Influence of rhizopheric bacteria on germination and initial growth of *Sporobolus cryptandrus* (Torr.) A. Gray

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In order to assess the effect of rhizobacteria strains on seeds of *Sporobolus cryptandrus*, a standard methodology was applied for the inocula preparation and seeds inoculation, according to that recommended in the international literature. A completely randomized experimental design with ten treatments and ten repetitions was used. The percent of germination, germination increase compared with the control, longitude of the radicle and plumule were assessed. In the germination, the treatments with the strains MSDJ865 (100 %) and ATCC9039 (100 %) had values higher than the rest, with increase of 40 % in respect to the control. In the plumule longitude, the treatments with ATCC9039 (19.10 mm), ATCC7486 (19.22 mm) and ORS534 (19.11 mm) were superior to the control. In the radicle longitude, the treatments HA1 (46.10 mm), ORS534 (45.33 mm) and ATCC9039 (44.30 mm) were superior to the control and to the majority of the treatments. It is concluded that the most outstanding strains in the variables studied were ATCC9039, ORS534, MSDJ865 and HA1. The longitude of the radicle was the variable with highest contrast of statistical values caused by inoculation. The strains belonging to *Bradyrhizobium sp*. showed different effects on the variables studied, in spite of their common taxonomic origin. Greenhouse tests with the strains ATCC9039, ORS534, MSDJ865 and HA1, are recommended, as well as similar studies in other meadow grasses.

Key words: *inoculation, rhizobacteria, meadow grasses.*

Sporobolus cryptandrus (Torr.) A. Gray is a meadow species belonging to the Gramineae family, Chloridoideae subfamily, Zoysieae tribe (Anon 2012), known in North America as the meadow grass, among other names. It is of great importance for its use as cattle pasture, mainly in temperate zones. Besides, it is a control factor of the soils erosion and is also part of the natural habitat of certain wild animals (Gleason and Cronquist 1991).

Nevertheless, the development of this plant species, or any other, depends on different factors such as the seed vigor for an efficient germination, as well as the rapid establishment of the plant (Mia *et al*. 2012). One of the alternatives applied to stimulate the plant development is the seeds inoculation with microorganisms, method used to modify the microbial populations next to the plants and promote, then, their development and productivity (Harper and Lynch 1979). According to Babalola *et al*. (2007), the composition of microbial associations in the root surface may be modified through the introduction of bacterial cells in the surface of seeds, roots and tubercles. The capacity of these microorganisms to stimulate the germination and improve the plants development has adapted from *in vitro* to *in vivo* conditions in plants of agricultural and ornamental importance (Tsavkelova *et al*. 2007).

The useful microorganisms, including the rhizopheric bacteria, promote the plant growth, as they have a determined number of physiological properties important for the colonization of radical surface and the improvement of growth and health of the plants (Cassán 2009). Among these properties, the production of indolacetic acid is considered the principal mechanism related with the promotion of plant growth (Mehnaz *et al*. 2010).

In Cuba, there are experimental data about the effect of bacterial inoculation on seeds germination. Tang (1995) carried out tests to assess the effect of *Azotobacter chroococcum* on legumes and meadow grasses seeds of Cuban ecosystems.

The objective of this experiment was to assess, under controlled conditions, the effect of different species of rhizopheric bacteria promoters of plant growth on *Sporobolus cryptandrus* seeds to select the most outstanding strains for the experimental phase of greenhouse.

Materials and Methods

Procedence of the bacterial strains. Strains from the strain bank of the Experimental Station of Pastures and Forages of Sancti Spíritus, Cuba were used. Others came from the Alberta Research Council of Agriculture and Agri-Food Canada, Canada (table 1).

Procedence and viability of the seeds of Sporobolus cryptandrus. They were donated by the Department of Native Phytogenetic Resources of the Alberta Research Council, Canada and had 60-70 % of germination.

Experimental procedure. All the rhizobia strains grew on solid yeast-manitol medium (Vincent 1970). They were resuspended in liquid yeast-manitol medium until achieving concentration of 10^7-10^8 CFU/mL. The strains of free-live dinitrofixer bacteria (Beijerinckia, Azospirillum and Azotobacter) grew in nitrogen-free medium (1L of medium 0.4 g KH_2PO_4, 0.1 g K_2HPO_4,

Table 1. Strains used in the experiment and their identification

Strains	Genre and species	Procedence
SP12	*Bradyrhizobium sp.*	Experimental Station of Pastures and Forages Sancti Spíritus, Cuba
TD1	*Bradyrhizobium sp.*	Experimental Station of Pastures and Forages Sancti Spíritus, Cuba
HA1	*Bradyrhizobium sp.*	Experimental Station of Pastures and Forages Sancti Spíritus, Cuba
MSDJ865	*Mesorhizobium loti*	Agriculture and Agri-Food Canada, Québec, Canada
ORS534	*Azorhizobium caulinodans*	Alberta Research Council, Alberta, Canadá
USDA191	*Sinorhizobiumfredii*	Agriculture and Agri-Food Canada, Québec, Canada
ATCC9039	*Beijerinckia indica*	Agriculture and Agri-Food Canada, Québec, Canada
ATCC7486	*Azotobacter chroococcum*	Agriculture and Agri-Food Canada, Québec, Canada
ATCC29145	*Azospirillum brasilense*	Agriculture and Agri-Food Canada, Québec, Canada

$MgSO_4.7H_2O$ 0,2 g; NaCl: 0,1 g; $FeCL_3$: 10,00 mg; $Na_2MoO_4.2H_2O$: 2,00 mg; sodium malate: 5,00 g; yeast extract: 50,00 mg), described by Dalton and Postgate (1969), with changes in the carbon source for Beijerinckia (2 % glucose) and Azotobacter (2 % manitol). The culture was used without changes for Azospirillum.

Disinfection of the seeds. The seeds were sterilized with ethanol 95 % (30 s), sodium hypochlorite 4-6 % (2-3 min) and successive rinses with sterile distilled water (Bécquer 2002).

Inocula application to the seeds. Ten seeds of each treamtent were placed in bags made of sterile gauze. They were submerged in the inocula at room temperature, in laminar flow for 4 h. They were immediately planted after that time (Harper and Lynch 1979). The seeds for the control were submerged in sterile distilled water.

Seeds germination in Petri plates. Ten seeds were planted in each sterile Petri plate of 15 cm Ø. Sterile agarized water was used as growth substrate (15 %). The plates were placed in the dark at 20 ºC. The assessment was conducted at 72 h, according to Harper and Lynch (1979). The germination percent was determined according to the formula described by Mia*et al*. (2012):

Germination percent: number of germinated seeds/ number of planted seeds x 100

Experimental design and statistical analysis. A one-way ANOVA analysis for inoculation experiments was conducted in a completely randomized design and ten repetitions (Somasegaran and Hoben 1994). The differences between means were determined by Duncan (1955). The percent data of the germination were transformed before the statistical analysis through arcsine $\sqrt{P}$, where P is the proportion value (Sigarroa 1985). The treatments were ATCC9039, ATCC7486, ATCC29145, MSDJ865, USDA191, ORS534, HA1, TD1, SP12 and control (growth in agarized water, without inoculation). The variables germination (%), longitude of the plumule (mm) and longitude of the radicle (mm) were assessed. The germination increase (%) of the treatments inoculated compared with the control were also calculated. The data were processed with the statistical software StatGraphics Plus, for Windows 2.0 (1994-1996).

Results and Discussion

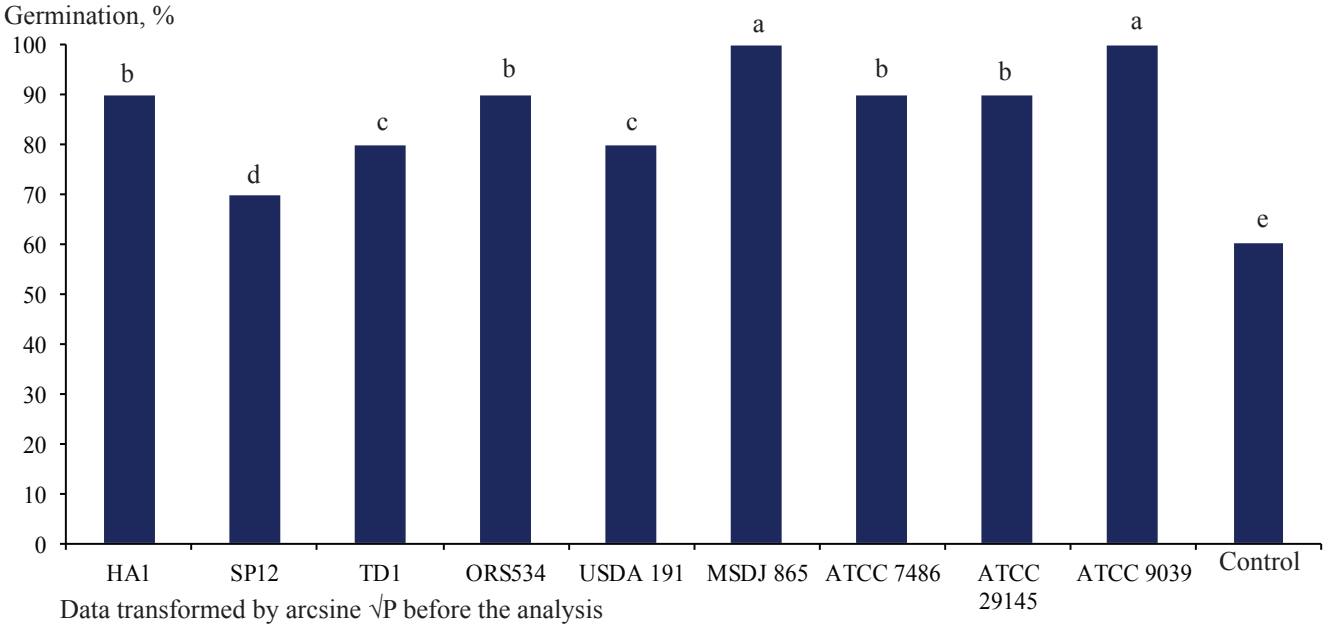
Figure 1 presents all the treatments inoculated with the strains MSDJ 865 (*Mesorhizobium loti*) (100 %) and ATCC9039 (*Beijerinckia indica*) (100 %). They had statistically values superior ($P < 0.001$) to the rest, with increase of 40 % in respect to the control (figure 2).

According to Mia*et al*. (2012), *B. indica* produces AIA, which is a stimulating factor of the seeds germination. Together with this, Thruler*et al*. (2003) proved that *B. derxii* produces bioactive substances, known as polyamines (putrescine and spermidine). Walters (2000) refers that these substances may modulate the functions of nucleic acids and proteins, as well as influence on the membrane stability, affecting the cell prolificacy and the differentiation in superior organisms. These characteristics make the Beijerinckia to be considered as feasible bacteria to achieve positive results in this experiment.

Although no specific differences were found about the effect of the *M. loti* on the seeds, the report of Santillana *et al*. (2005) in respect to the germination increase of at about 20 % of tomato seeds inoculated with *Rhizobium sp*, a genre taxonomically closed to Mesorhizobium (Bécquer 2002), could be referred.

In decreasing order, it was followed by the inoculated treatments with the strains HA1 (*Bradyrhizobium sp.*), ORS534 (*Azorhizobium caulinodans*), ATCC7486 (*Azotobacter chroococcum*) and ATCC29145 *(Azospirillum brasilense*) (90 % each). They increased their germination in 30 % in respect to the control. The rest of the treatments had inferior values, between 80 (USDA191-*Sinorhizobium fredii* and TD1 *Bradyrhizobium sp*.) and 70 % (SP12-*Bradyrhizobium sp*.), with light increase of 20 and 10 %, respectively (figure 2). The treatment inoculated with SP12 (70 %) showed the statistically inferior value compared with the rest, although superior to the control (60 %).

In any of the applied strain with positive effect on the *Sporobolus cryptandrus* germination is discarded that the gibereline production is one of the most probable causes in this result. Cassán *et al*. (2009) considered this premise



Data transformed by arcsine √P before the analysis
SE ± 0.005
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Figure 1. Response of *Sporobolus cryptandrus* in its germination when inoculated with different strains of rhizopheric bacteria

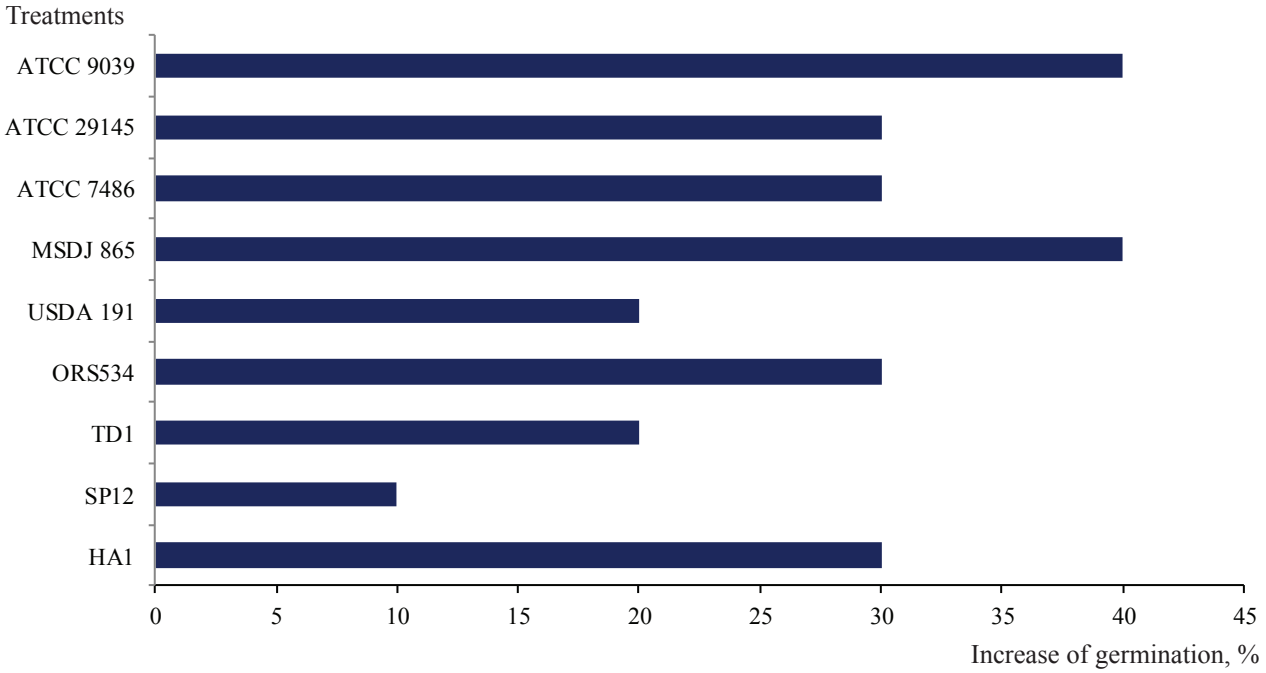


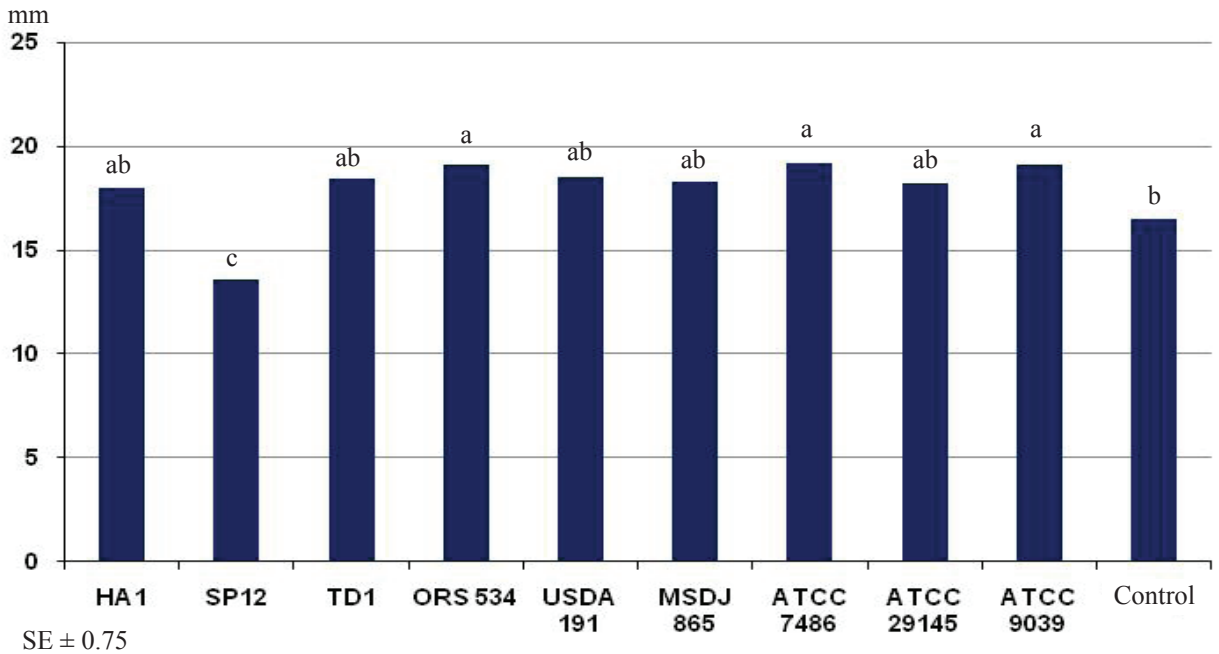
Figure 2. Increase of *Sporobolus cryptandrus* germination compared with the control in the treatments inoculated

as typical for explaining the germination increase of soybean and maize inoculated with *A. brasilense* and *B. japonicum*.

The low response of the seeds in the germination of the rest of the inoculated treatments could be attributed to the competence for oxygen by the microbial cells and seeds (Harper and Lynch 1979). This could be determined by the cell concentration used. Kurdish *et al*. (2008) stated that the seeds response to the bacterization with *A. vinelandii* depends also on the plant species used.

The treatments inoculated (figure 3) with the strains ATCC9039 (*Beijerinckia indica*) (19.10 mm), ATCC7486 (*Azotobacterchroo coccum*) (19.22 mm) and ORS534 (*Azorhizobium caulinodans*) (19.11 mm) were statistically higher (P < 0.001) than the control (16.50 mm) and SP12 (*Bradyrhizobium sp.*) (13.62 mm), although shared superscripts with USDA191 (*Sinorhizobium fredii*) (18.50 mm), TD1 (*Bradyrhizobium sp.*) (18.46 mm), MSDJ865 (*Mesorhizobium loti*) (18.30 mm), ATCC29145 (*Azospirillum brasilense*) (18.22 mm) and HA1 (*Bradyrhizobium sp.*) (18.00 mm).

The first three mentioned strains, belonging to different genres of dinitrofixer bacteria, stood out for their high values in the stem growth, but, when sharing superscripts with other relatively big group of strains, indicated great influence of the bacteria inoculated in



SE ± 0.75

[abc]Different letters differ statistically (P < 0.001)

Figure 3. Response of *Sporobolus cryptandrus* longitude of the plumule when inoculated with different rhizopheric bacteria strains

the development of this part of the plant. According to Davies (1995), the hormone gibereline (giberelic acid) is the responsible for the stem lengthening in the plants. Just some of the strains standing out their effect on this variable belong to the microorganisms group producing it, such as *Azotobacter spp*. (Martínez-Toledo *et al*. 1988) and *A. brasilense* (Janzen *et al*. 1992). According to Mia *et al*. (2012), the stem lenghtening in rice seeds, as inoculated with different rhizobium strains and dinitrofixer bacteria of free life, is due to the gibereline production by the bacteria in both groups.

In rhizobia, Atzorn *et al*. (1988) observed that *Rhizobium leguminosarum* also produces this hormone, so it is not discarded that the strains of *A. caulinodans*, *S. fredii*, *M. loti* and *Bradyrhizobium sp*., applied in the experiment, with superior value in the lengthening of the plumule, are potentially high producers of this compound.

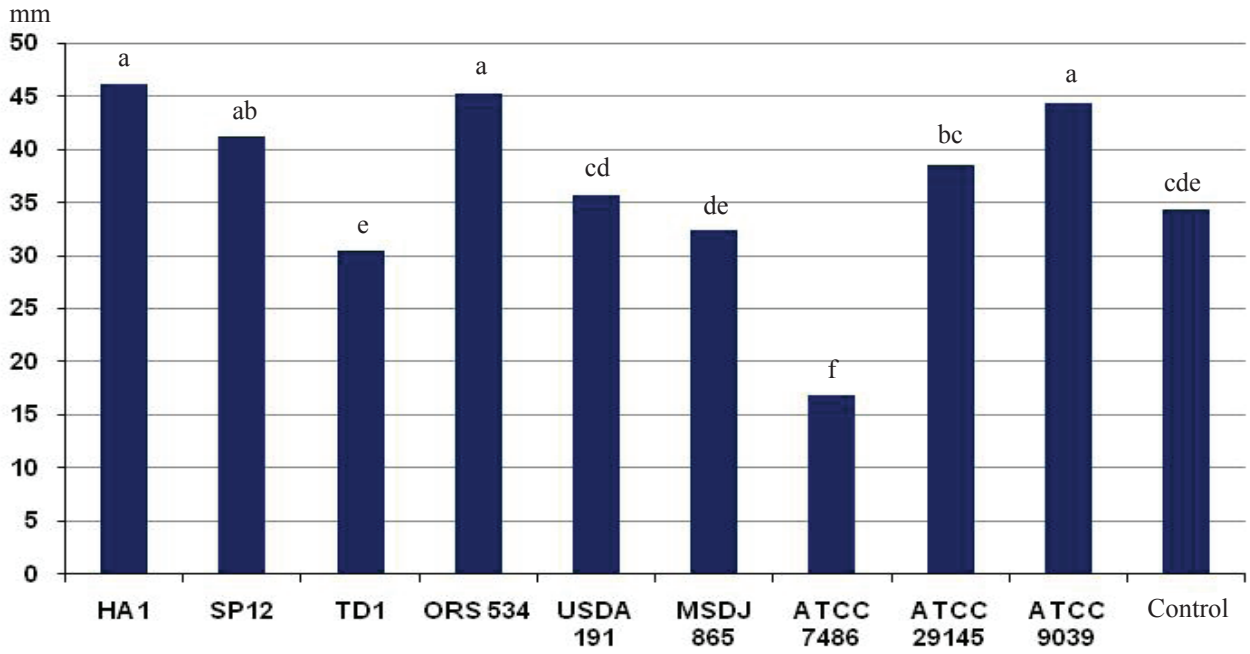
In respect to the radicle length (figure 4), statistically superior values (P < 0.001) were observed in the treatments with HA1 (*Bradyrhizobium sp*.) (46.10 mm), ORS534 (*Azorhizobium caulinodans*) (45.33 mm) and ATCC9039 (*Beijerinckia indica*) (44.30 mm). they, although having common superscripts with the treatment inoculated with SP12 (*Bradyrhizobium sp*.) (41.25 mm), were, at the same time, statistically superior (P < 0.001) to ATCC29145 (*Azospirillum brasilense*) (38.56 mm), USDA191 (*Sinorhizobium fredii*) (35.62 mm), control (34,32 mm), MSDJ865 (*Mesorhizobium loti*) (32.40 mm), TD1 (*Bradyrhizobium sp*.) (30.50 mm) and ATCC7486 (*Azotobacter chroococcum*) (16. 78 mm).

As in the previous variable, the influence of specific hormones produced by the inoculated bacteria could be the main mechanism of root stimulation instead of the fixation of atmospheric nitrogen. Bashan *et al*. (2004) considered that the effect of nitrogen fixation of Azospirillum in the plant is only carried out when it reaches more advanced phenological stages. García de Salomone *et al*. (2010) assured that hormonal effect of Azospirillum, and not that of the fixed nitrogen, improved the efficiency of N absorption in rice.

Cassán *et al*. (2009) assured that the AIA penetrates the seminal peel and accelerates the root development, with the consequent increase of water and minerals absorption for the plant. The high capacity of Azospirillum of producing AIA has been also reported by Reis Junior *et al*. (2004).

The low values of the treatment inoculated with *A. chroococcum* were inferior to that of other treatments inoculated as that of the control. Tang (1995), in an experiment with *A. chroococcum* inoculated in seeds of *Panicum maximum* and *Cenchrus ciliaris* determined that the germination and height of plantules decreased with this bacterium. This result could indicate a deficient production of stimulant hormones by the bacterium. It could also be attributed to the regulation of the radical morphogenesis by the bacterial AIA (Dobbelaere *et al*. 1999).

The highest increases of seeds germination were obtained with the strains MSDJ865 (*Mesorhizobium loti*) and ATCC9039 (*Beijerinckia indica*), while the highest positive effect of the plumule longitude was obtained with the strains ORS534 (*Azorhizobium caulinodans*), ATCC7486 (*Azotobacterchroo coccum*), ATCC9039 (*B. indica*), HA1 (*Bradyrhizobium sp*.), TD1 (*Bradyrhizobium sp*.), USDA191 (*Sinorhizobium fredii*) and ATCC29145 (*Azospirillum brasilense*). In the radicle longitude, the best effect was achieved with



SE ± 1.64

[abcdef]Different letters differ statistically (P < 0.001)

Figure 4. Response of *Sporobolus cryptandrus*, longitude of the radicle when inoculated with different rhizopheric bacteria strains

ORS534, ATCC9039, as well as with HA1 and SP12. It is obvious that the strain ATCC9039 was present in the four variables, as well as ORS534, MSDJ865 and HA, each coincided in two variables with statistically superior values.

In the strain ATCC9039 (*B. indica*), the high values in the variables longitude of the radicle and of the plumule could be due to not only the hormones this species also produces but to the high production of aminoacids like glutamine and alanine (Thuler*et al*. 2003). These compounds, apart from keeping low the intracellular N levels in the bacterium for a better N_2 fixation, are in the plant's disposal for its vegetative development.

The strains of *Bradyrhizobium sp*., in spite of their common taxonomical location, showed disparity in their effect on the variables assessed. This is explained by their different territorial precedence, factor that undoubtedly influences differently on the genotype and physiology of each of them (Bécquer 2002).

It is concluded that the most outstanding strains on the variables studied were ATCC9039, ORS534, MSDJ865, as well as HA1. It should be highlighted that the longitude of the radicle was the variable with highest contrast of statistical values caused by the inoculation. The strains belonging to *Bradyrhizobium sp*. showed different effects in all the assessed variables, in spite of their common taxonomical origin.

Greenhouse tests with the strains ATCC9039, ORS534, MSDJ865 and HA1 are recommended, as well as similar inoculation studies in other meadow grasses.

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