

Short communication

ACIDITY TOLERANCE OF RHIZOBIA FROM *Canavalia ensiformis* NODULES

Comunicación corta

Tolerancia a la acidez de rizobios provenientes de nódulos de *Canavalia ensiformis*

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ABSTRACT. The biofertilization of legumes with tolerant acidity rhizobia would allow a better establishment of these plants in acid soils. The objective of this study was to determine the acidity tolerance of rhizobia isolated from *Canavalia ensiformis* nodules. Five rhizobia isolates were used and their growth capacity at different acidity levels was performed. A second assay was performed where the pH influence on the viable cells number was determined. The statistical software Statgraphics version 5.0 was used to process the second test data. A simple classification variance analysis was performed and the means were compared for the Tukey test. All microorganisms grew at pH 3,5; 4,0; 4,5; 5,0 and 5,5. The isolates Can4 and Can6 had a higher viable cells number at pH 4,5 than pH 6,8. Both isolates are promising to improve the *Canavalia ensiformis* establishment on cuban acid soils.

Key words: forage legumes, simbiosis, acid soil

INTRODUCTION

Canavalia ensiformis (canavalia) is a forage legume that has high yields in grains and forage, in favorable soil and climatic conditions. In Cuba it is one of the species most commonly used as green manure (1,2). This legume is grown mainly in soils dedicated to livestock, which are characterized by their acidity,

RESUMEN. La biofertilización de leguminosas con rizobios tolerantes a la acidez permitiría un mejor establecimiento de estas plantas en suelos ácidos. El objetivo del presente trabajo fue determinar la tolerancia a la acidez de aislados de rizobios provenientes de nódulos de *Canavalia ensiformis*. Se emplearon cinco aislados de rizobios y se determinó la capacidad de crecimiento de los microorganismos en diferentes niveles de acidez. Se realizó un segundo ensayo donde se evaluó la influencia del pH en el número de células viables. El programa estadístico Statgraphics versión 5.0 se empleó para procesar los datos del segundo ensayo. Se realizó un análisis de varianza de clasificación simple y las medias se compararon a través del test de Tukey. Todos los microorganismos crecieron a pH 3,5; 4,0; 4,5; 5,0 y 5,5. Los aislados Can4 y Can6 presentaron un mayor número de células viables a pH 4,5 que a pH 6,8. Ambos aislados resultan promisorios para mejorar el establecimiento de *Canavalia ensiformis* en suelos cubanos afectados por la acidez.

Palabras clave: leguminosas forrajeras, simbiosis, suelo ácido

a limiting condition that affects more than 30 % of Cuban soils (3).

The capacity of canavalia to fix atmospheric nitrogen in symbiosis with the rhizobia favors the growth of other accompanying crops, increasing the nutritional value of the same (4-6). The biofertilization of canavalia with rhizobia obtained from nodules of this plant and adapted to acidic conditions in the soil would improve its establishment in the field. Based on the above, the objective of the present investigation was to determine tolerance to the acidity of rhizobia isolates from *Canavalia ensiformis* nodules.

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MATERIALS AND METHODS

Five isolates of rhizobia from *Canavalia ensiformis* nodules, effective in the Biological Nitrogen Fixation, were used. These plants were cultivated in acid soils of Cascajal, Villa Clara. The cultural, morphological and physiological characterization of the isolates made it possible to classify them as possible members of the *Rhizobiaceae* family (7).

TOLERANCE TO ACIDITY IN THE CULTURE MEDIUM

Two tests were carried out. In the first, the isolates were cultured by exhaustion in medium mannitol-solid yeast extract (LM) (8), at different pH (3,5; 4,0; 4,5; 5,0 and 5,5). PH 6,8 was used as control of the experiment. The plates were incubated at 28 ± 1 °C for five days and the presence of bacterial growth was determined. For the second test, inoculants were prepared with the isolates of rhizobia in liquid LM medium with pH 4,5 and pH 6,8 (control).

The number of colony forming units (UFC mL⁻¹) was determined at 20 h of culture. The count was carried out by the method of serial dilutions, which were cultured by dissemination on plates with solid LM medium.

The data from the first test did not have statistical processing because it was a qualitative analysis. Those from the second test were processed through the statistical package Statgraphics version 5.0, 2000 (9). A simple classification variance analysis was performed. The means were compared through the Tukey test for 5 % significance (10), after verifying that they fulfilled the normal distribution (Bartlett) and homogeneity of variances (Kormogorov-Smirnov) adjustment (11). The SigmaPlot 2001 program was used to graph the data (12).

RESULTS

The behavior of the isolates of rhizobia is shown in the Table. These microorganisms showed a similar growth in the different pH values.

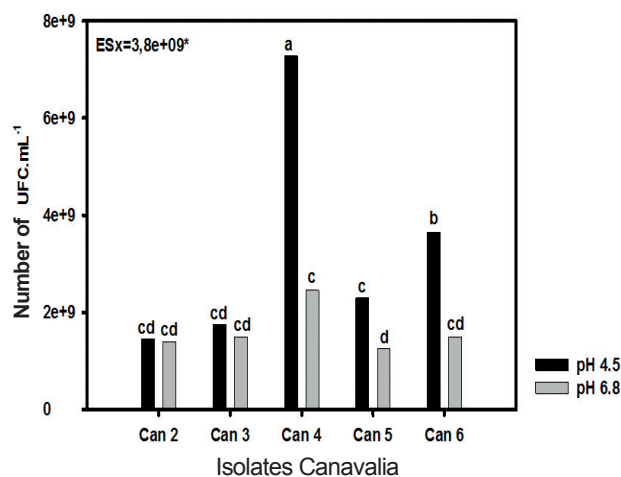
The effect of the acidity conditions on the number of viable cells of the isolates of rhizobia is observed in the Figure.

Only significant differences were observed between the number of viable cells of Can4 and Can5 at pH 6,8. However, both isolates together with Can6 had a significantly higher number of viable cells in pH 4,5 than in pH 6,8. The isolated Can 4 had the highest number of viable cells at pH 4.5.

Table. Growth of rhizobial isolates in AML medium with different levels of acidity

Microbial bateria	Acidity level (pH)					
	3,5	4,0	4,5	5,0	5,5	6,8 (control)
Can2	+	+	+	+	+	+
Can3	+	+	+	+	+	+
Can4	+	+	+	+	+	+
Can5	+	+	+	+	+	+
Can6	+	+	+	+	+	+

Legend: (+), growth



Common letters do not differ significantly (Tukey, $p < 0,05$)

Figure. Number of viable cells of the rhizobial isolates in LMA medium with pH 4,5 and 6,8

DISCUSSION

The five isolates of rhizobia were tolerant to the acidity conditions that were evaluated (3,5-5,5) since they presented a growth similar to the control (Table). These microorganisms belong to the *Rhizobiaceae* family, which groups the genera *Rhizobium*, *Sinorhizobium* and *Ensifer* (13,14). The literature has discussed the tolerance of some *Rhizobium* species to the conditions of acidity in the medium (15,16).

Soil acidity can limit the growth of the rhizobia communities in the rhizosphere and also their ability to infect the plant (17). Hence the importance of selecting strains whose growth is not negatively affected in soils with this limitation.

When analyzing the effect of acidity on the number of viable cells, Can4 and Can6 presented a greater capacity to remain viable at pH 4,5 than at pH 6,8 (Figure). This could indicate the presence of mechanisms that allow these microorganisms to survive in acidic conditions.

The soils of the Cascajal, Villa Clara region are characterized by their acidity with values close to pH 4,7 (3). This characteristic constitutes a decisive factor in tolerance to the acidity of the isolates studied. It has been reported about the selection that stressful conditions such as acidity in soils exert on populations of rhizobia, which favors those that are better adapted and competitive in the symbiosis (18).

CONCLUSION

The tolerance to acid pH of the Can4 and Can6 isolates makes them promising microorganisms to improve the establishment of *Canavalia ensiformis* in Cuban soils affected by acidity.

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Received: January 14th, 2016

Accepted: February 9th, 2017