# Evaluation of the Amount of Endophytic Bacteria Associated with Roots of Brachiaria Humidicola Cv. Humidicola (Rendle) Schweick in Relation to Ph Values, Organic Matter and Phosphorus Contents of Cattle Farm Soil

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## Abstract

The objective of this study was to isolate endophytic bacteria from roots of Brachiaria humidicola cv. humidicola (Rendle) Schweick and to relate their presence to pH, organic matter and phosphorus contents of the soil of cattle farms located in the municipality of San Benito Abad. Soil sampling was carried out with roots of B. humicola for the isolation and counting of the population density of endophytic bacteria and soil samples were also collected for the determination of pH, organic matter and phosphorus parameters. The results found indicate that there is an amount of endophytic bacteria in roots of B. humidicola when the soil contains optimum pH values of (6.1 ± 6.98); low to moderate phosphorus content (10.8 ± 16.28 mg/kg) and low to moderate organic matter content (1.7 ± 2.31%). The presence of endophytic bacteria was observed in a range of 3.0 ± 8.0 X 106 CFU/g of roots. There is a worldwide need to establish a relationship between endophytic bacteria and soil physical-chemical quality indicator parameters to predict a change in functional diversity under physical, chemical and biological conditions of the soil ecosystem.

**Keywords:** Endophytic bacteria, soil, pasture, abiotic parameters.

## 1. Introduction

As described by (Tapias and García, 2017), in the soil ecosystem, the main components of organic matter are Carbon (C), Nitrogen (N), P and Sulphur (S). P is the nutrient that must be supplied almost entirely by the weathering of the parent material, because it has a low atmospheric return.

According to (Silvia et al., 1998), micro-organism populations and their interactions are key components in the soil-plant continuum, affecting crop development. The biological fixation of atmospheric nitrogen by diazotrophic bacteria is the second most important process after photosynthesis in nature. In the absence of nitrogen fertilizers or animal waste, the dynamics of agroecosystems depend entirely on a group of microorganisms that have the ability to reduce atmospheric nitrogen to ammonium through the synthesis of an enzyme complex called nitrogenase.

White and Metcalf, (2007) point out that the element phosphorus (P) is essential for life, being fundamental in the metabolism of organisms. In addition to participating in countless metabolic pathways, P is a component of essential cell molecules, such as phospholipids, RNA, DNA and the main nucleotide cofactor (ATP), required for energy transfer and cellular catalysis.

As stated by (Mora, 2006), active organic matter, which represents about 10-20 % of the total soil organic matter, is constituted by soil microbiota, responsible for the decomposition processes of organic substrates and for the resynthesis of substances that give rise to other metabolic products such as mucilages, gums, acids, enzymes and extracellular polysaccharides, and of course $CO_2$.

Liu et al., (2013). and collaborators point out that there is evidence of the positive effect induced by endophytic microorganisms mediated by the expression of mechanisms such as antagonism, induced systemic resistance (ISR) and acquired resistance (AAR), plant growth promoters, and the induction of an adaptive response to environmental stress. Likewise, Higgins et al. (2007) indicate that the relationship between the host plant and the endophytic bacterial community reflects co-evolution in the colonization process influenced by genotype, growth stage, physiological state and plant tissue, as well as by soil characteristics, agronomic practices and environmental conditions such as temperature, water and nutrient supply.

According to the United Nations report (2022). The world's population is growing at an alarming rate, with a projected 8.5 billion inhabitants by 2030. This brings with it a clear increase in the demand for agricultural products to meet the needs of all inhabitants. At the same time, climate change on the planet generates flooding events, severe changes in temperature and prolonged periods of drought, among many others, which drastically impact the productivity of various crops. Given the unfavorable circumstances facing the agricultural sector, it is essential and urgent to find solutions to increase the production of economically important crops and to deal with the environmental impacts that may arise. In this search for strategies, the solution could be found where we least expect it Where? Perhaps endophytic bacteria hold the answer to climate change conditions and their importance for plant nutrition and changes in physical and chemical parameters as indicators of soil quality.

Based on the above hypothesis, the objective was to isolate endophytic bacteria from roots of Brachiaria humidicola cv. humidicola (Rendle) Schweick and to relate their presence to the pH, organic matter and phosphorus content of the soil of livestock farms located in the municipality of San Benito Abad.

## 2. Materials and Methods

Sampling. It was carried out in the soil of cattle farms in the municipality of San Benito de Abad, department of Sucre, sown with the grass species botanically identified as Brachiaria humidicola cv. humidicola (Rendle) Schweick.

Collection of soil samples. With the help of a PVC plastic tube of 3.8 cm in diameter and 25 cm in length, soil samples were collected at a depth of 0-20 cm, introducing, rotating and extracting the cylinder with the sample (soil and roots). Between 15-20 samples were taken from each farm, these were homogenized per farm to form one sample with a weight of 2000 grams. Two subsamples (1000 g each) were used to isolate endophytic bacteria from roots and one subsample of 1000 g for the determination of soil physico-chemical parameters (phosphorus ‘P’, pH and organic matter ‘OM’). The labelled samples in plastic bags were taken to the laboratory and stored at 4°C until further analysis (Pérez and Vertel 2010).

Isolation of endophytic bacteria. Isolation of endophytic bacteria from Brachiaria humidicola roots was carried out as follows:

Surface disinfection of roots. Tissue (root) separation was performed on each plant collected. Each root was subjected to a surface disinfection process using protocols described by Pérez et al. (2018).

Determination of population density. Once the disinfection process was completed, each root was deposited on porcelain plates, macerated with the help of liquid nitrogen until homogenized and serial dilutions (10-1 to 10-8) were prepared in triplicate, from which aliquots were taken and deposited on the surface of R2A agar, incubated at 32ºC for 72 hours. Bacterial population density per tissue (CFU/g tissue) was determined by direct colony counting on the surface of the plates (Pérez et al., 2018).

## 3. Results and Discussion

Figure 1 shows the distribution of the amount of endophytic bacteria in roots with respect to the parameters of pH, organic matter content and phosphorus in the soil. The figure indicates that between optimum pH values (6.1 ± 6.98); low to moderate phosphorus content (10.8 ± 16.28 mg/kg) and low to moderate organic matter content (1.7 ± 2.31%), the presence of endophytic bacteria was observed in a range of 3.0 ± 8.0 X $10^6$ CFU/g of roots.

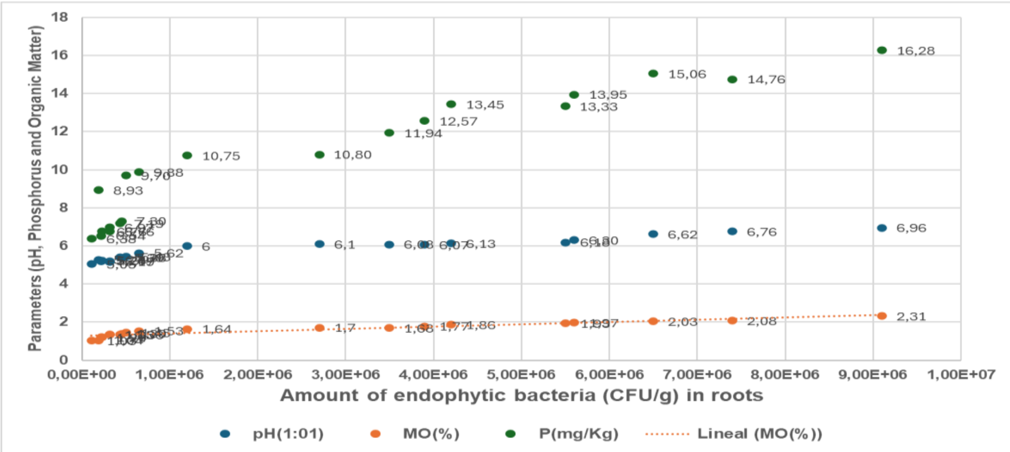


Figure 1. Quantity of endophytic bacteria in roots (CFU/g of tissue) as a function of pH values, phosphorus content and organic matter of cattle farms located in the municipality of San Benito de Abad, department of Sucre.

Figure 2 shows the relationship between the amount of endophytic bacteria as a function of pH values present in the soils of the cattle farms. The figure shows that the highest values of endophytic bacteria (CFU/g root) 1. 1 x 105 ± 9.1 x 106 found were observed under pH values of 6 ± 6.98; while the lowest amounts of bacteria (1.1 x $10^5$ ± 9.1 x $10^6$ CFU/g root) were present under pH values of (5.05 ± 5.62).

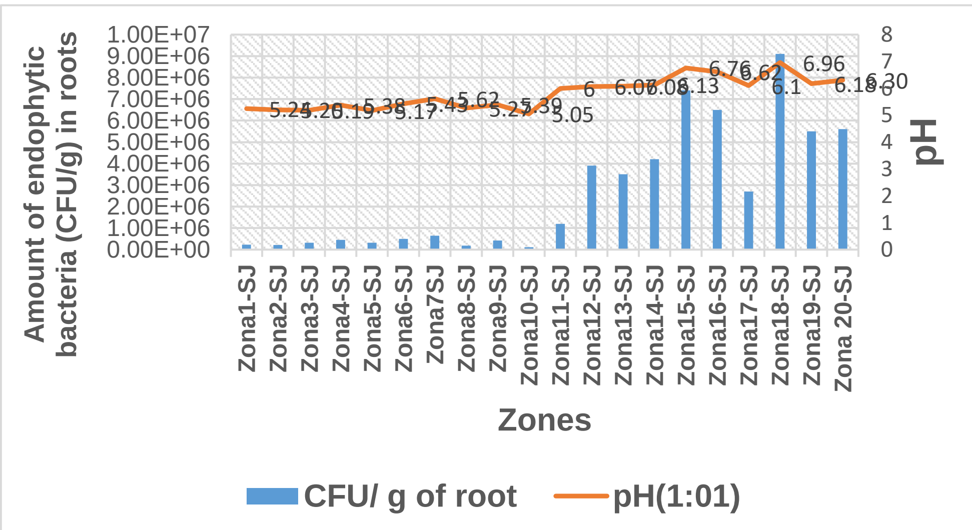


Figure 2. Amount of endophytic bacteria in roots (CFU/g of tissue) as a function of pH values, from livestock farms located in the municipality of San Benito de Abad, department of Sucre.

In figure 3, the relationship between the amount of endophytic bacteria associated with roots and the organic matter values present in the soil is described. The figure shows how the highest population densities of bacteria (1.1 x $10^5$ ± 9.1 x $10^6$ CFU/g of roots) occurred when the organic matter values oscillated in a range of (1.03 ± 2.31 %).

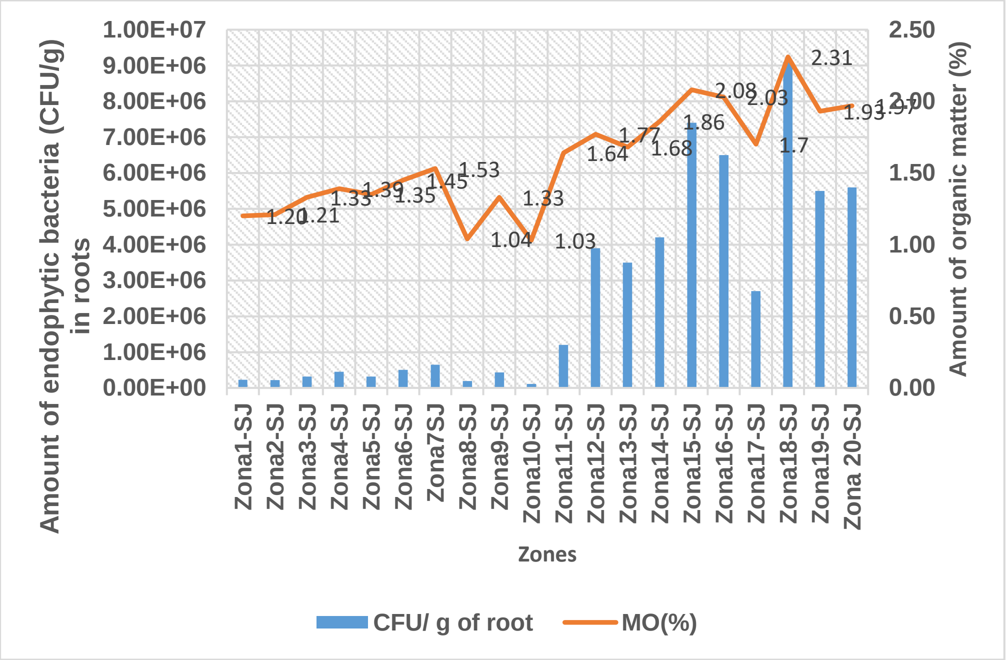


Figure 3. Amount of endophytic bacteria in roots (CFU/g of tissue) as a function of the amount of organic matter in livestock farms located in the municipality of San Benito de Abad, department of Sucre.

Figure 4 shows the relationship between the amount of endophytic bacteria associated with roots and phosphorus values present in the soil. The figure shows how the highest population densities of bacteria (1.1 x $10^5$ ± 9.1 x $10^6$ CFU/g of roots) occurred when the values of organic matter oscillated in a range of (6.38 ± 16.28 mg/kg).

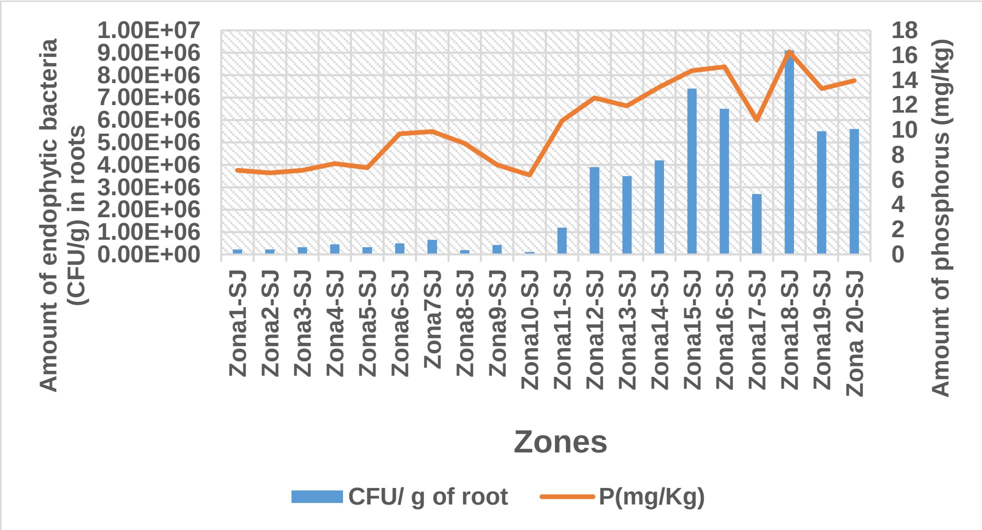


Figure 4. Amount of endophytic bacteria in roots (CFU/g of tissue) as a function of phosphorus content in livestock farms located in the municipality of San Benito de Abad, department of Sucre.

According to Harris (1992), it has been established that soils with high organic matter content tend to contain more organisms with complex demands, and that the soil fraction associated with plant roots (soil rhizosphere) has a higher level of organisms with simple demands, although subsequent applications appear limited. Similarly, the (IGAC, 1993), points out that, in the rhizosphere, where organic nutrients are more abundant than in the soil as a whole, algae are one of the few groups whose numbers are decreasing. The fungal flora generally releases less $CO_2$ per unit of aerobically transformed carbon than the other microbiological groups, as fungi are more efficient in their metabolism.

As stated by Viveros-Aguilar et al., (2023), endophytic bacteria seem to be the ideal solution for all problems related to plant nutrition, there are still many challenges to overcome in order to exploit the full potential of these microorganisms, because sometimes their high performance is not always maintained under field conditions. Some of the obstacles facing the development of strategies for the implementation of endophytic bacteria as biostimulants in plants are related to a) Differences in plant-bacteria interaction in different plant species (there is no general biostimulant on the market that promotes growth in all plant species) and b) The tolerance response to abiotic and biotic stresses, there is no bacterium or bacterial consortium so far that gives a uniform response for this purpose.

Studies related to the influence of physical-chemical parameters on the in situ establishment of endophytic bacteria in roots are scarce, which makes it difficult to make a comparison with the results obtained in the present work. There is evidence of high colonización of endophytic fungí in other pasture species such as Andropogon gerardi, when soils have low to moderate levels of phosphorus, nitrogen, organic matter and medium alkaline pH values (Barea and Jefries, 1995).

The establishment of endophytic fungi under field conditions is determined by various conditions such as: soil physico-chemical factors (pH, phosphorus content, temperature, aeration, texture and organic matter content), climatic conditions (light intensity and duration, temperatures, humidity, rainy and dry seasons) and by agronomic practices (soil preparation, pesticide application and cultural practices). In soils, the effect of pH is difficult to assess, because chemical properties change with pH, as does the solubility and availability of other elements to plant roots in the soil, including iron, manganese, copper, zinc and toxic amounts of aluminum (Perez and Vertel, 2010).

Also, according to Xin et al. (2005), the effects of phosphorus in the soil can be associated with other factors such as soil type, pH and nitrogen levels. High and low levels of phosphorus and nitrogen fertilization reduce the percentage of mycorrhizal colonization, while moderate levels of P increase nitrogen levels and mycorrhizal colonization. The form of inorganic nitrogen in the soil influences both the colonization rate, root length and the presence of colonizing structures such as arbuscules and vesicles in endophytic fungi.

It is also inferred that pH, soil moisture and nutrient availability influence not only colonization but also the number of spores produced by endophytic fungi (AMF). AMF are found in all types of soils and can colonize any plant that establishes symbiosis with them, however, the physicochemical conditions of the soil could be generating some kind of host plant specificity, depending on the responses that plants show to certain AMF species (Pérez and Vertel, 2010).

Finally, endophytic bacteria are considered to have emerged as an alternative for the development of ecological and sustainable agriculture. Due to their different growth-promoting mechanisms, such as phosphate solubilisation, nitrogen fixation and phytohormone production, these bacteria have promising uses in the development of biofertilisers, biopesticides and biostimulants (Viveros-Aguilar et al., 2023).

## 4. Conclusion

In the present study the presence of endophytic bacteria was found in roots of Brachiaria humidicola cv. humidicola (Rendle) Schweick, when the soils of the cattle farms have optimum pH values of (6.1 ± 6.98); low to moderate phosphorus contents (10.8 ± 16. 28 mg/kg) and low to moderate organic matter contents (1.7 ± 2.31%). The presence of endophytic bacteria was observed in a range of 3.0 ± 8.0 X $10^6$ CFU/g of Brachiaria humidicola cv. humidicola (Rendle) Schweick roots from cattle farms located in the municipality of San Benito Abad. In the search for more sustainable and environmentally friendly alternatives in pasture agriculture, the scientific community worldwide has turned its attention to endophytic microorganisms. These microorganisms not only establish a beneficial symbiosis with plants, but also offer an effective solution to protect your crops, as indicators of physical-chemical soil quality and to predict a change in functional diversity in the presence of abiotic parameters and changes due to climate variability.

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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.

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

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