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Isolation and characterization of endophytic bacteria from soybean (*Glycine max*) grown in soil treated with glyphosate herbicide

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Abstract

Endophytic bacteria are ubiquitous in most plant species influencing the host fitness by disease suppression, contaminant degradation, and plant growth promotion. This endophytic bacterial community may be affected by crop management such as the use of chemical compounds. For instance, application of glyphosate herbicide is common mainly due to the use of glyphosate-resistant transgenic plants. In this case, the bacterial equilibrium in plant–endophyte interaction could be shifted because some microbial groups are able to use glyphosate as a source of energy and nutrients, whereas this herbicide may be toxic to other groups. Therefore, the aim of this work was to study cultivable and noncultivable endophytic bacterial populations from soybean (*Glycine max*) plants cultivated in soil with and without glyphosate application (pre-planting). The cultivable endophytic bacterial community recovered from soybean leaves, stems, and roots included *Acinetobacter calcoaceticus, A. junii, Burkholderia* sp., *B. gladioli, Enterobacter sakazaki, Klebsiella pneumoniae, Pseudomonas oryzihabitans, P. straminea, Ralstonia pickettii,* and *Sphingomonas* sp. The DGGE (Denaturing Gradient Gel Electrophoresis) analysis from soybean roots revealed some groups not observed by isolation that were exclusive for plants cultivated in soil with pre-planting glyphosate application, such as *Herbaspirillum* sp., and other groups in plants that were cultivated in soil without glyphosate, such as *Xanthomonas* sp. and *Stenotrophomonas maltophilia.* Furthermore, only two bacterial species were recovered from soybean plants by glyphosate enrichment isolation. They were *Pseudomonas oryzihabitans* and *Burkholderia gladioli* which showed different sensibility profiles to the glyphosate. These results suggest that the application at pre-planting of the glyphosate herbicide may interfere with the endophytic bacterial community's equilibrium. This community is composed of different species with the capacity for plant growth promotion and biological control that may be affected. However, the evaluation of this treatment in plant production should be carried out by long-term experiments in field conditions.

Introduction

Plants may be considered complex microecosystems where different niches are exploited by a wide variety of bacteria. Such niches include not only the external surfaces of plants, but also the internal tissues which endophytic bacteria inhabit without apparent harm to the host or external structures (Azevedo et al., 2000). In most plant species, endophytic bacteria are ubiquitous, colonizing locally as well as systemically, and influ-

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encing plant health by suppression of disease, degradation of contaminants, and promotion of plant growth (Sturz et al., 2000). On the other hand, the plant-associated habitat is a dynamic environment in which many factors, such plant tissues, soil type, and interaction with other microorganisms, may affect the structure and species composition of the bacterial communities that colonize plant tissues (Araújo et al., 2001; 2002; Dalmastri et al., 1999; Lacava et al., 2004; Mocali et al., 2003).

The intensive use of herbicides has recently raised increasing concern due to their role in large-scale environmental pollution. Glyphosate [(*N*-phosphonomethyl) glycine] is a widely used herbicide known for its effective control of competing vegetation, rapid inactivation in soil and low mammalian toxicity (Busse et al., 2001). Although various microorganisms may degrade glyphosate, there have been studies that showed harmful effects of glyphosate to some microorganisms (Araújo et al., 2003; Levesque and Rahe, 1992; Santos and Flores, 1995). It is possible that glyphosate could induce a shift in the endophytic bacterial community of some plants. This aspect has not previously been explored.

Therefore, the aim of this work was to study the interaction between endophytic bacterial community from soybean and soil treatment (glyphosate herbicide application at pre-planting) by using cultivation based plating techniques and cultivation-independent methods involving PCR-generated 16S rRNA gene (rDNA) fragments and denaturing gradient gel electrophoresis (DGGE).

Materials and methods

Experimental field design

The soybean (*Glycine max* [L.] Merrill) cultivars Foscarin and Cristalina were cultivated in eutric nitosols type soil (pH 5.4 (water), 2.7 mmol kg^{-1} potassium, and 21.1 mg kg^{-1} total phosphorus) from fields with and without a reported history of glyphosate pre-planting application (1.44 kg ha^{-1}, Agrisato 480 CS, Alkagro). The cultivation practices were: irrigation and fertilization with phosphorus and potassium. Two plots (2 × 6 m) which were treated using a completely randomized block design of ten lines with fifteen plants per line were located at Escola Superior de Agricultura 'Luiz de Queiroz', University of São Paulo, Piracicaba, SP, Brazil (22°42′ S and 47°38′ W). Twelve plants from each treatment were collected after 35, 55 and 75 days from planting and were analyzed.

Total isolation and glyphosate enrichment isolation of cultivable endophytic bacteria

Soybean plants were removed from the soil with a trowel, placed in a plastic bag and immediately transported to the laboratory where they were washed in running tap water to remove soil. Leaves, stems and roots were used. Total endophytic bacteria were isolated after removing epiphytes by surface disinfection using serial washing in 70% ethanol for 1 min, sodium hypochlorite solution (2% available Cl^-) for 3 min, 70% ethanol for 30 s and two rinses in sterilized distilled water. The disinfection process was checked by plating aliquots of the sterile distilled water used in the final rinse onto 10% trypticase soy agar (TSA) supplemented by 50 μg mL^{-1} of the fungicide Imazalil (Magnate 500 CE, Agricur) and incubating the plates at 28 °C for 2–15 days. After surface disinfection, the leaf, stem or root tissues were cut and triturated in a 50 mL flask containing 10 mL of sterile phosphate buffered saline (PBS, containing [g L^{-1}] Na_2HPO_4, 1.44; KH_2PO_4, 0.24; KCl, 0.20; NaCl 8.00; pH 7.4), incubated at 28 °C with agitation (150 rpm) for 1 h. Following that, 100 μL aliquots from appropriate dilutions (10^{-1}, 10^{-2}, and 10^{-3}) were plated onto 10% TSA supplemented with 50 μg ml^{-1} of Imazalil fungicide and incubated at 28 °C for 2–15 days. After incubation, colonies were picked out, inoculated on 10% TSA slants, incubated at 28 °C for 2 days and stored at 4 °C. These isolated colonies were also cultivated on 10% TSA liquid, and incubated at 28 °C for 18 h. Each culture was suspended in 20% glycerol solution and stored at −70 °C.

Glyphosate enrichment isolation was accomplished by inoculating 1 mL of triturated tissue suspension in 10 mL of Dworkin–Foster (DF) liquid medium (Dworkin and Foster, 1958) supplemented with 3.38 g L^{-1} glyphosate (GY) (Agrisato 480 CS, Agricur) and incubated at 28 °C, under agitation (150 rpm) for 10 days. After incubation, 10% of the culture was subcultured

twice as described above. Appropriated dilutions were plated onto DF agar (medium containing 16 g L^{-1} of agar) and the plates incubated at 28 °C for 20 days. The colonies were then picked out, inoculated on DF agar slants, incubated at 28 °C for 2 days and stored at 4 °C. These isolated colonies were also cultivated on DF liquid, incubated at 28 °C for 18 h. Each culture was suspended in 20% glycerol solution and stored at −70 °C.

Extraction of total DNA from plant samples, PCR-DGGE analysis, and sequencing

After superficial disinfection of the roots, during the process of endophytic bacteria isolation, 2 mL of the triturated suspension were transferred to a new tube and centrifuged for 5 min at 12,000 × *g*, and the resulting pellet was dissolved in 500 μL of TE buffer (Sambrook et al., 1989). Total DNA was extracted as described by Araújo et al. (2002) and visualized by electrophoresis on a 0.8% (wt/vol) agarose gel (Sambrook et al., 1989).

The PCR mixture was made in a final volume of 50 μL containing: 1 μL (0.5 – 10.0 ng) of total DNA, 0.2 μ*M* of the primers U968GC (5′-CG CCCGGGGCGCGCCCCGGGCGGGGCGGG GCACGGGGGGAACGCGAAGACCTTAC-3′) and 1378R (5′-CGGTGTGTACAAGGCCCGG GAACG-3′), 200 μ*M* of each dNTP, 3.75 m*M* $MgCl_2$ and 0.1 U of *Taq* DNA polymerase (Invitrogen, Life Technologies) in 20 m*M* pH 8.4 Tris–HCl and 50 m*M* KCl. A negative control (PCR mixture without DNA) was included in all PCR experiments. The amplification protocol was accomplished according to Araújo et al. (2002). Five microliters of the PCR product were analyzed by electrophoresis in a 1.4% (wt/vol) agarose gel with 1 × TAE buffer (Sambrook et al., 1989) and stored at −20 °C for DGGE analysis. DGGE was performed as described previously (Muyzer et al., 1993) with the DGGE-1001 apparatus (C.B.S., Scientific Company, Inc., Del Mar, CA, USA). PCR samples were loaded onto 6% (wt/vol) polyacrylamide gels in 0.5 × TAE buffer. The polyacrylamide gels were made with denaturing gradients ranging from 45 to 80% (where the 100% denaturant contained 7 *M* urea and 80% formamide). The gels were run for 15 h at 100 V and 60 °C, after which the gels were soaked for 1 h in SYBR Green nucleic acid stain (1:10,000 dilution; Gold Nucleic Acid Gel Stain; Molecular Probes, Eugene, Oregon, USA) and immediately photographed under UV light.

Prominent bands were excised from the gels, reamplified, and subjected to DGGE as previously described. The new PCR products were purified with the GFX PCR DNA and gel band purification kit (Amersham Biosciences) to remove the unused deoxynucleoside triphosphates, and sequenced using the 1378R primer. Analyses of the sequences were performed with the basic sequence alignment BLAST program run against the BLAST database (National Center for Biotechnology Information website [http://www.ncbi.nlm.nih.gov]).

Genomic DNA extraction and RAPD (Random Amplified Polymorphic DNA) analysis

Genomic bacterial DNA was extracted from each isolate as described by Araújo et al. (2002) and RAPD analysis was carried out according to Araújo et al. (2001) using the primers AX17 (5′-TGGGCTCTGG-3′), AX19 (5′-CCCTGTCG CA-3′), C02 (5′-GTGAGGCGTC-3′), and CO8 (5′-TGGACCGGTG-3′) (Invitrogen).

Glyphosate sensibility test

Preliminary tests were accomplished with some isolates, but the isolates HG13 (*Burkholderia gladioli*) and HG37 (*Pseudomonas oryzihabitans*) were analyzed in detail. These isolates were grown separately in 50 mL flasks containing 10 mL of DF liquid medium supplemented with 3.38 g L^{-2} glyphosate and 0, 0.1, 1.0, and 10 g L^{-1} glucose. Growth was evaluated by measuring the optical density (OD) at 600 nm after 0, 24, 48, and 76 h incubation at 28 °C under agitation (150 rpm). A control was carried out for each isolate under the same conditions except that the medium contained no glyphosate. All experiments were accomplished in triplicate.

Identification of the isolates

Species identification was made by 16S rDNA sequencing and the fatty-acid methyl ester

(FAME) technique with whole-cell fatty acids derivatized to methyl esters analyzed by gas chromatography by the MIDI system (Microbial Identification System, Inc., Delaware, USA). Isolates that could not be identified by FAME analysis were additionally tested by Biolog (Biolog Inc., Hayward, USA) or with the analytical profile indexes API, AP 20E, and AP 50CHE (bioMérieux S.A., Marcy l'Etoile, France).

Data analysis

Analysis of the data was carried out using the SAS software package (Copyright (c) 1989–1996 by SAS Institute, Cary, NC, USA) with a completely randomized analysis of variances ($P < 0.05$). Bacterial counts were transformed using $\log_{10}$ of $X + 1$ before analysis of variance. The Tukey-test was used for comparison of the means (Steel and Torrie, 1980).

Dendrograms were constructed based on RAPD data and on the Jaccard coefficient and the unweighted pair group method with averages (UPGMA) cluster analysis using the NTSYS-PC program (Applied Biostatistics, Inc., USA) and a consensus tree was obtained using the Winboot program (Yap and Nelson, 1996) with 1000 replicate bootstrapping.

Results

Identification of cultivable endophytic bacteria by total and enrichment isolation

The interaction between endophytic bacteria and soybean plants was assessed in leaves, stems, and roots of soybean plants cultivated in soil with and without glyphosate herbicide treatment at pre-planting. The endophytic bacterial community recovered on 10% TSA medium included *Acinetobacter calcoaceticus, A. junii*, *Agrobacterium* sp., *Enterobacter sakazakii*, *Sphingomonas* sp. from plants that were cultivated in soil with glyphosate, *Ralstonia pickettii* from plants that were cultivated in soil without glyphosate and *Burkholderia* sp., *Erwinia* sp., *Klebsiella pneumoniae, Pantoea* sp., *Pseudomonas* sp., *P. oryzihabitans, P. straminea* from plants that were cultivated in both soils. The numbers of total cultivable endophytic bacteria were not significantly different among the soil treatments or among the cultivars, ranging from 10^2 to 10^4 CFU g^{-1} (fresh tissue weight basis), but was significant between leaves of plants cultivated in soil with and without glyphosate (Figure 1). On the other hand, the endophytic bacterial species recovered by enrichment isolation with DFGI medium included *Pseudomonas oryzihabitans* and *Burkholderia gladioli* (only from plants that were cultivated in soil without glyphosate).

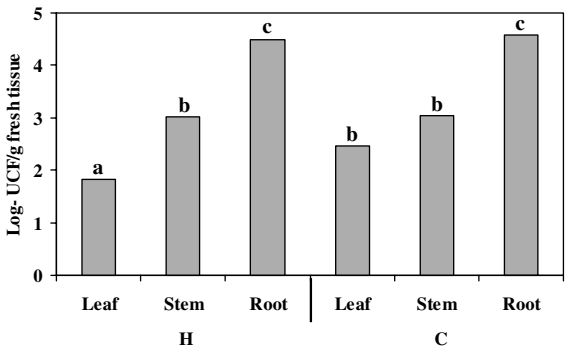

Figure 1. Total population density of endophytic bacteria from soybean leaves, stems and roots. Means with the different letters are significantly different by the Tukey test ($P < 0.05$). H (herbicide treatment): plants cultivated in soil with glyphosate herbicide application at pre-planting and C (control): plants cultivated in soil without glyphosate herbicide application at pre-planting.

DGGE analysis of the endophytic bacterial community from soybean roots

The DGGE patterns of the PCR products (amplified with universal primers for the 968-to-1387 16S rDNA fragment) from DNA extracted from soybean roots revealed that the communities were composed of groups of species that were not represented in all plant samples. Some groups were exclusive of plants that were cultivated in soil with glyphosate herbicide (pre-planting), such as *Herbaspirillum* sp., and other groups in plants that were cultivated in soil without glyphosate, such as *Xanthomonas* sp. and *Stenotrophomonas maltophilia* (Figure 2). Furthermore, *Pseudomonas* sp., *Erwinia* sp. and *Pantoea* sp. were observed by DGGE and plating analysis independently of the soil in which the soybean was cultivated (Table 1).

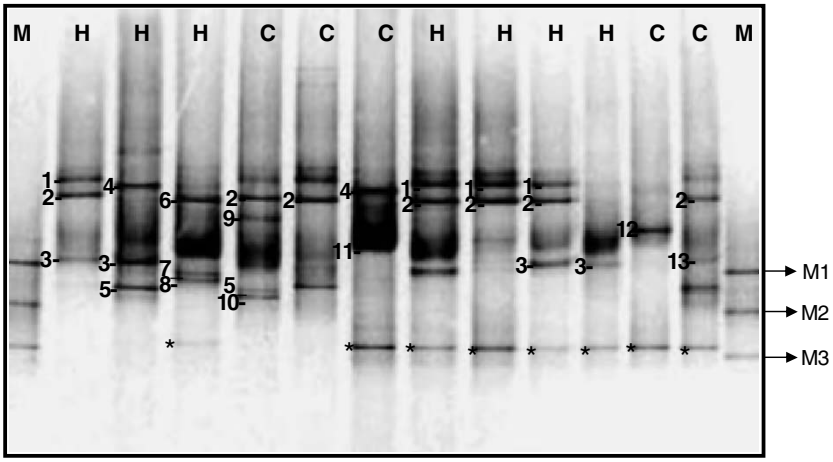

Figure 2. Fingerprinting of the endophytic bacterial community from soybean roots by DGGE separation and analysis of 16S rDNA fragments amplified by the bacterial primer set 968F (primer F968_GC without GC clamp) plus 1387R. Lanes: M, DGGE profile of *Curtobacterium flaccufaciens* (M1), *Methylobacterium mesophilicum* (M2), and *M. extorquens* (M3), C (control), plants cultivated in soil without glyphosate herbicide application at pre-planting, H (herbicide treatment), plants cultivated in soil with glyphosate application at pre-planting. Bands 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13 were excised and sequenced, and the sequence was shown to have between 80 and 100% sequence similarity to *Agrobacterium* sp. (90%), *Agrobacterium* sp. (80%), *Pseudomonas* sp. (82%), *Pantoea* sp. (99%), *Pseudomonas putida* (99%), *Herbaspirillum* sp. (98%), *Erwinia* sp. (97%), *Erwinia* sp. (97%), *Xanthomonas* sp. (93%), *Pseudomonas putida* (99%), *Pantoea* sp. (99%), *Stenotrophomonas malthophilia* (100%), and *Pantoea* sp. (89%), respectively. * Plastid DNA.

Table 1. Endophytic bacterial species obtained from soybean by total and enrichment isolation and DGGE analysis

Species observed by total and enrichment isolation[1]	Species observed by DGGE analysis[2]	Species observed by isolation and DGGE methods[3]
Acinetobacter calcoaceticus	*Herbaspirillum* sp.	*Agrobacterium* sp.
Acinetobacter junii	*Pseudomonas putida*	*Erwinia* sp.
Burkholderia gladioli	*Stenotrophomonas malthophilia*	*Pantoea* sp.
Burkholderia sp.	*Xanthomonas* sp.	*Pseudomonas* sp.
Enterobacter sakazaki		
Klebsiella pneumoniae		
Pseudomonas oryzihabitans		
Pseudomonas straminea		
Ralstonia pickettii		
Sphingomonas sp.		

[1]The species identified only by isolation in TSA and DF supplemented with glyphosate medium.
[2]The species identified only by DGGE analysis.
[3]The species identified by both techniques, isolation in (TSA and DF) culture medium and DGGE analysis.

RAPD analysis

Genomic DNA from 10 *B. gladioli* isolates from leaves, stems and roots of soybean plants cultivated in soil without glyphosate herbicide treatment at pre-planting and 12 *P. oryzihabitans* isolates from leaves, stems and roots of soybean plants cultivated in soil with and without glyphosate were amplified by the RAPD technique with four 10-mer primers, with the reproducibility of the results being verified by independent experiments. Amplification of DNAs from 10 *B. gladioli* and 12 *P. oryzihabitans* isolates recovered from enrichment isolation gave rise to 50 and 56 bands, respectively. The relationships between the different *P. oryzihabitans* isolates as well as those

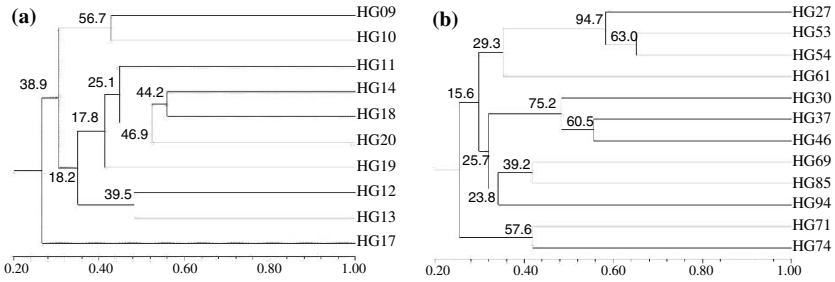

Figure 3. UPGMA Dendrogram, based on the coefficient of Jaccard, illustrating the genetic relationships among isolates of *Burkholderia gladioli* (a) and *Pseudomonas oryzihabitans* (b) isolated by the glyphosate enrichment method. The numbers in the dendrogram indicates the percentage of times the group to the right of the node occurred in the bootstrap trees.

between the *B. gladioli* isolates can be seen in the dendrograms based on the Jaccard coefficient (Figure 3). These results show that there was no correlation between the groups obtained by RAPD markers and the soil treatment or plant tissue from which the endophytic bacteria were isolated.

Glyphosate sensibility analysis

One isolate of *Burkholderia gladioli* (HG13) and one of *Pseudomonas oryzihabitans* (HG37) that were recovered by enrichment isolation were chosen to evaluate glyphosate sensibility. These isolates presented a different rate of growth after 76 h incubation (HG13-O.D.$_{600}$ 0.300 and HG37-O.D.$_{600}$ 0.090). Growth curves show that *B. gladioli* and *P. oryzihabitans* have a different sensitivity to glyphosate. Glyphosate (20 m*M*) inhibited growth of *P. oryzihabitans,* in spite of having the glucose as another source of carbon (Figure 4). However, *B. gladioli* adapted to growth in a medium containing glyphosate after 24 h and produced the same growth as in a glyphosate-free medium (Figure 5). This result shows *B. gladioli* to have a profile of resistance to glyphosate and *P. oryzihabitans* of sensitivity.

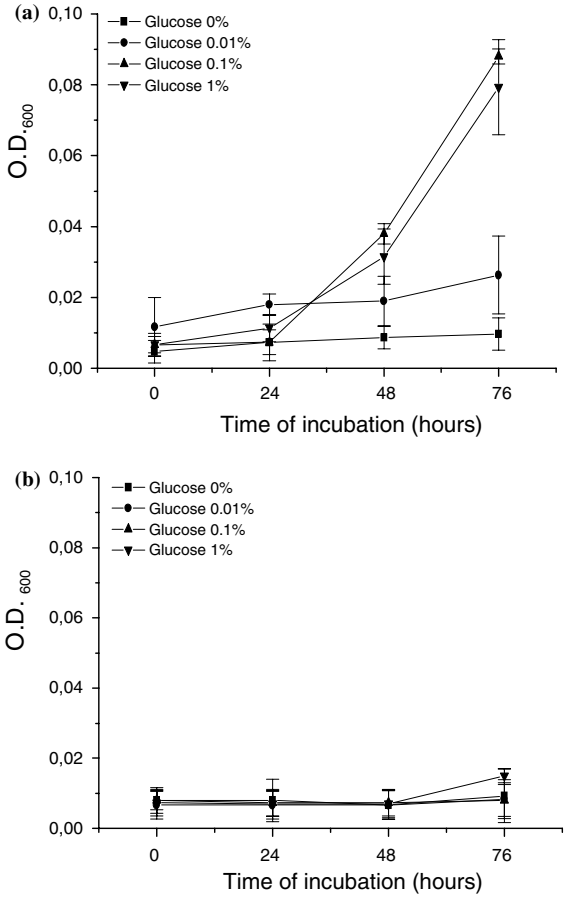

Figure 4. Growth of *Pseudomonas oryzihabitans* isolate HG37 in DF liquid medium containing different concentrations of glucose with (a) and without (b) glyphosate herbicide (3.38 gL^{-1}). Bars represent the standard errors of the mean.

Discussion

Glyphosate [(*N*-phosphonomethyl) glycine] is an effective non-selective herbicide that inhibits the enzyme 5-enolpyruvylshikimat-3-phosphate synthase (EPSPS) on the shikimic acid pathway, which is involved in the synthesis of aromatic amino acids (Penazola-Vazquez et al., 1995). The shikimic acid pathway is ubiquitous in microorganisms, but some microbial groups are able to use glyphosate as a source of energy and nutrients, whereas this herbicide may be toxic to other

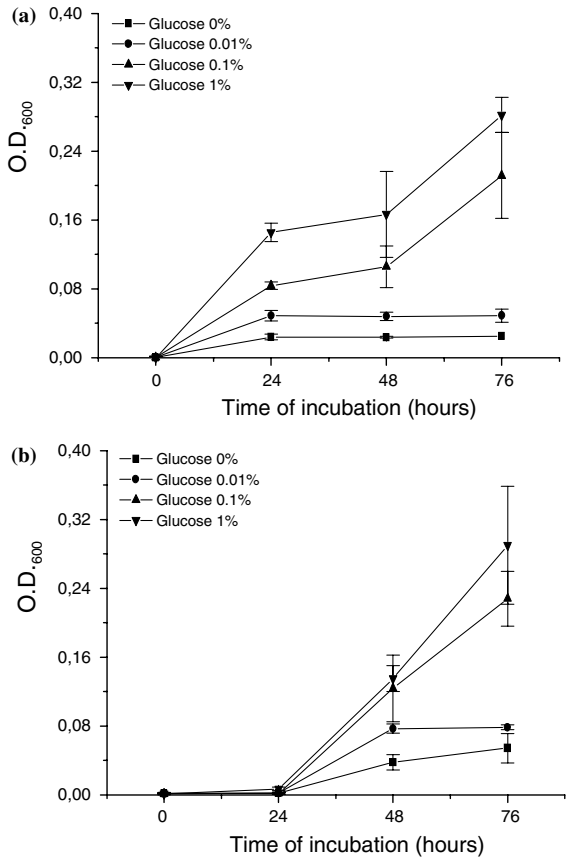

Figure 5. Growth of *Burkholderia gladioli* isolate HG13 in DF liquid medium containing different concentrations of glucose with (a) and without (b) glyphosate herbicide (3.38 gL^{-1}). Bars represent the standard errors of the mean.

groups (Busse et al., 2001; Santos and Flores, 1995; Dick and Quinn, 1995).

In this study, no influence of the pre-planting glyphosate application was observed on cultivable bacterial groups by using the total isolation method. However, the DGGE technique revealed that there were bacterial species exclusive of plants cultivated in soil with glyphosate, such as *Herbaspirillum* sp., or without glyphosate, such as *Xanthomonas* sp. and *Stenotrophomonas maltophilia*. We speculate that the glyphosate application at pre-planting could be selective for noncultivable bacterial groups of the endophytic community from soybean. The DGGE has been recently applied in studies on endophytic bacterial communities of potato (*Solanum tuberosum*) (Garbeva et al., 2001) and citrus plants (Araújo et al., 2002), suggesting its wide applicability for studies on endophyte–plant host interaction.

The study of endophytic bacteria is important, not only for understanding their ecological role in their interaction with plants but also for their possible biotechnological applications, such as bioremediation. From this point of view, an interesting interaction between the endophytic bacterial community and glyphosate herbicide was observed during enrichment isolation. Only two bacterial species were recovered from the culture medium supplemented by glyphosate, *Pseudomonas oryzihabitans* and *Burkholderia gladioli*. The bacterium *P. oryzihabitans* was also recovered from total isolation and presented sensibility to glyphosate. This species has been isolated from different samples, such as soil, water, zones of rice cultivation (Dussart et al., 2003), and moreover, soybean seeds (Oehrle et al., 2000). Furthermore, Belimov et al. (2001) observed that *P. oryzihabitans* isolates from *Pisum sativum* rhizoplane presented the capacity to produce the enzyme ACC (aminociclopropano-1-carboxilato) deaminase, evolved with the precursor (ACC) immediate of ethylene; and Kuklinsky-Sobral et al. (2004) observed that endophytic isolates from soybean were able to produce auxin and to fix nitrogen *in vitro*. Therefore, *P. oryzihabitans* has potential for plant growth promotion. In relation to *B. gladioli* that was only recovered from enrichment isolation and resistant to glyphosate, Shimosaka et al. (2000) observed quitinase and quitosanase production by this species that may also be interesting for biological control. Chiou and Wu (2001) observed that *B. gladioli* strains presented antagonistic effects against *Botrytis elliptica* on three lily cultivars in greenhouse trials. Furthermore, other *Burkholderia* species have potential application in biological control and plant growth promotion (Chen et al., 1995; Heydari and Misaghi, 1998; Heydari et al., 1997; Huang and Wong, 1998; Trân Van et al., 2000).

A more complete comprehension of the interaction between herbicides and plant-associated bacterial communities is an important factor for a more effective crop management. The results from this study indicated that pre-planting application of glyphosate herbicide influenced endophytic bacterial communities in soybean plants. Increased application of glyphosate may change endophytic populations, such as latent pathogens and plant growth promoting bacteria, which

could result in changes in plant production. However, the long-term effect of the application of this herbicide should be studied in field experiments to provide better knowledge.

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References

Araújo A S F, Monteiro R T R and Abarkeli R B 2003 Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere 52, 799–804.

Araújo W L, Maccheroni Jr W, Aguilar-Vildoso C I, Barroso P A V, Saridakis H O and Azevedo J L 2001 Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. Can. J. Microbiol. 47, 229–236.

Araújo W L, Marcon J, Maccheroni Jr W, van Elsas J D, van Vuurde J W L and Azevedo J L 2002 Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. Appl. Environ. Microb. 68, 4906–4914.

Azevedo J L, Maccheroni Jr W, Pereira J O and Araújo W L 2000 Endophytic microorganism: A review on insect control and recent advances on tropical plants. Electron. J. Biotechno. [on line]. Vol. 3, no. 1. Available from http://www.ejbiotechnology.info/content/vol3/issue1/full/4/index.html. ISSN 0717–3458.

Belimov A A, Safronova V I, Sergeyeva T A, Egorova T N, Matveyeva V A, Tsyganov V E, Borisov A Y, Tikhonovich I A, Kluge C, Preisfeld A, Dietz K J and Stepanok V V 2001 Characterization of plant growth promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. Can. J. Microbiol. 47, 642–652.

Busse M D, Ratcliff A W, Shestak C J and Powers R F 2001 Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biol. Biochem. 33, 1777–1789.

Chen C, Bauske E M, Musson G, Rodriguezkabana R and Kloepper J W 1995 Biological control of *Fusarium* wilt on cotton by use of endophytic bacteria. Biol. Control. 5, 83–91.

Chiou A L and Wu W S 2001 Isolation, identification and evaluation of bacterial antagonists against *Botrytis elliptica* on lyli. J. Phytopathol. 149, 319–324.

Dalmastri C, Chiarini L, Cantale C, Bevivino A and Tabacchiono S 1999 Soil type and maize cultivar affect the genetic diversity of maize root-associated *Burkholderia cepacia* populations. Microbial Ecol. 38, 273–284.

Dick R E and Quinn J P 1995 Glifosato-degrading isolates from environmental samples: Occurrence and pathways of degradation. Appl. Microbiol. Biotechnol. 43, 545–550.

Dussart L, Dupont J P, Zimmerlin I, Lacroix M, Saiter J M, Junter G A and Jouenne T 2003 Occurrence of sessile *Pseudomonas oryzihabitans* from a karstied chalk aquifer. Water Res. 37, 1593–1600.

Dworkin M and Foster J W 1958 Experiments with some microorganisms which utilize ethane and hydrogen. J. Bacteriol. 75, 592–601.

Garbeva P, Van Overbeek L S, Van Vuurde J W L and Van Elsas J D 2001 Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. Microbial Ecol. 41, 369–383.

Heydari A and Misagui I J 1998 Biocontrol activity of *Burkholderia cepacia* against *Rhizoctonia solani* in herbicide-treated soils. Plant Soil 202, 109–116.

Heydari A, Misagui I J and Mccloskey W B 1997 Effects of three soil-applied herbicides on populations of plant disease suppressing bacteria in the cotton rhizosphere. Plant Soil 195, 75–81.

Huang Y and Wong P T W 1998 Effect of *Burkholderia* (*Pseudomonas*) *cepacia* and soil type on the control of crown rot in wheat. Plant Soil 203, 103–108.

Kuklinsky-Sobral J, Araújo W L, Mendes R, Geraldi I O, Pizzirani-Kleiner A A and Azevedo J L 2004 Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ. Microbiol. 6, 1244–1251.

Lacava P T, Araújo W L, Marcon J, Maccheroni Jr W, Azevedo J L 2004 Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacteria *Xylella fastidiosa,* causal agent of citrus-variegated chlorosis. Lett. Appl. Microbiol. 39, 55–59.

Levesque C A and Rahe J E 1992 Herbicide interactions with fungal root pathogens, with special reference to glyphosate. Annu. Rev. Phytopathol. 30, 579–602.

Mocali S, Bertelli E, Celli F D, Mengoni A, Sfalanga A, Vilani F, Caciotti A, Tegli S, Surico G and Fani R 2003 Fluctuation of bacteria isolated from elm tissues during different seasons and from different plant organs. Res. Microbiol. 154, 105–114.

Muyzer G, De Waal E C and Uitterlinden A 1993 Profiling of complex microbial populations using denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes encoding for 16S rRNA. Appl. Environ. Microbiol 59, 695–700.

Oehrle N W, Karr D B, Kremer R J and Emerich D W 2000 Enhanced attachment of *Bradyrhizobium japonicum* to soybean through reduced root colonization of internally seedborne microorganisms. Can. J. Microbiol. 46, 600–606.

Penazola-Vazquez A, Mena G L, Herrera-Estrella L and Bailey A M 1995 Cloning and sequencing of the genes involved in glyphosate utilization by *Pseudomonas pseudomallei*. Appl. Environ. Microbiol 61, 538–543.

Sambrook J, Fritsch E F and Maniatis T 1989 Molecular Cloning: A Laboratory Manual. 2nd edition Cold Spring Harbor Laboratory Press, New York.

Santos A and Flores M 1995 Effects of glyphosate on nitrogen fixation of free-living heterotrophic bacteria. Lett. Appl. Microbiol. 20, 349–35.

Steel R G D and Torrie J H 1980 Principles and Procedures of Statistics: A Biometrical Approach. McGraw-Hill, Toronto, Ontario, Canada.

Shimosaka M, Fukumori Y, Zhang X Y, He N J, Kodaria R, Okazaki M 2000 Molecular cloning and characterization of a chitosanase from the chitosanolytic bacterium *Burkholderia gladioli* strain CHB101. Appl. Microbiol. Biotechnol. 54, 354–360.

Sturz A V, Christie B R and Nowak J 2000 Bacterial endophytes: Potential role in developing sustainable systems of crop production. CRC C.R. Rev. Plant Sci. 19, 1–30.

Trân Van V, Berge O, Ngô Kê S, Balandreau J and Heulin T 2000 Repeated beneficial effects of rice inoculation with a strain of *Burkholderia vietnamiensis* on early and late yield components in low fertility sulphate acid soils of Vietnam. Plant Soil 218, 273–284.

Yap I and Nelson R J 1996 Winboot: A program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. IRRI Discussion Paper Series 14.

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