to GR cultivar responses to glyphosate.

The effects of herbicide-resistant plants and glyphosate on soil microorganisms were reported in several studies (Zablotowicz and Reddy, 2004; Pline-Srnic, 2005; Cerdeira and Duke, 2006; Böhm and Rombaldi, 2010) and the conditions of each study affected the results. For instance, Böhm and Rombaldi (2010) showed that soil type and crop management can influence the effects of RR soybean on soil microorganisms, although the effects were dependent more on the herbicide than the transgenic trait. In conclusion, considering that limited variables were usually analyzed and experiments were usually conducted under greenhouse or in vitro conditions, the results suggested that transgenic trait does not affect soil microorganisms community.

The results of our study suggested the existence of significant differences in terms of diversity between groups of species associated with non-transgenic and transgenic glyphosate-resistant soybean. The management system of herbicide application used in the non-transgenic and GR soybean could be the reason for this difference. However, other factors such as field conditions, cultivar genetic background, environmental factors, temperature, soil characteristics, and the agricultural practices adopted could play important roles in the endophyte community composition. Moreover, the different abundance and diversity of selected bacteria could be due also to the laboratory cultivation media. Most studies that isolated and characterized endophytic bacteria from transgenic plants were conducted in greenhouses or plot trials, therefore, under alleviated stress conditions; in the present study we highlighted that, in order to identify the best PGP and competitive endophytic bacteria, we characterized genetically and phenotypically the bacterial endophytes recovered from commercial soybean varieties, in field trials from different localities and in the presence of abiotic stresses. There are no reports about the diversity of interaction between management system in non-transgenic and transgenic glyphosate-resistant soybean in the field. Studies involving field experiments and bacteria cultivation-independent analysis should be performed to provide complete comprehension of the interaction between herbicides and plant-associated bacterial communities for more effective crop management.

Data regarding the phenotypic characterization of the endophytic bacterial population associated with non-transgenic and transgenic glyphosate-resistant soybean including the PGPs characteristics, frequency and quality of antibiotic resistance and carbon patterns metabolism of the strains were clustered at a Euclidian distance of 10. In a similar way, the 20 endophytic bacteria isolated from non-transgenic soybean and the 22 endophytic bacteria recovered from glyphosate-resistant transgenic soybean were clustered into three groups at 57% similarity. Although the microbial diversity was often analyzed by methods revealing phenotypic features, this approach has several limitations, such as pre-cultivation in the laboratory or the fact that only a restricted part of the genetic information is revealed through phenotypic testing. A combination of phenotypic and genetic methods is known to provide a good estimate of soil bacteria diversity (Torsvik et al. 1990). Therefore, it is of great importance to characterize bacterial communities both genetically and phenotypically, and each characterization complements the other, leading to more reliable results.

Thirty-nine percent of 84 isolates yielded the best matches with bacteria of the family *Enterobacteriaceae* (*Enterobacter*, *Pantoea*, *Citrobacter* and *Serratia*) that has been previously reported as prevalent and widespread family associated with plant tissues (Bulgari et al. 2009; Markova et al. 2005; Khalifa et al. 2016); bacteria of the family *Enterobacteriaceae* were followed by *Pseudomonas*, *Bacillus*, *Acinetobacter*, among others.

Partial sequences of 16SrRNA of the recovered isolates in all field sites were aligned and the relationships between endophytic bacteria from non-transgenic and GR soybean were evaluated by neighbor-joining algorithm. The phylogenetic trees constructed from partial sequences of the 16SrRNA from both soybean system managements, non-transgenic and GR soybean, grouped the recovered strains into six groups of similarity at the genetic distance of 65.1. The 16SrRNA sequences of isolates from non-transgenic plants presented greater diversity than the sequences from endophytes isolated from transgenic plants. Most of 16SrRNA sequences from endophytic isolates obtained from transgenic soybean formed one large phenotypic group of similarity at the genetic distance of 45.1.

Taken together, cluster analysis of the phenotypes and 16SrRNA gene sequences did not reveal correspondence between bacterial isolates and transgenic character of plants; the heterogeneity of clustering suggests that various adaptive processes could have contributed to this variability to enhance endophytes overall fitness.

Discrepancies between the phenotypic and genotypic characterization of bacterial communities have already been observed by other authors and can be explained by the fact that certain characteristics that are highly dependent on environmental factors, such as antibiotic resistance, are usually given by local selection pressures (Lottmann and Berg, 2001; Davelos et al. 2004; Baines et al. 2007; Freitas et al. 2008; Sikorski et al. 2008; Sagova-Mareckova et al. 2015). The crop management adopted in each field locality suggests that local selection pressures are important in generating diversity in PGPs characteristics, antibiotic resistance and carbon source utilization pattern in endophytic communities.

Particularly in complex environments such as those influenced by specific crop management, the development of antibiotic resistance and carbon sources utilization pattern phenotypes may provide fitness benefits under a wide range of conditions. The evolutionary variation (horizontal transfer, mutation, recombination) and ecological forces (local, crop management, plant tissue) generate the variation. Thus, variations in local selection and ecological processes across the local sites, crop management and plant tissue are likely to be an important contributor to the generation and maintenance of phenotypic diversity. The lack of correspondence between genetic and phenotypic groups within soybean endophytes suggests that selection pressures and endophytes responses vary across plant tissue, crop management and local site.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Human and animal rights This article does not contain any studies with animals performed by any of the authors. This study used the GenBank genetic sequence database.

References

Andreote FD, Mendes R, Dini-Andreote F, Rossetto PB, Labate CA, Pizzirani-Kleiner AA, van Elsas JD, Azevedo JL, Araújo WL (2008) Transgenic tobacco revealing altered bacterial diversity in the rhizosphere during early plant development. Antonie Van Leeuwenhoek 93:415–424

Andreote FD, Rocha UN, Araújo WL, Azevedo JL, Van Overbeek LS (2010) Effect of bacterial inoculation, plant genotype and developmental stage on root-associated and endophytic bacterial communities in potato (*Solanum tuberosum*). Antonie Van Leeuwenhoek 97:389–399

Anzai Y, Kudo Y, Oyaizu H (1997) The phylogeny of the genera *Chryseomonas*, *Flavimonas*, and *Pseudomonas* supports synonymy of these three *genera*. Int J Syst Bacteriol 47:249–251

Assumpção LC (2008) Diversidade da comunidade bacteriana endofítica de sementes de soja e o seu potencial biotecnológico.p 93, Dissertation (Master in Agronomy)—Escola Superior de Agricultura Luiz de Queiroz, Piracicaba

Baines ALD, Xiao K, Kinkel LL (2007) Lack of correspondence between genetic and phenotypic groups amongst soil-borne streptomycetes. FEMS Microbiol Ecol 59:564–575

Barriuso J, Marín S, Mellado RP (2010) Effect of the herbicide glyphosate on glyphosate-tolerant maize rhizobacterial communities: a comparison with pre-emergency applied herbicide consisting of a combination of acetochlor and terbuthylazine. Environ Microbiol 12:1021–1030

Böhm GMV, Rombaldi CV (2010) Genetic transformation and the use glyphosate on soil microbial, biological nitrogen fixation, quality and safety of genetically modified soybean. Cienc Rural 40:213–221

Bulgari D, Casati P, Brusetti L, Quaglino F, Brasca M, Daffonchio D, Bianco PA (2009) Endophytic bacterial diversity in Grapevine (*Vitis vinifera* L.) leaves described by 16S rRNA gene sequence analysis and length heterogeneity-PCR. J Microbiol 47:393–401

Busse MD, Ratcliff AW, Shestak CJ, Powers RF (2001) Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biol Biochem 33:1777–1789

Cerdeira AL, Duke SO (2006) The current status and environmental impacts of glyphosate-resistant crops: a review. J Environ Qual 35:1633–1658

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678

Davelos AL, Xiao K, Flor JM, Kinkel LL (2004) Genetic and phenotypic traits of streptomycetes used to characterize antibiotic activities of field-collected microbes. Can J Microbiol 50:79–89

de Almeida Lopes KB, Carpentieri-Pipolo V, Oro TH, Stefani Pagliosa E, Degrassi G (2016) Culturable endophytic bacterial communities associated with field-grown soybean. J Appl Microbiol 120:740–755

Di Giovanni GD, Watrud LS, Seidler RJ, Widmer F (1999) Comparison of parental and transgenic alfalfa rhizosphere bacterial communities using biolog GN metabolic fingerprinting and enterobacterial repetitive intergenic consensus sequence-PCR (ERIC-PCR). Microb Ecol 37:129–139

Döbereiner J, Baldani VL, Baldan JI (1995) Como isolar e identificar bactérias diazotróficas de plantas não-leguminosas. EMBRAPA-SPI, Brasília, EMBRAPA-CNPAB, Itaguaí

Donegan KK, Palm CJ, Fieland VJ, Porteous LA, Ganio LM, Schaller DL, Bucao LQ, Seidler RJ (1995) Changes in levels, species and DNA fingerprints of soil microorganisms associated with cotton expressing the *Bacillus thuringiensis* var. kursmki endotoxin. Appl Soil Ecol 2:111–124

Duke SO, Lydon J, Koskinen WC, Moorman TB, Chaney RL, Hammerschmidt R (2012) Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. J Agric Food Chem 60:10375–10397

Egamberdiyeva D, Hoflich G (2003) Influence of growth- promoting bacteria on the growth of wheat in different soils and temperatures. Soil Biol Biochem 35:973–978

Embrapa (2006) Sistema Brasileiro de Classificação de Solos, 2nd edn. EMBRAPA-SPI, Rio de Janeiro, pp 306

Etesami H, Maheshwari DK (2018) Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: action mechanisms and future prospects. Ecotoxicol Environ Saf 156:225–246

Etesami H, Mirseyed Hosseini H, Alikhani HA (2014) In planta selection of plant growth promoting endophytic bacteria for rice (Oryza sativa L.). J Soil Sci Plant Nutr 14:491–503

Fischer RS, Berry A, Gaines CG, Jensen RA (1986) Comparative action of glyphosate as a trigger of energy drain in eubacteria. J Bacteriol 168:1147–1154

Fisher LM, Albery WJ, Knowles JR (1986) Energetics of proline racemase: tracer perturbation experiments using [^{14}C] proline that measure the interconversion rate of the two forms of free enzyme. Biochem 25:2538–2542

Franz JE, Mao MK, Sikorski JA (1997) Glyphosate: a unique global herbicide. American Chemical Society, Washington, DC, p 653

Freitas DB, Reis MP, Lima-Bittencourt CI, Costa PS, Assis PS, Chartone-Souza E, Nascimento AMA (2008) Genotypic and phenotypic diversity of *Bacillus* spp. isolated from steel plant waste. BMC Res Notes 92:1–11

Gaiero JR, McCall CA, Thompson KA, Day NJ, Best AS, Dunfield KE (2013) Inside the root microbiome: bacterial root endophytes and plant growth promotion. Am J Bot 100:1738–1750

Garipova SR (2014) Perspectives on using endophytic bacteria for the bioremediation of arable soils polluted by residual amounts of pesticides and xenobiotics. Biol Bull Rev 4:300–310

Germaine KJ, Liu X, Cabellos GG, Hogan JP, Ryan D, Dowling DN (2006) Bacterial entophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid. FEMS Microbiol Ecol 57:302–310

Glick BR, Stearns JC (2011) Making phytoremediation work better: maximizing a plant's growth potential in the midst of adversity. Int J Phytoremediation 13:4–16

Goes KCGP, Fisher MLC, Cattelan AJ, Nogueira MA, Carvalho CGPC, Oliveira ALM (2012) Biochemical and molecular characterization of high population density bacteria isolated from sunflower. J Microbiol Biotechnol 22:437–447

Guijarro KH, Aparicio V, De Gerónimo E, Castellote M, Figuerola EL, Costa JL, Erijman L (2018) Soil microbial communities and glyphosate decay in soils with different herbicide application history. Sci Total Environ 634:974–982

Hardoim PH, van Overbeek LS, van Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16:463–471

Hungria M, Mendes IC, Nakatani AS, Reis-Junior FB, Morais JZ, Oliveira MCN, Fernandes MF (2014) Effects of the glyphosate-resistance gene and herbicides on soybean: field trials monitoring biological nitrogen fixation and yield. Field Crops Res 158:43–54

Jaworski EG (1972) Mode of action of *N*-phosphonomethylglycine: inhibition of aromatic amino acid biosynthesis. J Agr Food Chem 20:1195–1198

Kado CI, Heskett MG (1970) Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. Phytopatology 60:969–976

Kang SH, Hyun-Soo C, Hoon C, Choong-Min R, Jihyun FK, Seung-Hwan P (2007) Two bacterial entophytes eliciting both plant growth promotion and plant defense on pepper (*Capsicum annuum* L.). J Microbiol Biotechnol 17:96–103

Khalifa AYZ, Alsyeeh AM, Almalki AM, Saleh FA (2016) Characterization of the plant growth promotiong bacterium, *Enterobacter cloacae* MSR1, isolated from roots of non-nodulating *Medicago sativa*. Saudi J Biol Sci 23:79–86

Kuklinsky-Sobral J, Araújo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL (2004) Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol 6:1244–1251

Kuklinsky-Sobral J, Araújo WL, Mendes R, Pizzirani-Kleiner AA, Azevedo JL (2005) Isolation and characterization of endophytic bacteria from soybean (*Glycine max*) grown in soil treated with glyphosate herbicide. Plant Soil 273:91–99

Kumar D, Kumar L, Nagar S, Raina C, Parshad R, Gupta VK (2012) Screening, isolation and production of lipase/esterase producing *Bacillus* sp. strain DVL2 and its potential evaluation in esterification and resolution reactions. Arch Appl Sci Res 4:1763–1770

Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, Chichester, pp 115–175

Liu Y, Feng Y, Cheng D, Xue J, Wakelin S, Li Z (2018) Dynamics of bacterial composition and the fate of antibiotic resistance genes and mobile genetic elements during the co-composting with gentamicin fermentation residue and lovastatin fermentation residue. Bioresour Technol 261:249–256

Loper JE, Schroth MN (1986) Influence of bacterial sources of indole-2-acetic acid on root elongation of sugar beet. Phytopathology 76:386–389

Lottmann J, Berg G (2001) Phenotypic and genotypic characterization of antagonistic bacteria associated with roots of transgenic and non-transgenic potato plants. Microbiol Res 156:75–82

Markova YuA, Romanenko AS, Dukhanina AV (2005) Isolation of bacteria of the family *Enterobacteriaceae* from plant tissue. Microbiology 74:663–666

Miliute I, Buzaite O, Baniulis D, Stanys V (2015) Bacterial endophytes in agricultural crops and their role in stress tolerance: a review. Zemdirbyste-Agriculture 102:465–478

Murray TS, Kazmierczak BI (2006) *FlhF* is required for swimming and swarming in *Pseudomonas aeruginosa*. J Bacteriol 188:6995–7004

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170:265–270

Oliveira RS, Dvoranen EC, Constantin J, Cavalieri SD, Franchini LHM, Rios FA, Blainski E (2008) Glyphosate influence on growth and nodulation of glyphosate resistant soybean cultivars. Planta Daninha 26:831–843

Padgette SR, Kolacz KH, Delannay X, Re DB, LaVallee BJ, Tinius CN, Rhodes WK, Otero YI, Barry GF, Eichholtz DA, Peschke VM, Nida DL, Taylor NB, Kishore GM (1995) Development, identification, and characterization of a glyphosate-tolerant soybean line. Crop Sci 35:1451–1461

Pline-Srnic W (2005) Technical performance of some commercial glyphosate-resistant crops. Pest Manag Sci 61:225–234

Reddy KN, Duke SO (2014) Soybean mineral composition and glyphosate use. In: Preedy VR (ed) Processing and impact on active food components in food. Elsevier Inc, London, pp 369–376

Rösch C, Bothe H (2005) Improved assessment of denitrifying, N2-fixing, and total-community bacteria by terminal restriction fragment length polymorphism analysis using multiple restriction enzymes. Appl Environ Microbiol 71:2026–2035

Sagova-Mareckova M, Daniel O, Omelka M, Kristufek V, Divis J, Kopecky J (2015) Determination of factors associated with natural soil suppressivity to potato common scab. PLoS One 10:1–13

Schulz B, Boyle C (2006) What are endophytes? In: Schulz B, Boyle CJ, Sieber TN (eds) Microbial root endophytes, vol 9. Springer, Berlin, pp 1–13

Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160:46–56

Siciliano SD, Germida JJ (1999) Taxonomic diversity of bacteria associated with the roots of field-grown transgenic *Brassica napus* cv. Quest, compared to the non-transgenic *B. napus* cv. *Excel* and *B. rapa* cv. Parkland. FEMS Microbiol Ecol 29:263–272

Siciliano SD, Fortin N, Mihoc A, Wisse G, Labelle S, Beaumier D, Ouellette D, Roy R, Whyte LG, Banks MK, Schwab P, Lee K, Greer CH (2001) Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. Appl Environ Microbiol 67:2469–2475

Sikorski J, Pukall R, Stackebrandt E (2008) Carbon source utilization patterns of Bacillus simplex ecotypes do not reflect their adaptation to ecologically divergent slopes in 'Evolution Canyon', Israel. FEMS Microbiol Ecol 66:38–44

Sturz AV, Christie BR, Nowak J (2000) Bacterial endophytes: potential role in developing sustainable systems of crop production. Crit Rev Plant Sci 19:1–30

Torsvik V, Salte K, Sorheim R, Goksoyr J (1990) Comparison of phenotypic diversity and DNA heterogeneity in a population of soil bacteria. Appl Environ Microbiol 56:776–781

Vela AI, Vázquez J, Gibello A, Blanco MM, Moreno MA, LiéBana P, Albendea C, Alcala B, Mendez A, Dominguez L, Fernández-Garayzábal JF (2000) Phenotypic and genetic characterization

of *Lactococcus garvieae* isolated in Spain from *Lactococcosis* outbreaks and comparison with isolates of other countries and sources. J Clin Microbiol 38:3791–3795

Vigani G, Rolli E, Marasco R, Dell'Orto M, Michoud G, Soussi A, Raddadi N, Borin S, Sorlini C, Zocchi G, Daffonchio D (2018) Root bacterial endophytes confer drought resistance and enhance expression and activity of a vacuolar H + -pumping pyrophosphatase in pepper plants. Environ Microbiol. https://doi.org/10.1111/1462-2920.14272

Wen CM, Tseng CS, Cheng CY, Li YK (2002) Purification, characterization and cloning of a chitinase from *Bacillus* sp. NCTU2. Biotechnol Appl Biochem 35:213–219

Xing D, Ren N, Wang A, Li Q, Feng Y, Ma F (2008) Continuous hydrogen production of auto-aggregative *Ethanoligenens harbinense* YUAN-3 under non-sterile condition. Int J Hydrogen Energy 33:1489–1495

Zablotowicz RM, Reddy KN (2004) Impact of glyphosate on the *Bradyrhizobium japonicum* symbiosis with glyphosate-resistant transgenic soybean: a minireview. J Environ Qual 33:825–831

Zhang J, Tang M, Viikari L (2012) Xylans inhibit enzymatic hydrolysis of lignocellulosic materials by cellulases. Bioresour Technol 121:8–12

Zinniel DK, Lambrecht P, Harris NB, Feng Z, Kuczmarski D, Higley P, Ishimaru CA, Arunakumari A, Barletta RG, Vidaver AK (2002) Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Appl Environ Microbiol 68:2198–2208

Zlosnik JE, Hird TJ, Frankel MC, Moreira LM, Henry DA, Speert DP (2008) Differential mucoid exopolysaccharide production by members of the *Burkholderia cepacia* complex. J Clin Microbiol 46:1470–1473