Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i13.7019

PREDICTION OF THE AMOUNT OF ENDOPHYTIC BACTERIA IN ROOTS OF DICHANTIUM ARISTATUM BENTH IN THREE LOCALITIES AS A FUNCTION OF PH

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Submitted: 2 February 2023 Accepted: April 2023 Published: 6 June 2023

ABSTRACT
The aim of this study was to evaluate the presence of endophytic bacteria in roots of *Dichantium aristatum* Benth present in cattle farms in three municipalities of the department of Sucre and to relate the presence and absence of these bacteria with respect to pH values. Isolation of endophytic bacteria by roots was carried out, counting was performed by serial dilution technique on R2A agar surface, quantity was determined with respect to municipality and presence of these bacteria with respect to pH values. The results show higher quantities of endophytic bacteria in roots of *D. aristatum* located in cattle farms in the municipality of Corozal (Colombia) (8.5 X 10^8 CFU/g root) and lower quantities in the municipality of Tolú (4.16 X 10^5 CFU/g root). Likewise, lower quantities of endophytic bacteria were found in livestock farms in the municipality of Tolú where pH values were acidic to moderately acidic. Conclusions. This is the first work at regional and national level on the presence of endophytic bacteria with respect to municipality and pH, which will allow in the future to infer how physical, chemical and biological parameters affect the presence of endophytic bacteria to determine the quality and sustainability of pasture agroecosystems in Colombia.

Keywords. Bacteria, rhizosphere, locality, density, chemical parameter.

1. INTRODUCTION

The main pasture species cultivated in the northern Caribbean region of Colombia are: *Botriochloa pertusa* (colosoana or kikuyo); *Branchiaria mutica* (Admirable); *Dichanthium aristatum* (Angleton) and *Pennisetum* sp (King grass). Angleton grass (*Dichantium aristatum* Benth) represents the third species with the largest area planted in Sucre, reaching an area of approximately 56,200 ha, distributed in 19 municipalities Pérez y Peroza (2013).

Associated with plant species are the endophytic bacteria, which can live inside the tissues during part of their life cycle without causing any damage to the host, establish symbiotic associations and produce great benefits for the plants. These bacteria fulfil a wide range of functions such as promoting

plant growth, biological control over a variety of phytopathogens, and improving the efficiency of phytoremediation processes of toxic compounds in the rhizosphere (Pérez and Chamorro, 2013).
Endophytic bacteria reside within pasture tissues without causing damage to their hosts (Perez et al., 2018), promote plant growth, remove contaminants, solubilize phosphates and fix nitrogen, and can be employed as a control of phytopathogens. The isolation and use of endophytic bacteria associated with different plant species to improve nutrient uptake in different plants is widely documented (Sá et al., 2019; White et al., 2019; Ochoa-Hueso et al., 2020). Among endophytic bacteria, species of the genus *Bacillus* spp. are promoters of plant growth by supplying nutrients and regulating water transport and indicate that they are an underexploited source of novel molecules of biotechnological interest (Lopes et al. 2018).
Between 5 to 50% of the biomass of soil microbes are considered as diverse and active biological communities essential for increasing the sustainability of agroecosystems. There is a knowledge gap regarding the relationship between endophytic bacterial diversity and physicochemical and biological parameters with respect to the presence and/or absence of bacterial populations and their functionality in livestock farming ecosystems.
Based on the above situation, the need arises to evaluate in situ the colonization of roots with arbuscular mycorrhizal fungi in relation to physical and chemical parameters of the soil. These facts justify further studies to determine the amount of endophytic bacteria in pasture agroecosystems and their relationship with soil pH. The aim of this study was to evaluate the amount of endophytic bacteria associated with *Dichantium aristatum* Benth in three municipalities in the department of Sucre and to relate the presence of these bacteria to the pH present in the rhizosphere of livestock farms.

2. MATERIALS AND METHODS

Sampling. Sampling was carried out randomly in a zig-zag fashion, collecting soil (1000 g) and 10 complete plants of the rooted pasture established on each livestock farm at the time of sampling from each site per municipality. The samples were identified with the respective variety and date of collection. They were stored and preserved for transport to the Microbiological Research Laboratory of the University of Sucre and processed within 24 hours after collection.

Isolation of endophytic bacteria associated with *Dichantium aristatum* roots. Plants collected from each pasture by municipality were subjected to a surface disinfection process. Roots of each plant were washed with sterile water and cut into approximately 1 cm segments. The surface disinfection process for each tissue was carried out according to the methodology recommended by Perez et al (2015, 2018). After the disinfection process, each tissue was placed in a porcelain dish and macerated with liquid nitrogen until a homogeneous sample was formed. Serial dilutions of 10-1 to 10-8 were prepared from each homogenate, from which 0.1 mL aliquots were taken and placed on the surface of R2A agar culture medium and incubated at 32 °C for 72 hours. The population density of bacteria per tissue (CFU/g tissue) was determined by direct colony counting on the surface of the agar plates. During counting, colonies that differed in shape, texture, color and size were observed and selected.

Determination of pH in livestock farms. Samples were collected in an area of one hectare for each municipality, marking the sampling points at random, covering the entire area. For sampling, the methodology recommended by IGAC (2015) was used. A 100g of soil was taken, spread on a plastic sheet and dried at room temperature in a dry place for four days; then, a 2 mm round sieve was used to obtain fine soil. The methodology of Olarte et al., (1979) was used to evaluate parameters such as pH.

3. RESULTS AND DISCUSSION

Figure 1 shows the quantity of endophytic bacteria associated with roots of *Dichantium aristatum* Benth in three municipalities of the department of Sucre. The highest density of bacteria was found in the municipality of Corozal (Colombia) in a range of 2.5 X 10^8 ± 9.6 X 10^8 CFU/ g of roots, followed by the municipality of Sampués with a density of 2.5 X 10^7 ± 9.1 X 10^7 CFU/ g of roots and finally the lowest amount was found in the municipality of Tolú (1.2 X 10^5 ± 6.7 X 10^5 CFU/ g of roots). The density of endophytic bacteria varied according to the area and environmental conditions existing in each municipality for the same pasture species. Studies carried out by Pillay and Norwark related to the population density of endophytic bacteria, associated with different plant species, consider that the presence of these bacteria is highly variable; this variation depended on the species of bacteria and the genotype of the host plant, as well as the stage of development of the plant, the inoculum density and the environmental conditions.

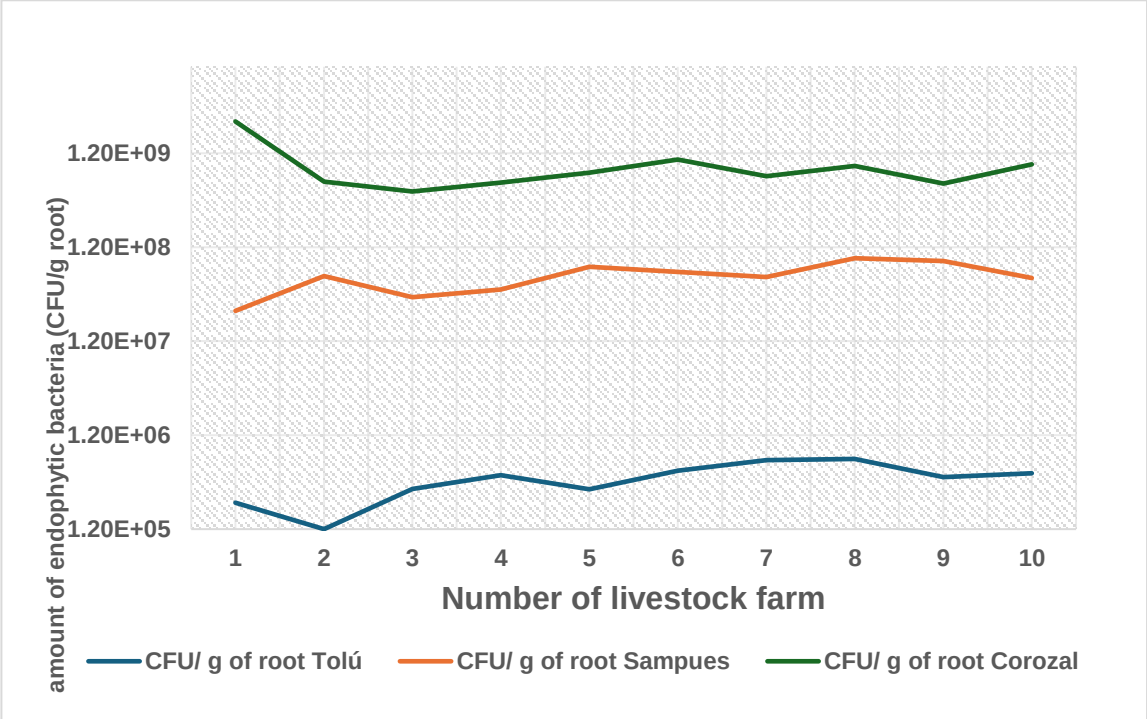

Figure 1. **Population density of endophytic bacteria isolated from *Dichantium aristatum* Benth grass roots in three localities in the department of Sucre, Colombia.**

Figures 2, 3 and 4 show the relationship between population density of endophytic bacteria and soil pH in livestock farms in the municipalities of Tolú, Sampués and Corozal (Colombia). In figure 2, it is observed that in the municipality of Tolú there is the lowest quantity of endophytic bacteria in roots (1.2 X 10^5 ± 6.7 X 10^5 CFU/ g of roots), when the pH values oscillated in the range of (5 to 6.7), while in the municipality of Sampués the density varied (2.5 X 10^7 ± 9.1 X 10^7 CFU/ g of roots) when pH values were between 6.10 to 6.9 (figure 3). Finally, in the municipality of Corozal (Colombia), the highest densities of endophytic bacteria in roots were found (2.5 X 10^8 ± 9.6 X 10^8 CFU/ g of roots), when the soils of the farms analyzed had pH values between 6.9 and 7.5 (figure 4).

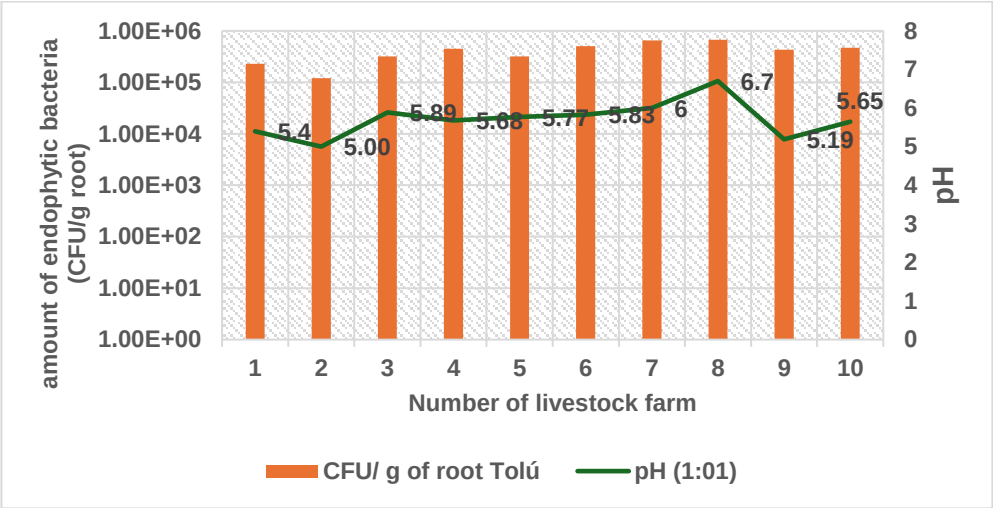

Figure 2. Quantity of endophytic bacteria in roots of *Dichantium aristatum* Benth in relation to pH of cattle farms in the municipality of Tolú, department of Sucre, Colombia.

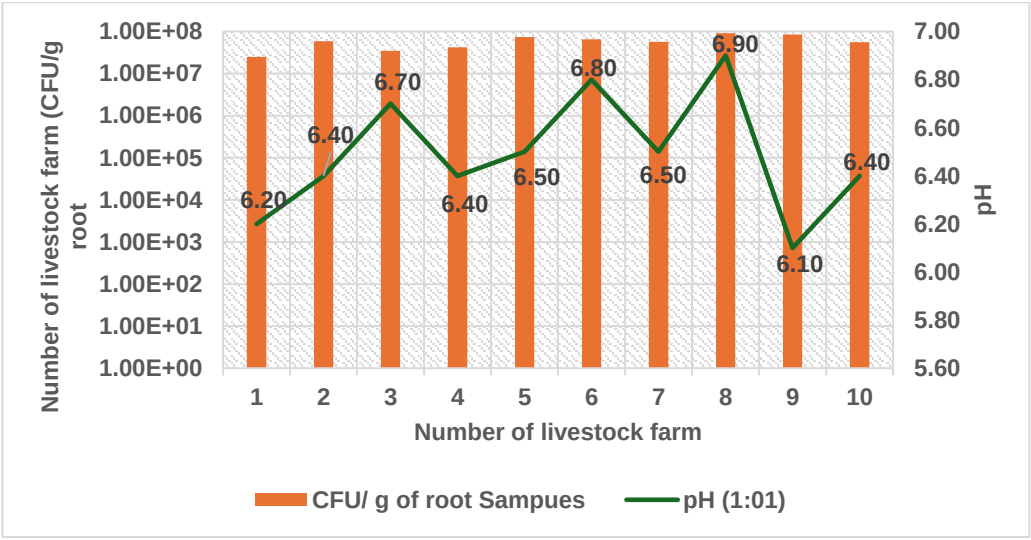

Figure 3. Quantity of endophytic bacteria in roots of *Dichantium aristatum* Benth in relation to pH of cattle farms in the municipality of Sampués, department of Sucre, Colombia.

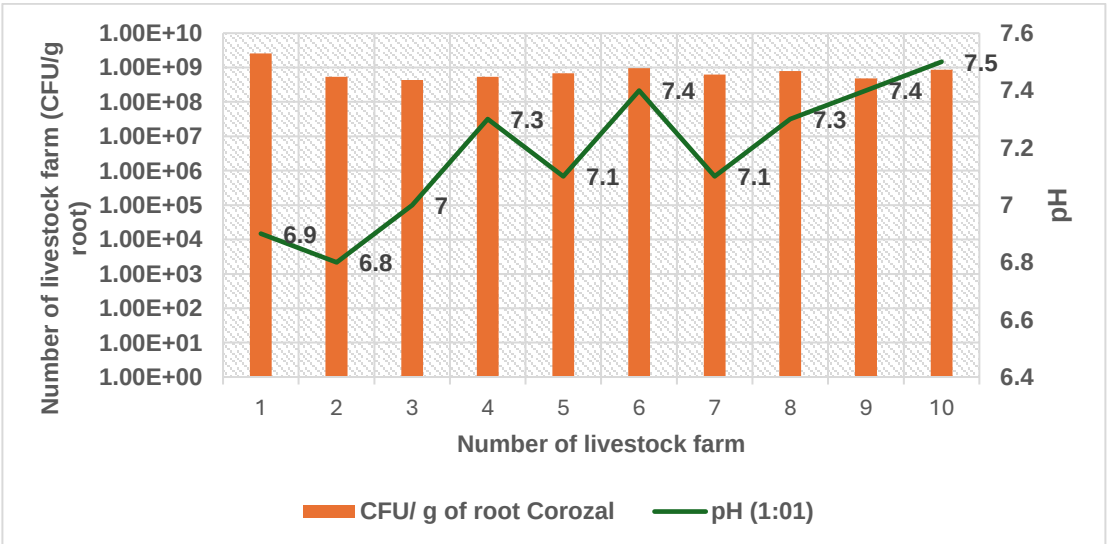

Figure 4. Quantity of endophytic bacteria in roots of *Dichantium aristatum* Benth in relation to pH of cattle farms in the municipality of Corozal, department of Sucre, Colombia.

The results found infer that when the pH values in the rhizospheres are at acidic or modernly acidic conditions, the density of endophytic bacteria is lower, while the highest densities of endophytic bacteria associated with *Dichantium aristatum* Benth roots were found at values close to neutral (6.9 to 7.5).

There are no conclusive studies on how physicochemical parameters affect the presence of endophytic bacteria? However, the present study related the pH factor to the presence of endophytic bacteria associated with roots of *Dichantium aristatum* Benth pasture in three locations in the department of Sucre, Colombia. According to Safir and Duniway (1991), pH influences the solubility of phosphorus, and the solubility and availability of other elements to plant roots in the soil, including iron, manganese, copper, zinc and toxic amounts of aluminum. In general, plant rhizosphere-associated micro-organisms are considered to be adapte to the soil pH of their origin and thus may become a limiting factor for the establishment of endophytic bacteria (Sylvia et al., 1993).

There are few research reports at global and national level that evaluate the integration between physical, chemical and biological parameters in relation to the quality of the soil ecosystem. The information that does exist is inconclusive and difficult to interpret, given the great complexity of the system under study, where several characteristics are evaluated, generating a larger number of data, which makes it proportionally more difficult to interpret. A strategy to evaluate the set of biological indicators associated with ecosystems commonly suggested is the analysis through biostatistical tools (multivariate analysis) that allows the conversion and interpretation of a set of variables with a high correspondence between certain indicators and a component, resulting in a high absolute weight of the indicator in a given component (Pérez and Vertel, 2010).

4. CONCLUSION

There is a high density of endophytic bacteria associated with roots of *Dichantium aristatum* Benth grass, adapted in three municipalities of the department of Sucre, Colombia, with varied edaphoclimatic characteristics. The results show that there is a direct relationship between population density of endophytic bacteria and pH, with lower densities in conditions of acid to moderately acid pH and high density of these bacteria when pH values are neutral. There are few research reports worldwide and nationally that evaluate the integration between physical, chemical and microbiological parameters in relation to the quality of the soil ecosystem of livestock farms, which is why it is proposed to continue conducting evaluative studies on the quantity of endophytic bacteria and physical, chemical and biological indicators of soil dedicated to tropical pastures.

5. ACKNOWLEDGEMENTS

The authors express their gratitude to the laboratory of microbiological research, attached to the Faculty of Agricultural Sciences of the University of Sucre for the development of this research.

6. AUTHOR CONTRIBUTION. Alexander Perez Cordero: experiment execution, data analysis. Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

7. CONFLICT OF INTEREST. All the authors of the manuscript declare that they have no conflict of interest.

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