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INTERACTION OF THE BACTERIA *Gluconacetobacter diazotrophicus* AND ROOT VEGETABLES

Interacción de la bacteria *Gluconacetobacter diazotrophicus* y hortalizas de raíz

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ABSTRACT. *Gluconacetobacter diazotrophicus* is endophyte bacterium that have among its metabolic characteristics, direct and indirect mechanism for plant growth promotion. There are positive results by its application in graminious and tropical tubers. Nevertheless, in vegetable researches of this plant-microorganism interaction are few. The present work had the aim to evaluate the effect of the application over growth of carrot (*Daucus carota* L.) and beet (*Beta vulgaris* L.), of four *G. diazotrophicus* strains isolated of Cuban agriculture ecosystem. It was showed that microorganism habitat have not direct relation with vegetable species that could be beneficiated with the interaction, due to that the best result was obtained with the strain isolated of mango. This strain was selected as promissory as active principle of bioproduct with positive effect over both vegetables. Besides, this strain has more plant growth promoting effect than patron strain of the bacterium specie (PAL5), indicating the importance of indigenous microorganisms. Results suggest that *G. diazotrophicus* could be used in plan growth promotion of root vegetables like carrot and beet and it also shows the complexity of plant-microorganism interaction.

Key words: biofertilizer, growth, stimulation, nitrogen fixation, microrganism

RESUMEN. *Gluconacetobacter diazotrophicus* es una bacteria endófita que presenta dentro de sus características metabólicas, mecanismos directos e indirectos de estimulación del crecimiento vegetal. Se han obtenido resultados positivos por su aplicación, fundamentalmente, en gramíneas y viandas tropicales. Sin embargo, en hortalizas, las investigaciones de esta interacción planta-microorganismo son escasas. El presente trabajo tuvo como objetivo evaluar el efecto de cuatro cepas de *G. diazotrophicus* aisladas de ecosistemas agrícolas cubanos, sobre el crecimiento de zanahoria (*Daucus carota* L.) y remolacha (*Beta vulgaris* L.). Se demostró que la procedencia del microorganismo no tiene una relación directa con la especie vegetal que puede beneficiar con su interacción, ya que los mejores resultados se obtuvieron con una cepa proveniente de los frutos del mango. Esta cepa se seleccionó como promisoria para constituir el principio activo de un producto con efecto positivo sobre ambas hortalizas. Además, presentó un efecto estimulador del crecimiento mayor que el patrón de la especie bacteriana (PAL5), lo que indica la importancia del empleo de microorganismos autóctonos. Los resultados sugieren que *G. diazotrophicus* puede ser utilizada en la estimulación del crecimiento de hortalizas de raíz como zanahoria y remolacha y evidencian cuan compleja es la interacción planta-microorganismo.

Palabras clave: biofertilizantes, crecimiento, estimulación, fijación de nitrógeno, microorganismo

INTRODUCTION

Gluconacetobacter diazotrophicus is an endophyte microorganism that has been successfully applied to different crops such as sugarcane (*Saccharum* spp.) (1), sorghum (*Sorgum bicolor* L. moench.) (2), taro (*Xanthosoma* spp.), sweet potato (*Ipomoea batata* L.) and cassava (*Manihot esculenta* Crantz.) (3). The stimulation of growth indicators and the yield of the plant species led to the realization of other investigations, from which different formulations were obtained where the bacterium constitutes the active principle (4, 5).

The growth stimulating effect of *G. diazotrophicus* is associated with different characteristics of the microorganism, where its potential for biological fixation of nitrogen (1) and its ability to produce different phytohormones,

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mainly indole acetic acid (6), are highlighted. The solubilization of mineral nutrients such as phosphorus, zinc, iron and manganese could also contribute to the stimulation of plant growth by the bacterium (1), as well as its antagonistic effect, which has been demonstrated against different phytopathogenic organisms (7).

However, studies with *G. diazotrophicus* demonstrate that not all strains have the same characteristics under *in vitro* conditions (5). It also happens in its interaction with the plant, so the effect that is achieved with its application is also variable (2). Research on the microorganism associated with horticultural species is scarce. In order to select a promising strain of this bacterium species for the growth stimulation of root vegetables, this work was carried out, whose objective was to determine the application effect of four strains of the microorganism isolated from Cuban agroecosystems on growth of carrot and beet.

MATERIALS AND METHODS

Bacterial strains: four strains of *Gluconacetobacter diazotrophicus* isolated from Cuban agroecosystems, conserved in the INIFAT Beneficial Bacteria Collection; in addition, the pathogenic microorganism of this bacterial species, PAL5 (ATCC 49037), was included. The provenance culture of each of the strains used is shown in Table I.

Table I. Strains of *Gluconacetobacter diazotrophicus* used in the research

Code of microorganism	Crops of origin
INIFAT Gd-19	Guava (*Psidium guajaba* L.)
INIFAT Gd-26	Beet (*Beta vulgaris* L.)
INIFAT Gd-42	Mango (*Manguifera indica* L.)
INIFAT Gd-46	Yuca (*Manihot esculenta* Crantz.)
PAL 5	Sugar cane (*Saccharum officinarum* L.)

Plant material: beet seeds of Detroit Roja cultivar and carrot, New Kuroda cultivars, which had 90 and 100 % germination, respectively. This percentage was determined in Petri plates of 90 mm with filter paper, from the emergence of the radicle between 7 and 10 days, using three plates for each plant species and 25 seeds per plate.

Application of the strains of *G. diazotrophicus* on carrot and beet: the experiment was carried out in semicontrolled conditions, in pots of two kilograms capacity with Red Ferralitic Compacted Leachate, Glicine and Ferruginous Nodular soil (8). The soil characteristics are shown in Table II.

A cell suspension in sterile distilled water from each of the strains of *G. diazotrophicus* was inoculated by spraying the soil, at a rate of 10 mL per pot, after germination of the seeds, this being understood as the emergence of the plant above the surface of the soil. Evaluations: at 60 days for beet and 70 days for carrot were done, the following growth indicators were evaluated; Height of the plant (cm); Long (cm) and diameter (cm) of the root; Number of leaves, fresh weight of leaves (g) and fresh weight of roots (g).

The experiment was run in duplicate, with 30 plants per treatment (strain), including one variant without bacterial inoculation (control). A completely randomized design was used. Statistical processing of the data was performed using the STATGRAPHICS Plus version 5.0 (9) program, which verified the normality and homogeneity of variances according to the Kolmogorov-Smirnov, Cochran C, Hartley and Bartlett tests. When there were significant differences among treatments, the means were compared, according to the Duncan test at 5 % significance.

RESULTS AND DISCUSSION

The endophyte bacterium *Gluconacetobacter diazotrophicus* presents different stimulation mechanisms of plant growth, taking into account that it fixes nitrogen, solubilizes nutrients and has an antagonistic effect against phytopathogenic organisms (1). However, not all the strains described for this species have the same characteristics (5), nor do they produce the same effect when applied to crops (2).

In the case of the bacterium interaction with vegetables, the studies are scarce. For these reasons, and with the long-term objective of selecting promising biological materials for the stimulation of carrot and beet growth, this research was conducted where the effect of the application of four strains of the microorganism isolated from Cuban agroecosystems and the pattern of this bacterial species, on the growth of both vegetables.

Table II. Characteristic of the used soil during the study

pH	Organic matter (%)	Phosphorus assimilable (p.p.m)	Ca^{++}	Mg^{++} (c mol kg soil^{-1})	Na^{+}	K^{+}
7,3	2,58	209	19,1	4,2	0,11	0,38

Figure 1 shows the results of indicator associated evaluation of the aerial part of the crops under study. Note that the response depends not only on the microorganism, but also on the species of the vegetable present in the interaction. Thus, the bacteria action on the plant height is superior for beet, where four of the five strains evaluated allow to reach values of this indicator higher than those obtained in the control treatment, lacking bacterial application. However, for the number of leaves and their fresh weight, the cultivation of the carrot stands out. The greatest effect of *G. diazotrophicus* was on fresh leaf weight, a marker stimulated by almost all the microorganisms under study.

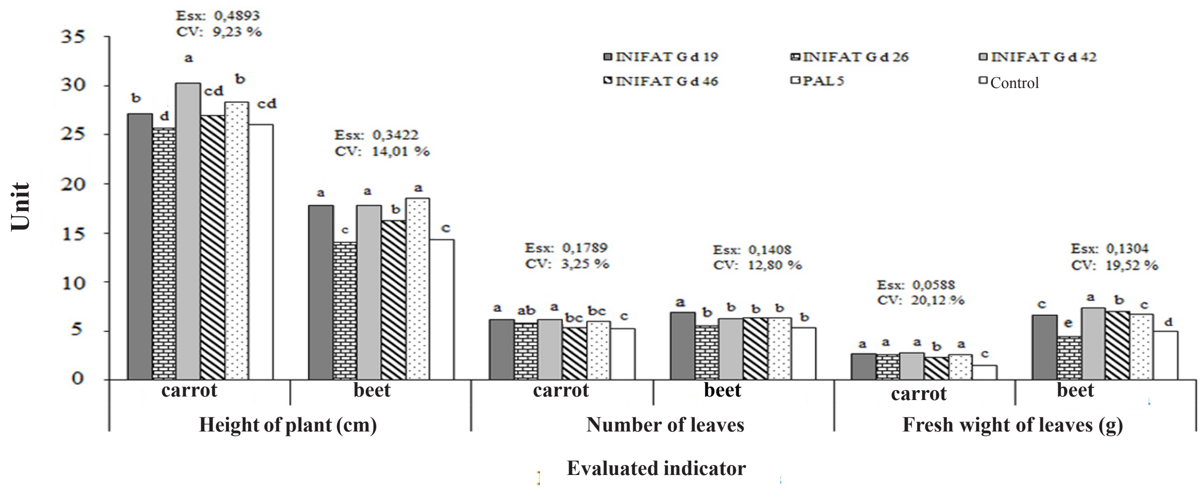
The INIFAT Gd 19 strain, which increased the three indicators evaluated for both horticultural species and the INIFAT Gd 42 strain, was noted for its effect on the aerial part of the plant, which had a positive action on all the indicators, in the case of the carrot and on two of them for the beet. The microorganisms caused a greater response than the standard strain of the bacterial species (PAL 5), which shows that autochthonous strains can allow better results. This is an aspect commented by several authors, which include permanently, the experimental stage of work, the isolation of microorganisms and their selection in interaction with the crop to benefit (10).

The advantages of using strains from plant species planted under Cuban conditions were even more evident in the case of indicators associated with the roots of both crops, according to the results shown in Figure 2.

Most of the inoculated microorganisms stimulate the diameter of the beet root. However, the inhibition provoked in some of the indicators by the INIFAT Gd 19, INIFAT Gd 26 and PAL5 pathogens is highlighted. In the literature it is described that many bacteria show *in vitro* mechanisms to stimulate plant growth, although their application does not always produce the expected effect (10).

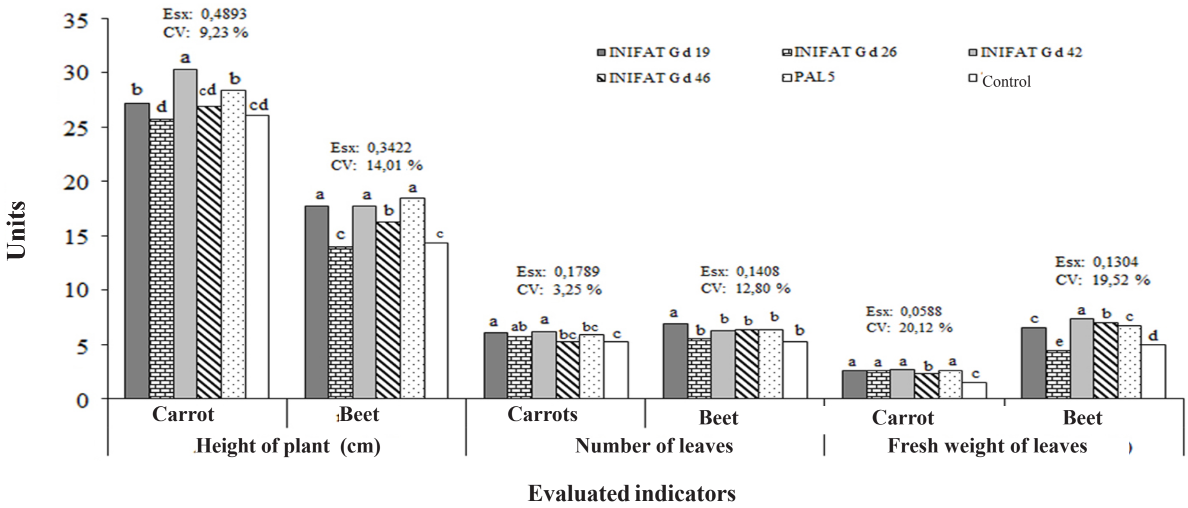
In stimulating growth by microorganisms different factors are involved. The increase in nutrient availability for the plant and the net hormonal balance produced by the interaction among phytohormones released by the microorganism and those present in the plant are some of the most relevant aspects (10). Among the phytohormones produced by growth-promoting bacteria, indole acetic acid (11) stands out, with differences among the more and less efficient strains in these and other characteristics (12, 13). This could explain the stimulation of the radical development of the INIFAT Gd 42 strain and the inhibition of the above mentioned indicators, since the bacterial species used in the study is known to release auxins such as indole acetic acid (6) and gibberellins (11).

The application of *G. diazotrophicus* strains produces differences in dry weight, leaf nitrogen content, sugar content and chlorophyll content in the case of sorghum (2), which would be interesting to determine for the vegetables under study, taking into account few studies with the bacterium in association with this type of crop and its influence on the degree of plant growth stimulation.



The results correspond to the average of 60 plants for each treatment. Different means differ significantly, according to Duncan's test at 5 % significance

Figure 1 Effect of application of *G. diazotrophicus* strains on aerial part of carrot (*Daucus carota* L.) and beet (*Beta vulgaris* L.)



The results correspond to the average of 60 plants for each treatment. Means with different letters differ statistically for a 5 % significance, according to the Duncan Multiple Rank test. N = 30

Figure 2. Effect of the application of G. diazotrophicus strains on beet roots (Beta vulgaris L.) on the inoculation of different strains of G. diazotrophicus

During this research the INIFAT Gd 42 strain was highlighted, not only for its effect on the carrot and beet root, but also for the stimulation of indicators associated with the aerial part of the plants. This microorganism was isolated from mango fruits, so the result suggests that there is no direct relationship between the origin culture of the *G. diazotrophicus* strain and the plant species that can benefit from its application, an aspect previously shown for grasses and tropical vegetables (2, 3).

The result shows the complexity of the plant-microorganism interaction, a process that depends not only on the characteristics of the bacteria, but also on the success of the colonization process (14), its ability to release exopolysaccharides (15) and forming biofilms (16), among other factors. The plant also intervenes, with strict regulation of the microorganism with which it interacts. From the research, the INIFAT Gd 42 strain was selected as promising for future studies, where its effect on both crops is evaluated in systems that allow the maximum development of its roots, consumption organ in this case, taking into account, in addition, the relevant results obtained for the rest of the evaluations carried out.

CONCLUSIONS

- The endophytic bacterium *Gluconacetobacter diazotrophicus* can establish a positive interaction with root vegetables such as carrots and beets, depending on their stimulatory growth effect on the applied strain and the plant species.
- The INIFAT strain Gd 42, isolated from the fruits of the mango, is a promising microorganism to stimulate the growth of carrots and beets.

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Note:

During the editing process it was not possible to access the work of retouching and improvement of images, so they have been inserted with the same quality as the ones sent by their authors.

The editorial