Biofertilizing potential of a fertilizer based on cienego and native microorganisms in corn seeds

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Abstract. In the development of the productive process of stone aggregates, during the stage of washing and extraction of sludge from the decanter wells, a “cienego” sand is produced in an approximate quantity of 24.75 tons per day, of which only 10% per day is commercialized, the remaining 90% must be discarded, causing financial detriment, impact on the environment in terms of pollution by handling the product that is discarded, occupation of useful areas and landscape degradation, among other impacts negatively affecting the companies in the sector, and their environment. The objective of this research was to evaluate a fertilizer based on cienego and three native microorganisms (Azotobacter sp, 1 Azotobacter sp, 2 and Pseudomonas sp) in corn seeds. For this purpose, three native strains of diazotrophic bacteria were isolated from soil samples of oil palm crops in Tibú, located at Norte de Santander, Colombia, which were biochemically identified using traditional culture media. These isolates were inoculated into corn seeds in trays with sterile soil plus cienego at 70/30 ratio respectively, and the plant growth-promoting effect was recorded every 4 days, by measuring morphological variables such as height, number of leaves, number of roots, length and germination rate. The results showed that Azotobacter sp, 2 treatment presented a higher production of roots, reached greater height (cm), number of leaves and germination rate in comparison to control treatment after 12 days of monitoring, besides directly influencing the percentage of organic matter in the substrate and elements such as calcium, potassium and phosphorus, allowing greater productivity and corn seed yield once these were germinated.

1. Introduction
Colombia is one of the agricultural producers with a diversity of plantations throughout its territory, obtaining a multitude of products and raw materials in all thermal floors. According to data from the “Departamento Administrativo de Estadística (DANE)” [1], the national area used for agricultural activity was 7.6% by 2015; of this percentage, 62.26% was used for permanent crops, 34.74% for transients and fallow land, and the rest for fallow land. The crops with long useful life have stood out in recent years, due to their expansion and high technology in the Colombian national territory, as is the case of fruit trees and African palm. The latter is the second largest crop (450.131 Ha in 2014) after corn, has better oil yields (3.14 ton/ha per year in 2014) against oilseeds, and is of great importance in the Caribbean region, along with bananas and plantains. However, inadequate soil management has brought ecological consequences that have substantially affected the productivity of the land, which has
led to the intensive use of agrochemicals of great polluting potential in the production of these crops, which has affected soil quality, environmental deterioration and the health of animals and humans [2].

In this sense, the systems of inoculation and cultural management of microorganisms with biofertilizing properties become a rational and integral technological option; an efficient and promising productive practice in the agricultural sector due to the fact that microorganisms in association with crops are important inputs for the improvement of production and environmental control [3], as well as allowing the maintenance of biodiversity and the sustainability of ecosystems [4]. Such is the case of the bacteria of free life of the genera Azotobacter sp and Pseudomonas sp which are found in the rhizosphere and can stimulate plant growth through different processes [5] such as: synthesis of plant growth regulators [6], nitrogen fixation [7], nutrient solubilization [8], production of siderophores and control of soil phytopathogens [9].

However, the biological fertilization is only one of the strategies to maintain productivity in the agricultural sector, where not only adequate nutrition is needed but also the reduction of fertilizer costs and obtaining maximum production per unit of applied nutrient [10] in an environmentally friendly way.

For this reason, the use of natural materials such as the marsh, a grit obtained in the development of the productive process of stone aggregates during the stage of washing and extraction of sludge from the decanter wells, would contribute to the strengthening of the science and engineering of materials by providing new knowledge about a material that until a few years ago was considered a waste but that according to chemical analysis shows organic and physical characteristics to be constituted as a fertilizer. However, of this daily production of cienego, only 10% of the day is sold, the remaining 90% must be discarded, causing financial detriment, impact on the environment in terms of pollution by handling the product that is discarded, occupation of useful areas and landscape degradation, among other impacts negatively affecting the organization, stakeholders and their environment.

Therefore, the aim of this research was to prove the biofertilizing effect of diazotrophic strains native of Azotobacter sp and Pseudomonas sp in Norte de Santander, Colombia on corn plants planted in a substrate based on a cienego compared to a control, since this is an area of agricultural potential by excellence, where these biotechnological tools would contribute to the reduction of adverse impacts of agrochemicals.

2. Materials and methods

The experimental work was developed in the laboratories of the Universidad de Santander, San José de Cúcuta, Colombia. The cienego used for the experiments was supplied by the company Transmateriales S.A. Table 1 specifies the composition of the substrate (cienego).

| Element          | Expression | Results (%) |
|------------------|------------|-------------|
| Total nitrogen   | NT         | ND          |
| Nitric nitrogen  | N-NO3      | ND          |
| Ammoniacal nitrogen | N-NH4    | ND          |
| Ureic nitrogen   | N-NH2      | ND          |
| Total phosphorus | P2O5       | 0.12        |
| Total potassium  | K2O        | 0.13        |
| Total calcium    | CaO        | 2.03        |
| Total magnesium  | MgO        | 0.1         |
| Total sulphur    | S          | 0.05        |
| Total manganese | Mn         | 0.01        |
| Total copper     | Cu         | ND          |
| Total zinc       | Zn         | 0.01        |
| Total sodium     | Na         | 0.01        |
| Total silicon    | SiO2       | 88.8        |
2.1. Selection of isolates
The isolates were obtained from soil samples from oil palm crop in Tibú, Norte de Santander, Colombia. For this, 500 g of sterile soil were taken with shovels per sample from different points of the crop in duplicate at a depth of 10 cm - 20 cm [11]. Of the isolates obtained, three were selected that showed excellent growth in Ashby agar and Winogradsky broth (Azotobacter sp (AZT1), Azotobacter sp (AZT2) and Pseudomonas sp) and were biochemically identified using traditional culture media.

2.2. Obtaining inoculants
From each of the selected isolates, an inoculation was carried out in 250 mL of soy trypticase broth with constant agitation at 165 r.p.m., at room temperature for 24 hours, until obtaining a liquid inoculant with a minimum concentration of $10^8$ cells/mL, using the McFarland 5 standard.

2.3. Inoculation
For inoculation, the protocol proposed by Moreno [2] was followed with some modifications. 500 grams of soil plus 500 g of sterile cienciego were placed in aluminum trays and 15 corn seeds per tray were sown with three replicates for a total of 180 seedlings per treatment. The whole surface of the soil was sprayed with 25 mL of the liquid inoculum with the bacteria to be evaluated. They were immediately watered and moved to an open place where the sunlight reached them and water was supplied daily.

2.4. Monitoring and evaluation of inoculants
The number of plants germinated in the trays at 3, 6, 9 and 12 days was determined, also recording their height, number of leaves, length and number of roots. A completely random design was established consisting of four treatments with five repetitions each and at a concentration of cienciego and biofertilizing microorganisms 30/70, respectively. The comparisons between each treatment were made by Tukey's method using an analysis of variance (ANOVA).

2.5. Soil analysis
To observe the effect of the treatments on the chemical properties of the soil, two soil subsamples were taken before and after planting corn seeds, were homogenized to obtain a significant sample and processed to determine organic matter, phosphorus, potassium, calcium, magnesium and sodium among others.

3. Results and discussion

3.1. Evaluation of the plant growth-promoting effect of native microorganisms
Figure 1 shows the results obtained in the test on the effect of inoculants on stem length, leaf length, number of leaves and number of roots. The AZT2 treatment showed significant differences with respect to the other treatments for the above-mentioned parameters. In terms of stem length, the AZT2 treatment was followed by the treatment (Pseudomonas sp) and in terms of leaf length and number of leaves, this was exceeded by the AZT1 treatment, being evident the stimulating effect of inoculants against the parameters mentioned. These results coincide with Sánchez et al., [12], Arellano et al., [13], Armenta et al., [14] Piromyou et al., [15], who demonstrated that Azotobacter sp and Burkholderia sp at corn root level convert their exudates into plant growth-promoting substances for greater formation of secondary roots that optimize soil nutrient absorption and consequently there is an increase in length, number of secondary grass roots [16] and stem growth [14,17].

Figure 2 shows the germination percentage achieved by the different treatments at day 12 of sampling, observing 85% of germination for the T2D treatment (AZT1), followed by T1C (Pseudomonas sp.) reaching 80%. On day 9 the T1C treatment showed a germination of 70% followed by T2D (AZT1) with 60%, being evident the effect of native microorganisms such as AZT1 and AZT2, according to Arellano, et al., [13] suggest that Azotobacter sp. converted the exudates of the spermosphere of the corn seed into a vegetable plan growth promoting substance (PGPS), to end the
latency of the embryo and accelerate its germination speed, the investigations suggests that there are organic compounds of the amino acid type such as tryptophan that the seed of the grass exudes and transformed it in PGPS. Rojas and Moreno [18] have found that Azotobacter sp. has beneficial effects on the germination and growth of the crops, due to the integral stimulation of the components of the growth in these plant through the production of great amounts of capsular slime, concluding that its application can increase the stability of the same one, this proves the assertion given by Barea [19] who state that microorganisms that grow in the rhizosphere have greater metabolic activity from which plants benefit and the number of organisms in this is determined by the intensity of rooting of the plant, the rate of germination of microbial propagules in the soil and their movement to the root, as well as the rate of growth of microorganisms, likewise depends on soluble substrates present in the rhizosphere.

![Figure 1. Effect of native microorganisms evaluated Azotobacter sp (AZT1), Azotobacter sp (AZT2) and Pseudomonas sp on height (stem length), number of roots, number of leaves and leaf length.](image1)

![Figure 2. Effect of inoculation on the germination percentage of corn seeds.](image2)

According to Moreno [1], soil organic matter is a key indicator of soil quality, both in its agricultural functions and in its environmental functions, including carbon capture and air quality, as well as being the main determinant of its biological activity; this allows us to deduce the presence of Azotobacter sp inoculated in the soil sample from the AZT2 treatment since the variations in the chemical and physical properties of the soils depend on its influence [20] giving a value of 4.15%, above the value obtained in the soil sample from the control treatment 2.93% (see Table 2). Similarly, organic matter mineralized releases organic phosphorus in ways easily exploited by plants or can absorb phosphorus in its organic radicals, in this sense, the highest content was presented by the control soil sample against the AZT2 treatment, however, this also allows deducing its uptake by the microorganism reflected in the positive
results obtained according to the morphological variables analyzed in the seedlings once these germinated, which places the cienego as an optimal material for use as fertilizer.

Likewise, it is to appreciate the development of strategies by corn plants in the presence of the inoculant *Azotobacter sp* the established by Patiño and Sanclemente [21] who affirm that as an evolutionary response to the low se to the low availability of phosphorus (P) in soils, plants develop different morphological, biochemical and symbiotic adaptive strategies to increase the acquisition of inorganic phosphorus (Pi) or to improve the efficiency of its internal use, being one of the most important the association of the root with soil microorganisms, able to mineralize or solubilize organic and inorganic P sources, respectively.

| Table 2. Physicochemical analyses performed on the substrate plus the inoculant *Azotobacter sp* 2 and control substrate. |
|---|
| Analytes | Soil + Cienego 70/30 + microorganism-free control | Substrate +Azotobacter (AZT2) | Measurement |
| pH | 7.89 | 7.90 | % |
| Organic matter | 2.93 | 4.15 | % |
| Ca | 1.70 | 2.41 | % |
| P | 247.00 | 210 | ppm |
| K | 1.97 | 2.40 | mEq/100g |
| Ca | 15.20 | 14.50 | mEq/100g |
| Mg | 1.45 | 1.74 | mEq/100g |
| Na | 0.31 | 0.30 | mEq/100g |
| Fe | 16.50 | 22.10 | ppm |
| Mn | 16.20 | 16.60 | ppm |
| Cu | 0.42 | 0.39 | ppm |
| Zn | 2.35 | 2.39 | ppm |
| S | 44.30 | 43.40 | ppm |
| B | 0.71 | 0.56 | ppm |

The Potassium levels were low (1.97), which can affect the development of the plant and microorganisms, because potassium and phosphorus catalyze important processes such as respiration, photosynthesis, chlorophyll formation and regulation of water content in leaves, as well as is linked to the transport and accumulation of sugars within the plant [22]. As for calcium (2.41%), it is observed that it is high in comparison with the control substrate since they are associated with low contents of magnesium (1.74 mEq/100 g) and potassium (1.97 mEq/100 g), which causes an imbalance and therefore low availability for plants.

4. Conclusions

According to the results of the bromatological analysis, organic matter must be taken into account as a parameter that makes the difference, allowing the soil inoculated with biofertilizing microorganisms to show a higher productivity of the corn crop allowing rapid growth, related to the capacity of the corn seed to exchange with the soil the nutrients it requires, corroborate the positive value that bacteria of this genus have in fundamental biological processes such as nitrogen fixation and, as a consequence, the potential to be used as biofertilizers.

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References
[1] Departamento Administrativo Nacional de Estadística (DANE) 2016 Estadísticas agropecuarias: Encuesta nacional agropecuaria ENA 2015 (Colombia: Departamento Administrativo Nacional de Estadística) p 2
[2] Moreno L 2013 Caracterización de Azotobacter spp y otras bacterias diazótrofas para su posible uso como biofertilizante (Colombia: Universidad Francisco de Paula Santander)
[3] Carvajal J, and Mera A 2010 Fertilización biológica: técnicas de vanguardia para el desarrollo agrícola sostenible Produción + Limpia 5 1
[4] Lynch M and Whipp 1990 Substrate flow in the rhizosphere Plant and Soil 2 15
[5] Zaidi A, Ahmad E, Khan MS, Saif S and Rizvi A 2015 Role of plant growth promoting rhizobacteria in sustainable production of vegetables: Current perspective Scientia Horticulturae 193 231
[6] Rivera-Cruz M, Trujillo M, Córdova G, Kohler J, Caravaca F, and Roldán A 2008 Poultrymanure and banana waste are effective biofertilizer carriers for promoting plant growth and soil sustainability in banana crops Soil Biol & Bioch 40 3092
[7] Álvarez C, Scianca C, Barraco M and Klappenbach J 2007 Impacto de fertilizantes biológicos sobre la productividad del cultivo de girasol (Córdoba: Instituto Nacional de Tecnología Agropecuaria)
[8] Torriente D 2010 Aplicación de bacterias promotoras del crecimiento vegetal en el cultivo de la caña de azúcar Tropical Crops 31 19
[9] Cline M 1944 Principles of soil sampling Soil Sci 58 275
[10] Sanchez J, Lopez I, Villegas J, and Montaño N 2014 Respuesta del maíz (Zea mays L) a la inoculación con Azotobacter sp y Burkholderia sp a dosis reducida de fertilizante nitrogenado Scintia Agropecuaria 5 17
[11] Arellano Y, García E, and Vázquez J 2008 Estimulación de la síntesis de ADN y de proteínas de ciclo celular por auxinas durante la germinación de maíz Agrociencia 42 637
[12] Armanta A, García C, Camacho J, Apodaca M, Montoya L, and Nava E 2010 Biofertilizantes en el desarrollo agrícola de México Revista Sociedad, Cultura y Desarrollo Sustentable 6 51
[13] Piromyou P, Buranabanyat B, Tantasawat P, Tittabutr P, Boonkerd N and Teaumroong N 2011 Effect of plant growth promoting rhizobacteria (PGPR) inoculation on microbial community structure in rhizosphere of forage corn cultivated in Thailand European J of Soil Biol 47 44
[14] Wong A and Caballero J 2010 Rapid identification of nitrogen fixing and legume nodulating Burkholderia species based on PCR 16S RNA species-specific oligonucleotides Syst Appl Microbiol 33 35
[15] Camelo M, Vera S and Bonilla R 2011 Mecanismos de acción de las rizobacterias promotoras del crecimiento vegetal Revista Ciencia y Tecnología Agropecuaria 12 159
[16] Rojas V and Moreno B 2010 Eficiencia del Azotobacter chroococcum en la producción de posturas de Coffea Arabica L Cultivos tropicales 15 58
[17] Barea J 1998 Biología de la rizosfera Investigación y Ciencia 256 74
[18] Roberts T and Henry J 2000 El muestreo de suelos: los beneficios de un buen trabajo Informaciones Agronómicas 42 4
[19] Patiño C and Sanclemente O 2014 Los microorganismos solubilizadores de fósforo (MsF): una alternativa biotecnológica para una agricultura sostenible Entramado 10 288
[20] Otero J 2011 Aislamiento, selección e identificación de actinomicetos, bacterias fotosintéticas no sulfurosas y bacterias acidolácticas con potencial biofertilizante a partir de suelos asociados al cultivo de plátano en la costa atlántica colombiana (Bogotá, Universidad Nacional de Colombia)