RELATIONSHIP BETWEEN SOIL PHYSICOCHEMICAL CHARACTERISTICS AND NITROGEN-FIXING BACTERIA IN AGRICULTURAL SOILS OF THE ATLÁNTICO DEPARTMENT, COLOMBIA.

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SOIL MICROBIOLOGY IN THE ATLÁNTICO DEPARTMENT

Microbial richness of agricultural soils is an indicator of its health and fertility with a significant impact on crop yields. Present study analyzed the relationship between nitrogen-fixing bacteria and physicochemical characteristics of agricultural soils in the southern department of Atlántico, Colombia. Soil samples were collected from 10 sites of Repelón irrigation district, for physicochemical analysis (pH, organic matter, texture, moisture and available phosphorus) and isolation of nitrogen-fixing bacterial' strains using nitrogen-free culture media. Results demostrated the higher previlance of nitrogen–fixing bacteria in northern zone and central zone of the Repelón irrigation district (1.63×10^8 CFU g^{-1} for strain-1, 5.2×10^7 CFU g^{-1} for strain-2 and 4.5×10^7 CFU g^{-1} for strain-3). On the other hand, the physicochemical characteristics of soil show the adequacy to sustain nitrogen-fixing bacteria. The findings of the present research may serve as a baseline to identify soil micorganisms and defining strategies for sustainable management of agricultural soils in the region because these are integral component in ecosystem for nutrient recycling.

Keywords: native microorganism, fertilization, sustainable management, conservation.

In agriculture, nitrogen is the principal nutrient for plant growth (García, 2011) and productivity. Nevertheless, nitrogen mineralization is very slow process (1 to 3% of the total N of the soil) (Escobar *et al*., 2011). In nitrogen-deficient soils, it is necessary to use nitrogenous fertilizers to increase yield of the crops. However, indiscriminate use of these fertilizers and losses after their application (through erosion, leaching and volatilization) can cause serious soil contamination problems. These problems are mainly related to the increase in soil salinity, decrease in soil pH (mostly of nitrogenous fertilizers exert an acidifying action on the soil), and the contamination of groundwater (Martínez-Mera *et al*., 2016; Iturri and Buschiazzo, 2016). At the same time, these alterations in the physicochemical characteristics of soils, depending on the climatic conditions of the zone, can bring changes in the distribution, diversity and abundance of soil microorganisms (Mahmood *et al*., 2006; Gupta and Roper, 2010). The identification of alternative sources of fertilization that reduce impacts on soils is necessary for the development of sustainable agriculture and sustainable soil management practices (Martínez-Mera *et al*., 2016).

Nitrogen-fixing bacteria are among important microorganisms in agricultural soils which are recognized free-living or non-symbiotic (e.g. *Clostridium*, *Beijerinckia*, and *Azotobacter* genera) and mutualistic or symbiotic (e.g. genera of *Rhizobium*, *Frankia*, and certain species of *Azospirillum*) associated with different species of plants. Nitrogen-fixing bacteria are prominent because of their capability to transform inert nitrogen from atmosphere into bioavailable form for plants (Philippot and Germon, 2005). Biological nitrogen fixation is critical for ecosystem productivity (Dahal *et al.*, 2017). In addition, these bacteria have the ability to promote plant growth through synthesis of plant growth regulating hormones (e.g. indole acetic acid), inhibition of growth of plant pathogens and decrease in incidence of disease through secretion of antibiotic like substances and increase in P nutrition through phosphates solubilization (Escobar *et al*., 2011).

In order to promote the establishment of sustainable agricultural systems, it is necessary to have a fundamental knowledge of different components that comprise it (Ferrera and Alarcón, 2001). In Colombia, few studies have been carried out on this subject (e.g. Mantilla-Paredes *et al*., 2009), particularly agricultural areas in the south of Atlántico Department. Qualitative and quantitative information concerning soil microflora is very scarce and there are no reports on this aspect. Thus, it is necessary to generate information on the health of agricultural soils of the south of Atlántico Department represented by populations of nitrogen-fixing bacteria. Finally, this research may serve as a baseline to define strategies for sustainable management of agricultural soils of south Colombian region.

The study was carried out in agricultural soils of the Irrigation District of Repelón (10º 29 'N and 75º 08' O) located south of Atlántico Department, Colombia (Figure 1). The Irrigation District of

Repelón records an average annual temperature of 28.2°C. The annual precipitation varies and dry season is characterized by average rainfall of 39 mm (January to July), and rainy season gives 117.2 mm rainfall that extends from August to December (Climate, 2017). The soils belong to the order inceptisols, subgroups *Fluventic Haplustepts* and *Typic Haplustepts* (IGAC, 2008). Sampling was carried out in June 2016 i.e during dry season. Sampling sites were selected at random and collected soil samples were named according to characteristics zone beginning in the north to the south of irrigation district. At each sampling site, a plant was taken as axis and at 1 m distance, three points were established. At each point the vegetation cover was removed and deepened to 20 cm. Composite soil samples were prepared by mixing the three sub-samples to get representative soil sample for each site (IGAC, 2006). The samples were stored in Ziploc® bags and transported in polyethylene coolers with gel packs, maintaining a temperature of 2 ± 1°C. These samples were maintained under these conditions for 24 h, till processing initiated.

A portion of each sample was stored for the determination of potential nitrogen-fixing bacterial population and another part for physicochemical analyses. All analysis were carried out in the Microbiology and Environmental laboratories of the University of the Costa CUC. The physicochemical parameters evaluated were: pH, humidity, organic carbon (Walkley and Black, 1934) to calculate organic matter and total phosphorus (Olsen *et al*., 1954) (IGAC, 2006). Soil texture was evaluated with Bouyoucos-Densimeter Hydrometer, IGAC method adapted to Colombian soils at certified laboratory.

On the other hand, during the sampling, the study area was surveyed and primary information was collected through interviews with farmers about the agricultural practices. They were asked about general aspects about crop production practices such as area planted, type of crop frequency of pests and use of fertilizers and pesticides (type of pesticide or fertilizer, characteristics, amount applied, method and frequency of application).

For the isolation of nitrogen-fixing bacterial strains, N-selective culture media as asbhy mannitol agar, congo red agar and yeast extract mannitol agar, were used, respectively. Ten grams of composite sample (inoculum) was used to prepare (10^{-1} to 10^{-5}) dilutions. Fifty microliters (50 µL) aliquots of three dilutions (10^{-3}, 10^{-4} and 10^{-5}) were seeded into each of the culture media. For each dilution and for each sampling point, three replicates were made. Additionally, two controls (positive and negative) were maintained to avoid errors due to contamination. After inoculation, culture media were incubated at 37°C for 7 days (Aquilanti *et al*., 2004; Mantilla-Paredes *et al*., 2009). Observations and descriptions of the macroscopic morphological characteristics i.e. color, border, elevation, texture and shape were made. Finally, the isolation of nitrogen-fixing bacteria was confirmed with staining: bromothymol blue staining and Gram staining (Perez *et al*., 2011; Flores-Gallegos *et al*., 2012). The colonies of microorganisms were evaluated by counting viable cells, the CFU g^{-1} soil (number of colonies × dilution factor / volume of inoculum) was determined (Ilyas *et al*., 2008).

The spatial distribution of potentially nitrogen-fixing bacteria in the Irrigation District of Repelón was determined by generating maps of CFU g^{-1} soil with the Surfer program v 23 (developed by Golden Software). In addition, a correlation analysis was performed between the soil physicochemical characteristics and CFU, using the Spearman correlation coefficient. In the statistical tests, the differences were significant with a $P<0.05$. All statistical analyses were performed with the Infostat program (Di-Rienzo *et al*., 2012).

According to field observations, few soils with agricultural activity were found due to scarce precipitation (Table 1). In the study zone, we found nursery with forest trees of neem (*Azadirachta indica*) and annual crops (banana, maize, cassava and bean). In the soils 6 and 8, the crops were cassava, maize, banana and bean, and cassava and banana, respectively. Additionally, the sampling sites without agricultural activity, were listed as rest area with vegetal cover (grass). Only in one place, the soil was in preparation for cultivation. Although farmers depend on supply of irrigation water to crop development, during dry season the irrigation district does not function. However, we found some crops where water supply was very low due climatic conditions. Moreover, the surveys confirmed the use of chemicals such as Lorsban^{TM} 4E (insecticide), glyphosate (herbicide) and NPK 15-15-15 (fertilizer-Triple 15)[1] when the agricultural activity is active. However, at the time of sampling these were not applied due the areas with the crops were small (approximately 25m^2). The farmers communicated that in the dry season, the agricultural activity is low, and they have small areas planted due the irrigation district does not work. In this context, is possible to

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[1] Manufactures name are: Dow (U.S.), Monsanto (U.S.) and Fercon (Colombia), respectively.

carry out manual pest control and no fertilization is done because the crops are for themselves and not for commercialization.

Table 2 shows the physicochemical characteristics of the soil for each sampling point. In the study area, the pH showed variations between slightly acidic (6.4) to slightly alkaline (7.26). Soils presented three textures: silty clay loam, clay and silty clay. On the other hand, soil moisture was generally low, particularly point 7 had the lowest moisture content (0.91%); points 3, 8 and 10 were characterized by higher moisture contents (5.79% on average). The content of organic matter varied in the evaluated soils; point 8 had the highest percentage (6.45%) and point 1 exhibited the lowest value (2.90%); however, according to the soil texture content of this parameter, it is high (Murphy *et al.*, 2002). Similarly, the available soil phosphorus was high, emphasizing that points 6 and 10 were characterized by values greater than 110 mg/kg.

In selective culture media, it was observed that in controls (positive and negative) microorganisms did not grow, guaranteeing that the isolated bacteria were in soil samples. The characteristics of colonies of the strain-1 exhibited red color, lanceolate, filamentous and irregular shape, and flat elevation. In contrast, isolated strains-2 the colonies had white color, irregular shape with filamentous edges, low convex elevation and smooth surface. Finally, in the strains-3 isolates showed yellow, blue-green and cream coloration, lanceolate shape, irregular border, convex elevation and smooth surface. Similarly, the pink staining obtained on gram staining indicated gram-negative bacteria and the yellow staining resulting from the bromothymol blue test allowed us to confirm that the isolated colonies corresponded to potential nitrogen-fixing bacteria.

The 10^{-5} dilution was the most appropriate for CFU counting. The total population of strain isolated of agricultural soils is showed in Table 3. The population of isolated bacteria showed variation between the sampled points (Figure 2), comparing the isolated genera, the CFUs varied in the following order strain-1 > strain-3 > strain-2. In general, in the south, the CFU g^{-1} soil at points 9 and 10 was low. Specifically, average populations were found for the strain-1 isolates of 2.0×10^7, strain-3 of 1.6×10^7 and strain-2 of 1.1×10^7 CFU g^{-1} soil. On the contrary, in the central zone (points 5, 6, 7 and 8) CFU g^{-1} varied. The CFU of the strain-1 isolates remained low, with a small increase to the north, thus reaching 3.15×10^7 CFU g^{-1} soil (points 7 and 8) and 6.55×10^7 CFU g^{-1} of soil (points 5 and 6). In the strain-3 isolates point 6 was the lowest in this area with 1.9×10^7

CFU g^{-1} soil and point 8 showed the highest population for this group with 5.2 x 10^7 CFU g^{-1} soil. As in the central zone, in the north (points 1, 2, 3 and 4), there was variation in populations. In the north zone, strain-1 (points 3 and 4) and strain-2 (points 2) showed the highest population, with an average of 1.33 x 10^8 CFU g^{-1} soil and 4.40 x 10^7 CFU g^{-1} soil, respectively. In contrast, as for the CFU of the isolates strain-3 in this zone, the lowest populations were observed, on average 6.16 x 10^6 CFU g^{-1} soil. The highest population in south zone was in point 8 (5.26 x 10^7 CFU g^{-1} soil).

The correlation analysis between the soil physicochemical parameters and the CFU of isolates compatible with the nitrogen-fixing bacteria showed a significant correlation ($p \leq 0.05$) between the available phosphorus and the strain-3, and between the soil texture with the strain-1 and strain-2 (Table 4).

Free living nitrogen-fixing bacteria naturally fertilize the soil by providing a bioavailable form of nitrogen, which can be used by plants. The presence of this type of bacteria in soils also allows the soil to maintain a constant exchange of nutrients and minerals, thus avoiding erosive effects and loss of soil fertility (Philippot and Germon, 2005). In the present investigation, bacterial strains were isolated on nitrogen-free selective media for potential nitrogen-fixing bacteria. Nitrogen-fixing bacteria commonly inhabit the rhizosphere soil for a long period, colonizing different species of plants with a vast geographical distribution worldwide and other species live independently of the other organisms (Barassi *et al*., 2007). Soil is considered as the natural medium for the growth of plants and microorganisms however, the physicochemical parameters condition their growth (Horneck *et al*., 2011).

Different studies have evaluated the correlation of soil physicochemical parameters and microbial populations. The diversity and density of soil bacterial communities are determined by biotic and abiotic factors. In fact, the growth of microbial populations is dependent on soil type, plant species and soil use, and management activities, because these factors influence the structure of bacterial community (Viera and Nahas, 2005; Horneck *et al*., 2011; Hamid-Dar *et al*., 2012; Mohammad, 2015). Most crops grow best with a pH between 6.0 and 8.2 (Horneck *et al*., 2011). In addition, low-pH soil bacteria decrease their populations and even inhibit N-fixation and nitrification processes. The optimum pH is 7.0, but ranges 5.0-9.0 are tolerable (NRCS, 2011). In this study, the highest population densities (isolates' strains-1) were found in the northern and central part of the Repelón irrigation district, while the southern part presented the lowest population densities for the three strains analyzed. This could be due to the fact that in these soils the lowest values were found in pH and organic matter. The variability in pH could be related to the agricultural activity that has been developed, since the practices of fertilization and pest management using chemicals alter the pH of soil. In soil samples evaluated, the pH average was 6.93. Considering this aspect, the pH of the agricultural soils of the irrigation district of Repelón presents suitable conditions for the establishment of cultures and the populations of microorganisms but the content of organic matter is a factor that is possibly influencing the population density of nitrogen-fixing bacteria.

On the other hand, dry season in the south of the Department was associated with the low soil moisture content and high content of organic matter. The majority of soils were rest soils only with vegetal cover (grass) facilitating the accumulation of organic residues that are finally transformed into organic matter. In the same way, the scarce precipitation avoids the washing of the organic matter (higher precipitations in soils without cover are susceptible to water erosion) therefore this is maintained in the soil (Appelhans *et al.,* 2016). In relation with this factor, soil texture influence some soil properties as organic matter retention, microbial biomass and distribution of minerals (Scott and Robert, 2006). In agricultural systems where the increase of the organic matter content has been favored, microbial biomass and the activity of the phosphatase enzymes increased, hence, the mineralization of organic phosphorus increased (Appelhans *et al.,* 2016). It is probable that texture and phosphorus had positive correlation with CFU because they influenced microbial biomass. Similarly, the low agricultural activity associated with non-use of chemical inputs is a factor that favors the soil physico-chemical properties during the dry season.

Although, no genetic identification of isolated strains was reported in this research, the reported literature on soils and similar environmental conditions in the Colombian Caribbean Region, could suggest different isolated genera compatible with those found in this evaluation. Obando-Castellanos *et al*. (2011) in the Cesar Department, characterized ashybiotic diazatrophic bacteria associated with eucalyptus (*Eucalyptus* spp.). Finding that population diversity, among these genera compatible with *Azospirillum, Herbaspirillum, Burkholderia, Gluconacetobacter, Azotobacter, Beijerinckia* y *Derxia* showed homogeneous population density when comparing times of rain and drought. This response is possibly associated with edaphic alterations where they modify metabolic functions for the availability of carbon, nitrogen and moisture content. It is probable that some of the strains isolated will be compatible with some of the genera due wide adaptability of these bacteria with environmental conditions. On the other hand, some species of the genus *Azotobacter*, *Azospirillum*, *Rhizobium* and *Bradyrhizobium* which in addition to being nitrogen-free fixers, are capable of promoting plant growth through solubilization of inorganic phosphates (Fernández et al., 2005). In this evaluation, in soil 8, where the largest population of CFU was present, the total phosphorus content was found among the highest values. This feature is another reason that probably explains the positive correlation between CFU and available phosphorus.

This research represents the first report on microbiological studies in soils of south of the Atlantic Department. Emphasizing the importance of the municipality of Repelón as an agricultural pantry, it is imperative to know and identify the microbiota, especially nitrogen fixing bacteria, to reduce the use of inorganic fertilizers and to implement environmentally sustainable agricultural practices, such as bioinoculants that maintain and increase soil fertility, thus improve crop yields by lowering production costs.

Conflict of interest

The authors do not have any conflict of interest to declare.

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Table 1. Description of the land uses during the dry season in the Repelón Irrigation District.

Sampling point	Types of plantations				
	Forestry trees	Fruit trees	Annual crops	Rest area	Soil preparation
1. Rest area	+	-	-	+	-
2. Rest area	-	-	-	+	-
3. Rest area	-	-	-	+	-
4. Annual crops	-	-	+	-	-
5. Annual crops	-	-	+	-	-
6. Annual crops	-	-	+	-	-
7. Rest area	-	-	-	-	+
8. Annual crops	-	-	+	-	-
9. Rest area	-	+	-	+	-
10. Rest area	-	-	-	+	-

+ Presence, - Absence

Table 2. Physicochemical parameters during the dry season in the soils of the Repelón Irrigation District.

Sampling point	pH	Texture	Humidity (%)	Organic matter (%)	Total phosphorus (mg/kg)
1. Rest area	7.26	sicl	4.25	2.90	76.2
2. Rest area	6.80	sicl	3.95	5.20	90.6
3. Rest area	6.97	c	5.77	4.05	94.4
4. Annual crops	7.23	c	3.71	6.00	98.5
5. Annual crops	7.20	sic	1.48	5.60	101.6
6. Annual crops	7.22	sic	4.81	5.97	113.0
7. Rest area	6.55	sic	0.91	3.42	106.3
8. Annual crops	7.20	sic	5.99	6.45	108.5
9. Rest area	6.44	c	2.43	3.83	102.8
10. Rest area	6.41	c	5.61	3.58	111.3

Soil texture: sicl (silty clay loam), c (clay), sic (silty clay).

Table 3. Total population of strain isolated of agricultural soils during the dry season in Repelón Irrigation District.

Soil sample	CFU g^{-1} soil		
	Strain-1	Strain-2	Strain-3
1. Rest area	6.13×10^{7}	2.06×10^{7}	9.33×10^{6}
2. Rest area	$2. 80 \times 10^{7}$	4.40×10^{7}	4.66×10^{6}
3. Rest area	$1. 62 \times 10^{8}$	3.40×10^{7}	4.00×10^{6}
4. Annual crops	1.04×10^{8}	1.60×10^{7}	6.66×10^{6}
5. Annual crops	7.53×10^{7}	2.80×10^{7}	2.86×10^{7}
6. Annual crops	5.06×10^{7}	1.60×10^{7}	1.93×10^{7}
7. Rest area	4.86×10^{7}	3.06×10^{7}	3.80×10^{7}
8. Annual crops	1.66×10^{7}	2.80×10^{7}	5.26×10^{7}
9. Rest area	2.93×10^{7}	9.33×10^{6}	1.86×10^{7}
10. Rest area	1.33×10^{7}	1.26×10^{7}	1.33×10^{7}

Table 4. Correlation of CFU g^{-1} soil of nitrogen-fixing bacteria´s strain with the physicochemical parameters of the soil.

		Strain-1	Strain-2	Strain-3	pH	Humidity	OM	P available	Texture
		P-value							
Strain-1	r				0.0804	0.5795	0.8287	0.1615	0.0243
Strain-2					0.8799	0.9466	0.906	0.1905	0.0038
Strain-3					0.8543	0.6032	0.6271	0.0375	0.9716
pH		0.5775	0.0550	-0.0668					
Humidity		-0.2000	0.0243	-0.1878					
OM		0.0787	0.0426	0.1757					
P available		-0.4787	-0.4512	0.6606					
Texture		-0.0261	0.8174	-0.0129					

OM= Organic Matter. $P<0.05$ Significant correlation.

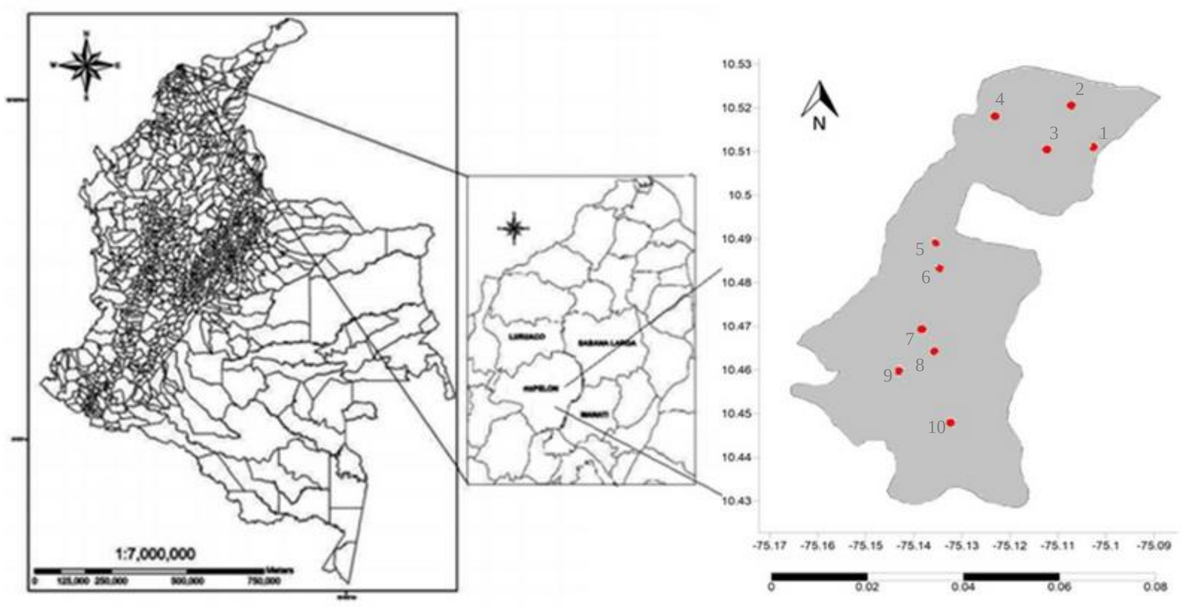
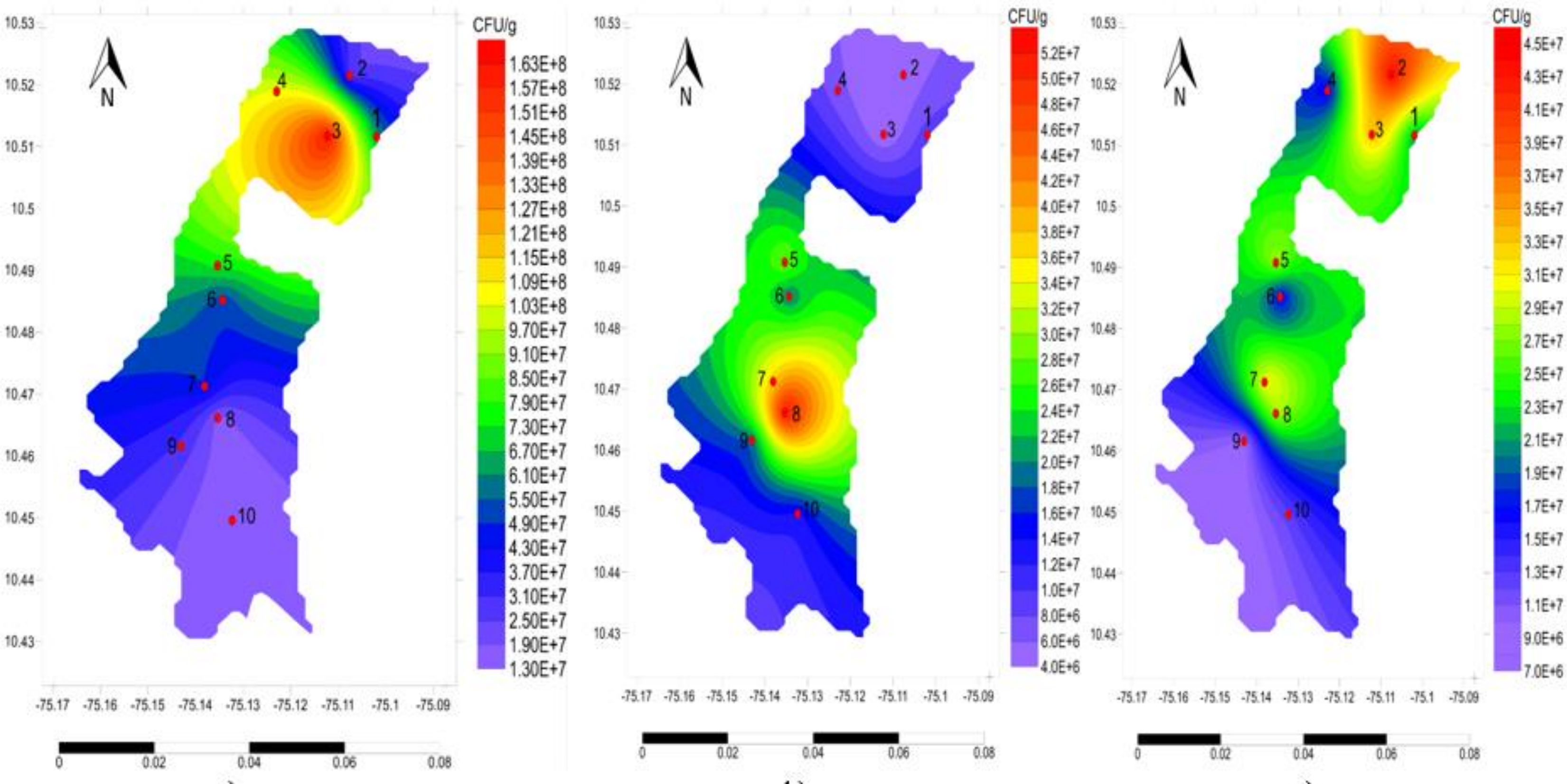


Figure 1. Location of the Repelón Irrigation District (Modified by Torres-Bejarano *et al.*, 2014).



a) b) c)

Figure 2. Distribution of potentially nitrogen-fixing bacteria in the Repelón Irrigation District a) Strain-1, b) Strain-3, and c) Strain-2.