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Full Length Research

Isolation and selection of growth-promoting bacteria of the genus *Bacillus* and its effect on two varieties of lettuce (*Lactuca sativa* L.)

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The objective of this study was to assess rhizobacteria isolates of the genus *Bacillus* and check which are more promising for the production of seedlings of two varieties of lettuce (*Lactuca sativa* L.). Seeds of two varieties of lettuce (Babá de Verão Manteiga (smooth) and Monica SF 31 SF 31 (crisp) were inoculated in styrofoam trays where each cell received substrate with 5 ml of bacterial suspension containing *Bacillus* spp. adjusted A_{580} = 0.7, and taken to a greenhouse for 25 days as being variables: weight of dry matter of aerial part (WDMAP), root (WDMR), total (WDMT), leaf area (LA), length leaf (LL), length root (LR) and number of leaves (NL). For Babá de Verão Manteiga variety the isolated 8 highlighted variables WDMAP, WDMR and WDMT and increase index (II) 62.3% 38.5% 59.1% respectively while for what LA and LL and the isolate 7 showed better results with II of 73.3% and 28.4%. The variety Monica SF 31 the best results for WDMAP and LL were obtained with the strain 1 having II of 31.0% and 29.1% respectively, while for WDMR and WDMT, isolate 4 showed II 66.7% and 33.7% respectively. To variable LR the isolate 6 highlighted for both varieties.

Keywords: *Lactuca sativa* L., rhizobacteria, *Bacillus* spp., PGPR

INTRODUCTION

Lettuce (*Lactucca sativa*), a leafy vegetable most commonly consumed among Brazilians, is sensitive to the adverse conditions of temperature, humidity and rain, requiring special attention regarding the control of pests and diseases, polluting the environment, enhancing the product and can harm the health of consumers.

Plant growth is influenced by the interaction of these with the environment to the root system. This environment is represented by the soil or the planting substrate. The microflora interacts with the roots and plays an important role in the growth and survival of plants. The bacteria that promote plant growth represent an important segment of these microorganisms by promoting growth, increasing productivity and health of plants (Bettiol, 1995).

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Among the most studied groups of bacteria and of great potential for use in agriculture are plant growth promoting rhizobacteria (PGPR), representing a diverse subset of bacteria that colonize the roots. The study of these microorganisms comes with prominence in recent years. Due to great demand for technology 'clean', ie the viability of sustainable agriculture can be expected that in future, a greater percentage of these bacteria is used in food production.

The rhizobacteria are bacteria that inhabit the rhizosphere, ie the region that is influenced by the roots. These bacteria can be beneficial, for example, PGPR, neutral or pathogenic to plants. Among the key PGPR include: *Pseudomonas* fluorescent spp., *Bacillus* spp. *Azospirillum* spp. and *Azotobacter* spp (Gomes et al., 2003; Silveira et al., 2004; Coelho, 2006).

The PGPR can increase plant growth by promoting the mineralization of nutrients, the mineral phosphate solubilization, production of growth hormones like auxin

Table 1. Characterization of isolates of *Bacillus* spp

Isolates	Origin
1, 2, 3	Lettuce roots gathered in the garden of the School Agrotécnica Federal de Satuba in the municipality of Satuba - AL.
4,5,6	Lettuce roots collected in growing organic business in the city of Arapiraca - AL.
7, 8	Collection of plant pathology laboratory UFAL.

and gibberellin (Asghar et al., 2002; Joo et al., 2004). Moreover, PGPR are important biological control agents because they can eliminate pathogens from the rhizosphere, the production of beta-1, 3-glucanase, chitinases, antibiotics, hydrogen cyanide, and siderophores, which are composed of low molecular weight iron chelators produced by most bacteria under limiting conditions of this element. (Owen and Zdor, 2001; Pidello, 2003; Coelho, 2006.) Can also act as bioremediation of contaminated areas by degrade xenobiotics.

The inoculant production of low cost rhizobacteria promote plant growth (PGPR) is an alternative to reduce the use of pesticides and chemicals, which, if used wrongly, can reach the water table and contaminate water resources. Furthermore, the use of such inoculants can increase agricultural production, making the product more competitive and differentiated as well as reduce the costs to the producer, the less need for inputs.

Within this context, the objective of this study was to isolate rhizobacteria of the genus *Bacillus* and check which rhizobacteria isolates were more promising in the production of lettuce.

MATERIALS AND METHODS

The experiments were conducted at the Federal University of Alagoas (UFAL) in the laboratory of Agricultural Microbiology and Experimental Field in the city Rio Largo situated in the geographic coordinates 9°27'57,3" S and 35°49'57,4" W.

Roots and stems of healthy lettuce were collected in commercial crops of vegetables. To make the insulation used the method of the World Health Organization (World Health Organization, 1985). One gram of soil and macerated roots of each sample were placed in sterile tubes with 10 ml of saline and agitated in vortex for 2 minutes. Then 1.5 ml were transferred to sterile tubes and subjected to heating for 12 minutes at 80° C and 5 minutes on ice.

The samples were then diluted 1000 times in sterile saline solution, seeded in Petri dishes containing nutrient agar, spread with the aid of handle Drigalski and incubated at 30° C. After 48 hours logged to an examination of the colonies and isolated to those typical of the bacilli with absence of pigmentation, wavy edges, opaque and positive reaction for amylolyse were isolated.

The seedlings were grown in sterilized by autoclaving substrate at 121°C for an hour, contained in tray polystyrene, seeds of cultivars Babá de Verão Manteiga (smooth) and Monica SF 31 (crisp) were bacterized, depositing aliquots of 5ml of a tuned suspension with a colorimeter at 580 nm, control consisted of a solution of 0.1 M. of $MgSO_4$. After 25 days the following variables were analyzed: weight of dry matter of aerial part (WDMAP), root (WDMR), total (WDMT), leaf area (LA), length leaf (LL), length root (LR) and number of leaves (NL). It was used the formula II (%) = [(bacterized-control) / control] X 100, to verify the increase index (II).

Leaves and stems of lettuce plants were placed in an oven at 65° C until they had constant weight and weighed in the balance of precision WDMAP determined. Performing the same procedure was determined WDMR and the sum of two-and results WDMAP and WDMR was observed the WDMT.

LA was determined woodpecker "weighing method of discs" of Blackman and Wilson (1951), cited by Luchesi (1987). Is to perforate the sheet with a punch of known area, where this part is placed, along with the rest of the sheet that was perforated to dry in oven at 600° C until constant weight present to be weighed in the balance of precision. Is subsequently used the formula: LA = (known area of perforated sheet X part of the remaining leaf weight) / weight of the area known part of the perforated leaf.

LL was established by choosing the two largest leaves of each plant and with the aid of a ruler held measurements. The roots were washed carefully to avoid loss of roots, and with the aid of a ruler held the measurements for the LR.

The design was a randomized block design with four replications and experimental units consisted of 16 plants. Data were submitted to analysis of variance and test F (5% of probability) and averages compared by Scott Knott at 5% probability. The isolates are caracterized in of Table 1.

RESULTS

The eight isolates were obtained from roots of lettuce from the garden School Agrotecnica Federal Satuba, city of Satuba - AL (Figure 1) and commercial organic farming in the city of Arapiraca - AL (Figure 2) showed rod-shaped cells and clusters of cells in pairs or chains characteristic of bacteria of the genus *Bacillus.*

For data on weight of dry matter of aerial part (WDMAP) is the F test at 1% probability detected significant differences between treatments. There were significant differences at the level of 1% in the F test between the two varieties of lettuce (*Lactuca sativa* L.) among isolates of *Bacillus* spp. and the interaction (Variety x Bacteria).

The values ranged from 0.053 g WDMAP 0.086 g for variety Babá de Verão Manteiga and the mean equal to 0.073 g while the variety Monica SF 31 values ranged from 0.050 g to 0.076 g with an average 0.061.

Figure 3 presents the data WDMAP. Isolates of *Bacillus* spp. 1, 2, 5, 6, 7 and 8 did not differ statistically and

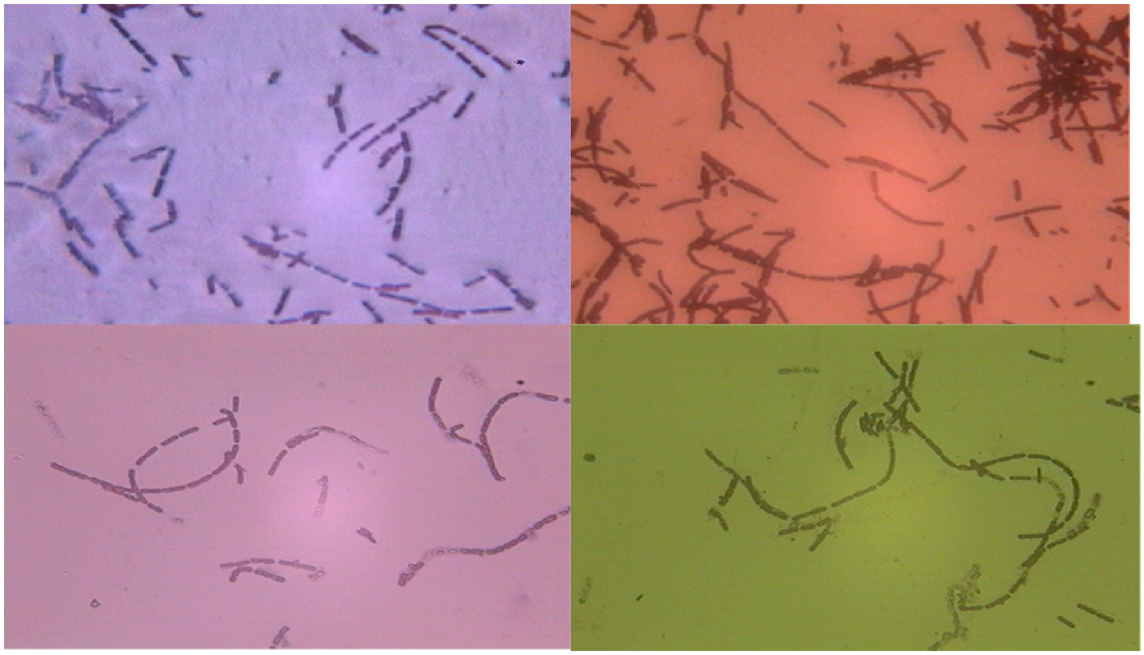

Figure 1. Isolates of *Bacillus* spp. obtained from the roots of lettuce Horta School Agrotécnica Federal Satuba.

Figure 2. Production of seedlings of two varieties of lettuce in a greenhouse unit of agricultural sciences academic center ((A) BABÁ SUMMER BUTTER (smooth) (B) MÔNICA SF 31 (crisp)).

provided the best results for a variety Babá de Verão Manteiga with an II averaged 47.2%, however, the 8 isolate presented the highest result, with II from 62.3% to WDMAP compared to control. The isolates 3 and 4 differed from isolates 1, 2, 5, 6, 7 and 8, WDMAP demonstrating the variety below for Babá de Verão Manteiga.

For variety Monica SF 31 (crisp) isolates of *Bacillu*s spp. 1, 3 and 4 did not differ statistically and the best results on average a II of 24.1% with isolate 1 showed the highest result, increasing 31.0% over the WDMAP the control.

Isolates 2, 5, 6, 7 and 8 differ from the isolated 1, 3 and 4, with lower results for the variety Monica SF 31.
Isolates 2, 5, 6 and 7 were lower than the control on average 8.6% for WDMAP.

The isolated 1, not statistically different in the two varieties of lettuce showing good results in an average II of 36.2% for both varieties in comparison with the controls, showing an interaction with this isolate varieties. It was also noted that the variety Babá de Verão Manteiga differed significantly from the variety Monica SF 31, being more than Monica SF 31, a difference of 19.7% II of WDMAP.

For WDMR significant differences were detected at 1% probability among the varieties of lettuce and among isolates of *Bacillus* spp. in relation to WDMR. For the interaction (Variety x bacteria) were detected at significance level of 5% by F test.

In figure 4 are the data WDMR, where isolates of

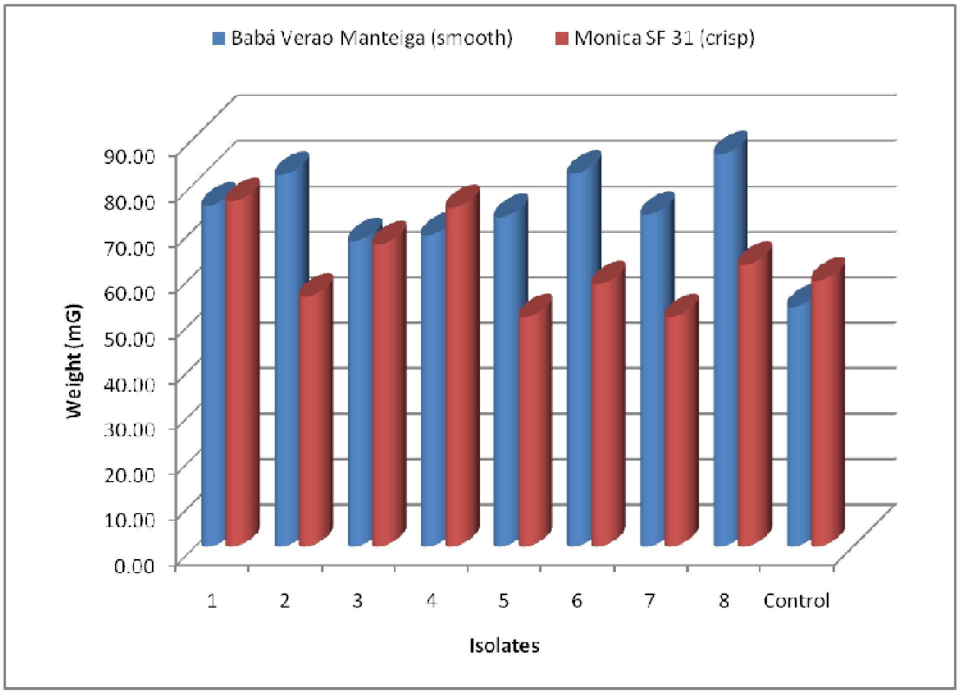

Figure 3. Weight of dry matter of aerial part of seedlings of two varieties of lettuce (*Lactuca sativa*) inoculated by different strains of rhizobacteria *Bacillus* spp

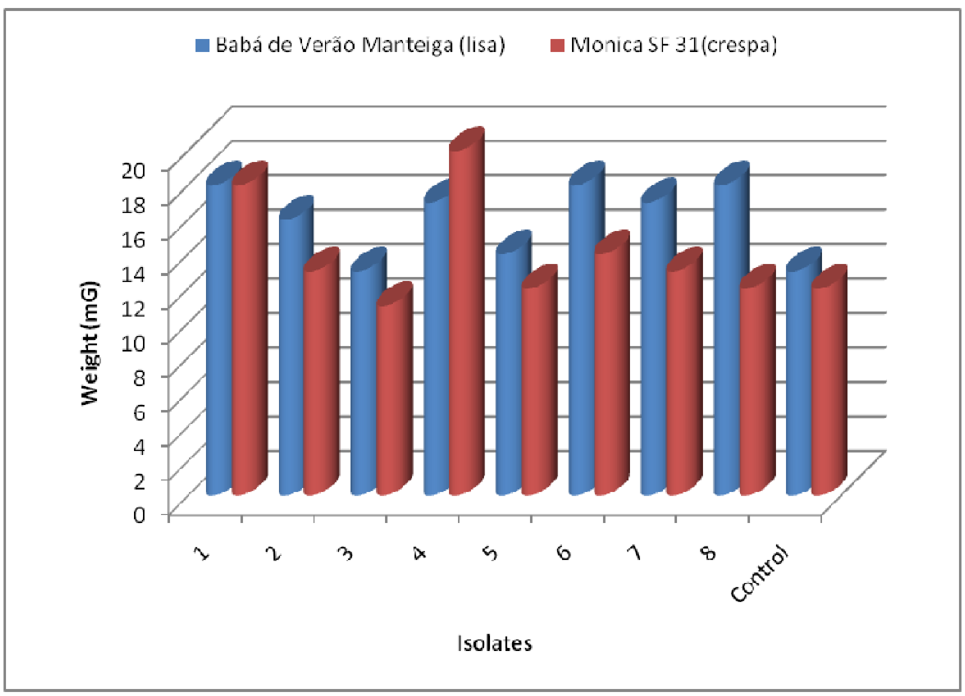

Figure 4. Dry weight of roots of seedlings of two varieties of lettuce (*Lactuca sat*iva) inoculated by different strains of rhizobacteria *Bacillus* spp.

Bacillus spp. 1, 2, 4, 6, 7 and 8 are not statistically different from each other, presenting the best results with respect to the variety Babá de Verão Manteiga, with an average of 30.8% increment, however, isolated 8 showed the best result, II of 38.5% compared to control. The isolates 3 and 5 differ from the strains 1, 2, 4, 6, 7 and 8,

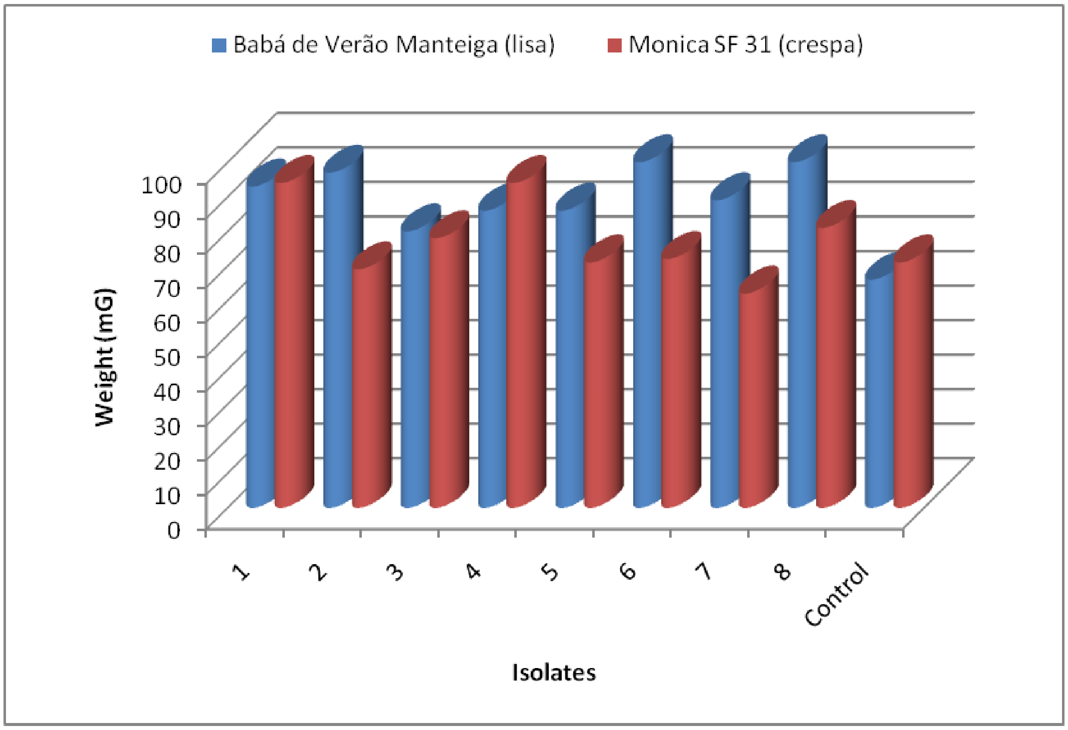

Figure 5. Dry weight of total seedlings of two varieties of lettuce (*Lactuca sativa* L.) inoculated by different strains of rhizobacteria *Bacillus* spp.

demonstrating the variety below for Babá de Verão Manteiga.

The isolates of *Bacillus* spp. 1 and 4 did not differ statistically and showed better results with respect to WDMR with II of 58.3% above control for the variety Monica SF 31, and isolate 4 showed the best result with respect to WDMR with II of 66.7%. Isolates of *Bacillus* spp. 2, 3, 5, 6, 7 and 8 differ from the strains 1 and 4, with lower results for WDMR for the variety Monica SF 31.

For varieties Babá de Verão Manteiga and Monica SF 31, the isolates of *Bacillus* spp. 1 and 4 are not statistically different, showing good results for the two varieties, with an II averaged of 46.4% in both varieties compared in relation to WDMR.

For the data of WDMT, the F test at 1% probability detected significant differences among treatments. The values ranged from 0.08 g to 0.10 g for the variety Babá de Verão Manteiga, averaging 0.091 g and the variety Monica SF 31 data range from 0.062 g to 0.094 g and averaged 0.078 g.

In figure 5, presents data from WDMT. The isolated *Bacillus* spp. 1, 2, 6 and 8 did not differ significantly showing the best results for a variety Babá de Verão Manteiga an average of 49.5% in II, but the best result was found in isolate 8, II 59.1% compared with the control. Isolates 3, 4, 5 and 7 differed from isolates 1, 2, 6 and 8, showing lower values, with II on average 28.9% compared to control.

LA in two varieties of lettuce, the F test at 1% significance detected between the varieties of lettuce and 5% in isolates of *Bacillus* spp., the interaction (Variety x bacteria) was not significant.

The Figure 6 presents data LA of varieties of lettuce in function of 8 isolates of *Bacillus* spp. The values of LA for isolates of *Bacillus* spp. ranged from 15.95 cm^2 to 20.55 cm^2 and the average equal to 18.25 cm^2. The isolate 7 was statistically different from all other treatments featuring the best IA, and 73.3%, compared with control. The isolates 2 and 8 did not differ among themselves, showing intermediate result to isolate 7, with an average of 46.15% in II, compared with control.

In the variety Monica SF 31 isolates 1 and 4 do not differ statistically for WDMT, showing the best results in an average AI of 33.3%, the best result was observed in 4 isolated II 33.7% compared with the control. The lowest values were observed in 2, 3, 5, 6, 7 and 8 isolates which differed significantly from isolates 1 and 4. Isolate 1 was not statistically different in the two varieties of lettuce to WDMT good results for the two varieties.

For data LL in two varieties of lettuce the F test at 1% significance detected significant differences between treatments. In figure 7, presents data from LL, 2, 3, 5, 6, 7 and 8 isolates did not differ significantly, demonstrating the best results for the LL, on average AI of 21.6%. The best result was observed for isolate 7 II of 28.4% compared to the control variety Babá de Verão Manteiga. Isolates 1 and 4 isolates differed from 2, 3, 5, 6, 7 and

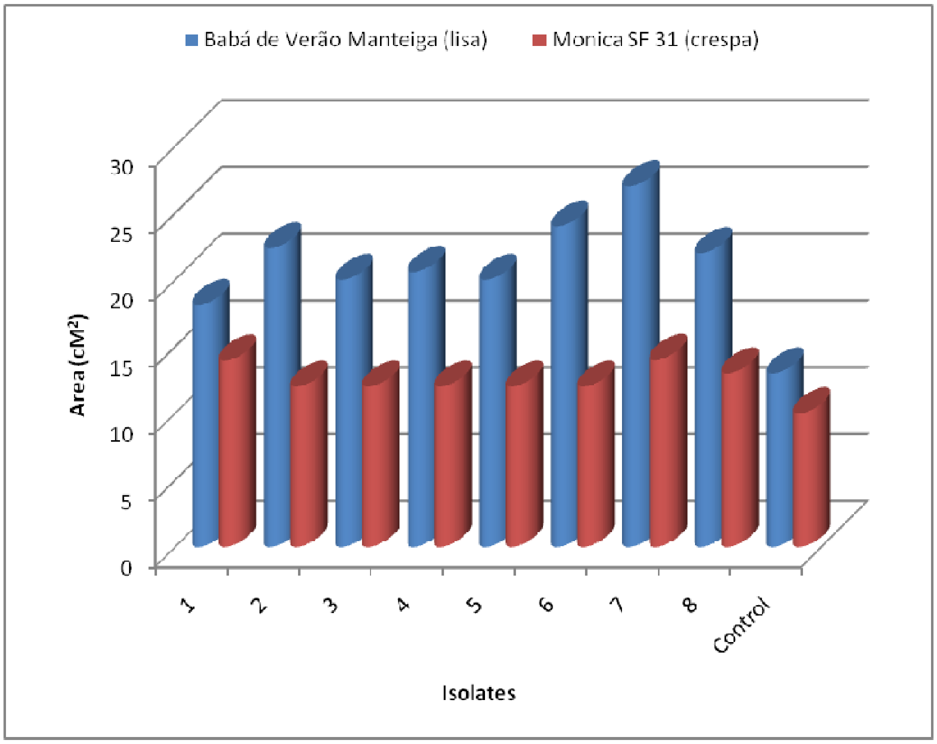

Figure 6. Leaf area of seedlings of two varieties of lettuce (*Lactuca sativa L.*) inoculated by different strains of rhizobacteria *Bacillus* spp.

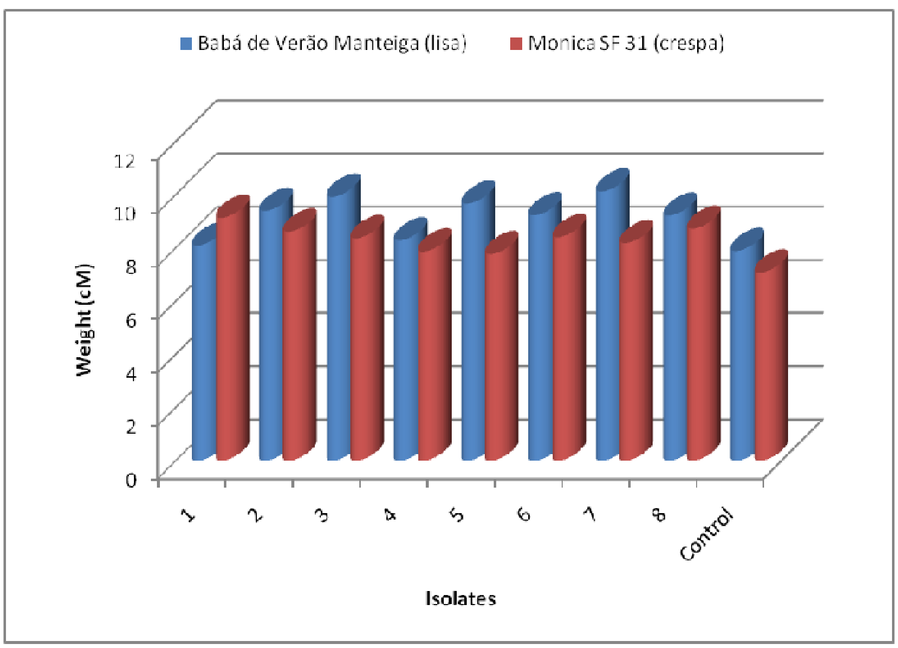

Figure 7. Leaf length of seedlings of two varieties of lettuce (*Lactuca sativa*) inoculated by different strains of rhizobacteria *Bacillus* spp.

8, with lower values.

In the variety Monica SF 31 isolates 1, 2, 3, 6, 7 and 8 did not differ statistically by observing the best results on average AI of 21.1% and the isolated one with the highest score II 29.1%. The isolates 4 and 5 differed from the others and showed inferior results, 2, 3, 6, 7 and 8

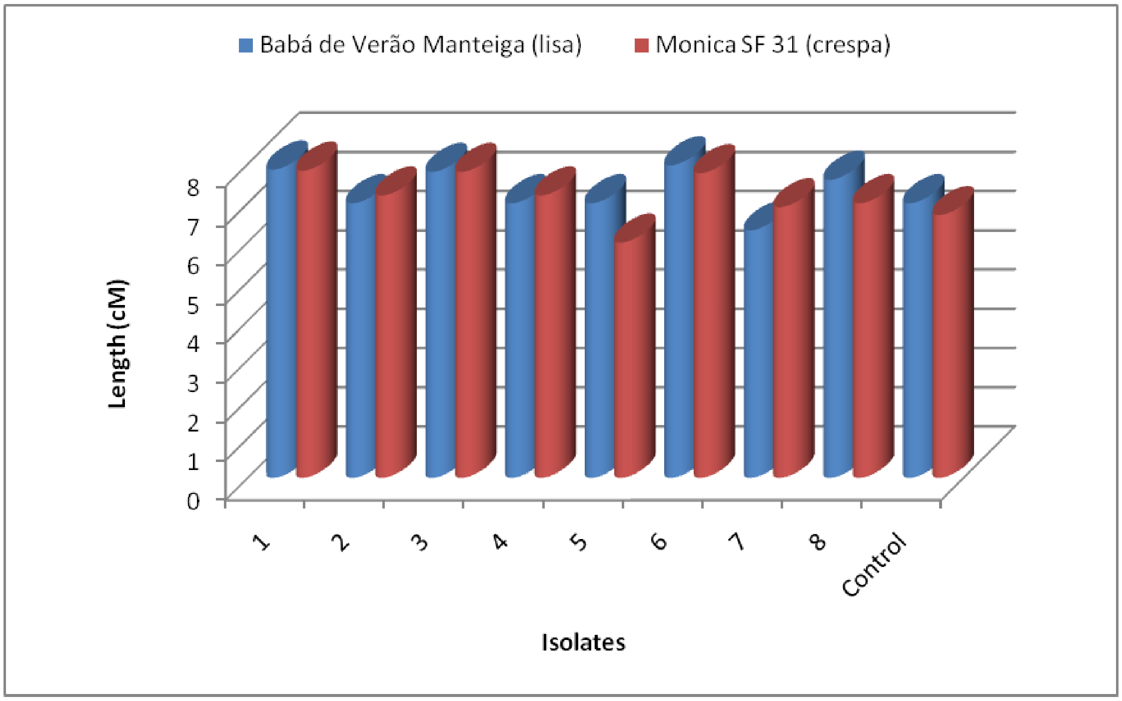

Figure 8. Root length of seedlings of two varieties of lettuce (*Lactuca sativa*) inoculated by different strains of rhizobacteria *Bacillus* spp.

isolates did not differ in the two varieties of lettuce for the LL showing the best results for the two varieties. The variety Babá de Verão Manteiga differs from that variety Monica SF 31, presenting the best result in LL.

For data LR in two varieties of lettuce the F test at 1% significance detected significant differences between treatments for the isolates of *Bacillus* spp. And there is no significant difference for interaction and variety (variety x bacteria) indicating no dependency between the two factors.

In figure 8, presents data LR. The isolated 1, 6 and 8 did not differ in showing the best, an average II of 11.7%, the highest result was observed in isolated 6 II of 14.4% compared to the control. The isolates 2, 3, 4, 5 and 7 differed from strains 1, 6 and 8 was inferior results.

For data of NL, the F test at 1% significance detected significant differences between treatments for the isolates of *Bacillus* spp. and variety, with no significant difference for interaction (varieties x bacteria).

For the smooth variety, only isolate 7 presented results inferior to the other isolates, since the curly variety, isolates 1, 4, 5 and 8 did not differ, showing better results for the number of leaves LN (Figure 9).

DISCUSSION

For WDMAP, Freitas et al. (2003), Santos (2005) and Sottero et al. (2006) obtained similar results, but in the work of the first author was no death of plants. According to Coelho (2006) it is interesting to note that some rhizobacteria can be considered deleterious (RD) and inhibit the growth of plants. The mechanism could be involved in this process would be the production of HCN. These rhizobacteria have been studied as important agents in controlling weeds (Kremer and Souissi, 2001). Superior results with respect to WDMAP was observed by Silveira et al. (2004) production in cucumber seedlings, IA where she obtained 55.5% compared to control.

In the present study 8 isolates were significant for the varieties Babá de Verão Manteiga and 6 for Monica SF 31. Three isolates were beneficial surpasses the number of isolates found beneficial for by Freitas et al. (2003) that working with *Citrus* spp. used strains of *Bacillus* spp. and *Pseudomonas* spp, getting just one isolated from Bacillus spp. and 7 of *Pseudomonas* spp, which promoted increased to WDMAP with beneficial results of *Citrus* spp.

Sottero et al. (2006) also obtained positive results in increasing the dry weight of roots of lettuce seedlings were inoculated compared to control. Noting also in his experiment had isolated beneficial when considered the WDMR, when the number of leaves. Freitas et al. (2004) obtained good results for WDMR in citrus seedling production, but the isolates used only one satisfactory.

The values of WDMT for variety Babá de Verão Manteiga found in this study were higher than those obtained by Silveira (2004), which is to cucumber seedlings.

The roots interact with a complex of microorganisms

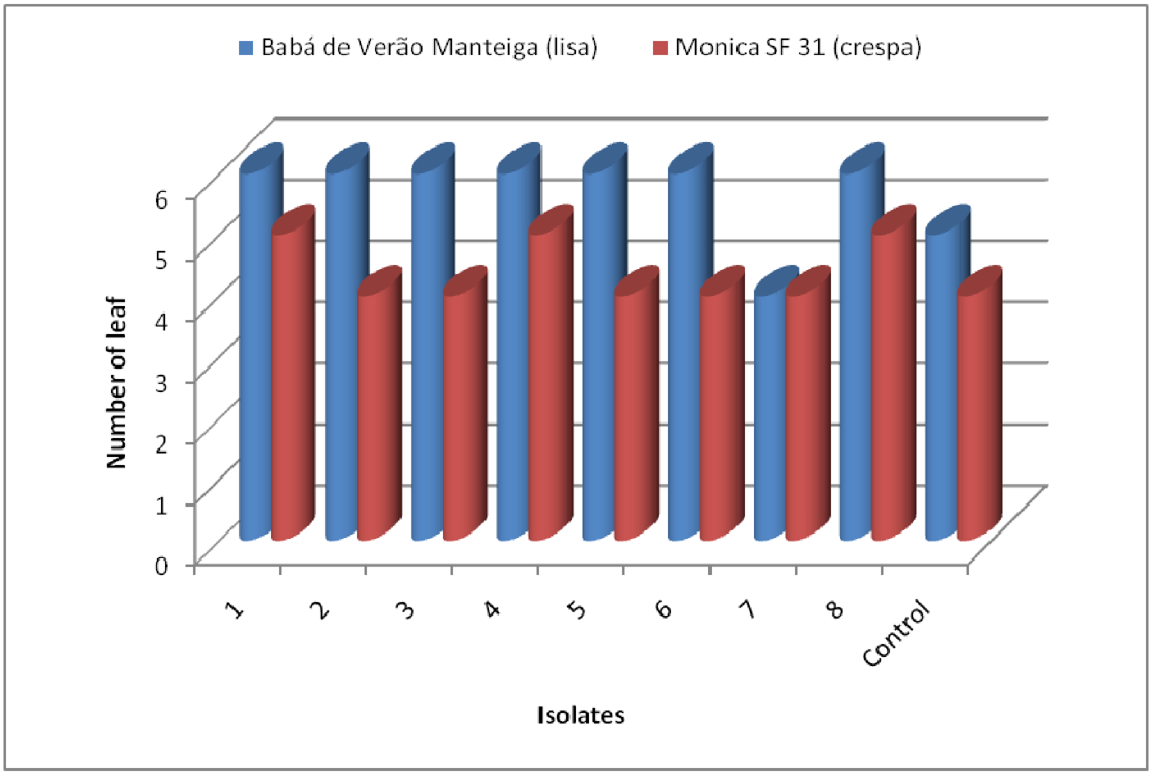

Figure 9. Leaf number of seedlings of two varieties of lettuce (*Lactuca sativa*) inoculated by different strains of rhizobacteria *Bacillus* spp.

that play an important role in growth and survival of plants. The root is a site of contention among many microorganisms, where the inoculation of beneficial Rhizobacteria can reduce or eliminate the action of pathogens through competition, release of antibiotics or stimulation of defense mechanisms in plants.

The bacteria that promote plant growth, act indirectly by suppression of diseases and production or directly by altering the concentration of phytohormones, nitrogen fixation, phosphate solubilization minerals or other nutrients from the soil, sulfur oxidation, increased permeability of roots and siderophore production (Catellani, 1999; Gomes et al., 2003).

In an experiment conducted by Benizri et al. (1997), with isolates of *Pseudomonas* rhizosphere of maize, inoculated on corn and tomatoes, there was better rhizosphere colonization in the first plant species, where according to Cole (2006) can be stated that the isolate of maize, having been inoculated in the same species from which it originated, has characteristics that make it more adapted to the corn plant than the isolate obtained from the rhizosphere of tomato.

However, Gomes et al. (2003) using *Bacillus pumilus* and *Bacillus thuringiensis* from the rhizosphere of cabbage observed that the isolates stimulated significant growth in lettuce plants, indicating that there was no specificity to the host. In this case, the bacteria can readily colonize hosts of different species, with even greater intensity, and promote their growth. *Bacillus* isolates from cabbage, beans and radish promoted growth in pineapple plantlets, demonstrating the lack of specificity regarding the promotion of growth (Melo et al., 2002).

A study conducted with lentil and pea showed differences in the ability of growth promotion mediated by two strains of rhizobacteria, which was attributed in part to the effect of plant genotype (Chanway et al., 1989).

Even with respect to specificity, differences in the quantity and quality of root exudates of different plant species and cultivars and genotypes of the same species, have been reported as the cause of these variations (Baldani and Dobereiner, 1980; Shishido and Chanway, 1999).

CONCLUSION

For the variety of summer butter (smooth) stood isolated 8 for WDMAP, WDMT, and WDMR, NL, isolated 7 for LA and LL, while the isolate 6 to LR. For variety Monica SF 31 (curly) stood the isolate 1 LL and WDMAP, 4 for WDMT and WDMR 6 for LR and 8 to for NL.

The variety Babá de Verão Manteiga (smooth) showed superior results in most of the variables analyzed in comparison to the variety Monica SF 31 (crisp).

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