AGRO PRODUCTIVIDAD

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Determination of bacteria morphotypes associated with the rhizosphere of organic coffee plantations

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ABSTRACT

Objective: To determine the bacterial diversity in the rhizosphere of coffee (*Coffea arabica* L.) in coffee plantations in Oaxaca, Mexico.

Design/methodology/approach: Soil samples collected from organic arabian coffee plantations in the Loxicha region of Oaxaca were analyzed to isolate and characterize bacterial populations associated with the rhizosphere of those plantations. Samples were collected from six sites in three altitude ranges (two sampling sites per each range): low (≥1,200, ≤1,400 masl), medium (≥1,700, ≤1,800 masl), and high (≥1,900 masl). Tukey's test was used to compare the bacteria population distribution per altitude range.

A multivariate analysis (Principal Component Analysis and Hierarchical Cluster Analysis) was performed considering four morphological —shape, surface, border, and color— and two microscopic —type and Gram— characteristics of the colonies.

Results: Forty-three bacterial colonies were isolated and purified; their population distribution showed a significant difference (Tukey α=0.5) with respect to the altitude range in which they were collected. The Principal Components Analysis showed that the first three principal components accounted for 74.19% of the total variation of the 43 bacterial colonies, indicating that the evaluated characteristics were widely distributed. The Hierarchical Cluster Analysis determined eight groups and divided them into subgroups, based on the semi partial correlation coefficient (0.05).

Study limitations/implications: The environmental conditions where bacteria grow allow changes in the interspecific variation of each species.

Findings/conclusions: The morphological and microscopic characterization of the bacterial colonies shows a high variability that is expressed in characteristics, indicating a high diversity of bacterial species in organically-managed coffee soils in Oaxaca.

Keywords: *Coffea arabica*, diversity, microorganisms.

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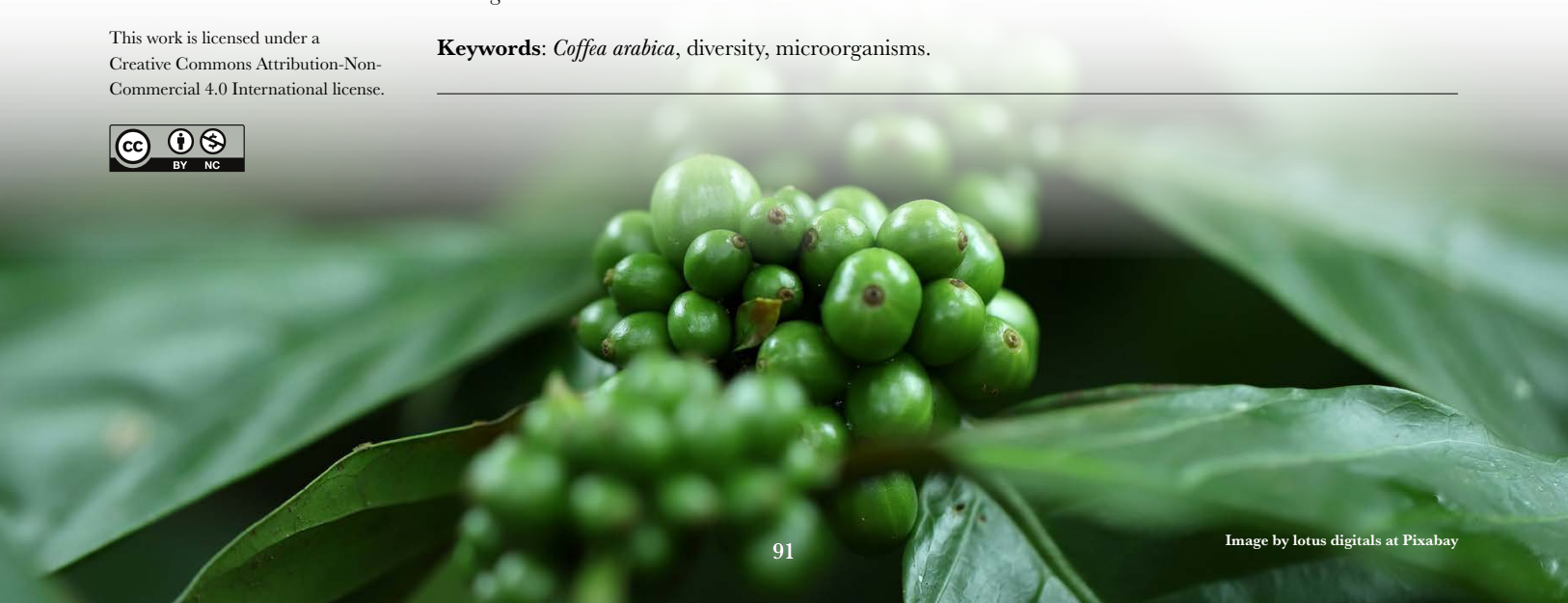

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INTRODUCTION

Coffee (*Coffea arabica* L.) production in Mexico is a fundamental strategic activity (Escamilla *et al.*, 2005), whose importance lies in the fact that it generates multiple social, economic, and environmental benefits (Ruiz-García *et al.*, 2020). In Mexico, coffee is grown in 16,020,284 ha (SIAP, 2021), out of which 519,375 ha are located in the state of Oaxaca (SIAP, 2021). Oaxaca sells coffee in market niches such as organic production, organic coffee, fair trade, and shade-grown coffee (CEDRSSA, 2018). Coffee production systems in Oaxaca are characterized by being shade-grown wooded agroecosystems (Sánchez and Schwentesius, 2015), with a wide tree diversity that includes timber and non-timber forest species, as well as shrubs (Román *et al.*, 2016). The management type and intensity of these production systems directly affects the abundance and composition of the biodiversity that is housed in coffee plantations (Rapidelt *et al.*, 2015).

The soil is a key element within coffee agroecosystems, due to the wide microbial biodiversity it houses (Raaijmakers *et al.*, 2009), which includes: bacteria, rhizobacteria, mycorrhizal fungi, mycoparasitic fungi, and protozoa (Mendes *et al.*, 2003). Rhizospheric bacteria include those that can improve growth and health of plants (Molina-Romero *et al.*, 2015); however, they also include pathogenic bacteria (Beneduzi *et al.*, 2012). The main growth-promoting genera are: *Acidithiobacillus*, *Aminobacter*, *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Clostridium*, *Enterobacter*, *Gluconoacetobacter*, *Pseudomonas*, *Serratia*, and *Sphingomona* (Velasco-Jiménez *et al.*, 2020). The nitrogen-fixer genera are: *Azospirillum*, *Azotobacter*, *Gluconoacetobacter diazotrophicus*, and *Azocarus* (Bhattacharyya and Jha, 2012). Finally, the potassium solubilizers genera include: *Acidithiobacillus*, *Aminobacter*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Cladosporium*, *Enterobacter*, *Paenibacillus*, and *Sphingomonas* (Etesami *et al.*, 2017).

Despite the importance of rhizospheric bacteria, there are few studies about bacterial diversity in coffee soils, which reduces the possibility of taking advantage of this diversity in coffee production systems, both in disease management and in production and yield improvement (Velasco-Jiménez *et al.*, 2020; Granda-Mora *et al.*, 2020). Therefore, the objective of this work was to determine the bacteria morphotypes associated with the rhizosphere of three coffee growing areas located at different altitudes in southern Oaxaca.

MATERIALS AND METHODS

Soil samples were collected in six organic coffee plots, in three altitude ranges (two sampling sites per range): low (≥1200, ≤1400 m), medium (≥1700, ≤1800 m), and high (≥1900 m). A representative sample of 200 g of soil was obtained from each sampling site using the five diagonal point sampling method. The samples were labeled with a key, protected in polypaper bags, and transported to the phytopathology laboratory of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) - Campo Experimental Rosario Izapa, located in Tuxtla Chico, Chiapas, Mexico.

Sampling sites 1 (SBL01) and 2 (SBL02) were located in San Bartolomé Loxicha; sites 3 (SAL01) and 4 (SAL02), in San Agustín Loxicha; and sites 5 (AL01) and 6 (AL02), in El Aguacate Loxicha (Table 1).

Table 1. Locate of sampling sites according to altitudinal range.

Site	Locality	Geographical coordinates (w, n)	Altitudinal range	Organic management (years)
1	San Bartolomé Loxicha	15° 58' 51.4" – 96°42'9.92"	Low (1215)	15
2	San Bartolomé Loxicha	15° 57' 51.29" – 96° 42' 31.59"	Low (1298)	3
3	San Agustín Loxicha	16° 5' 7.224" – 96° 12' 49.2"	Medium (1774)	10
4	San Agustín Loxicha	16° 1' 17.67" – 96° 36' 56.3"	Medium (1729)	5
5	El Aguacate Loxicha	16° 03' 07.4" – 96° 35' 38.0"	High (1919)	12
6	El Aguacate Loxicha	16° 03' 07.6" – 96° 35' 38.3"	High (1913)	2

Isolation of bacterial colonies

Bacterial colonies were obtained by the soil dilution method in Petri-dishes (Waksman, 1927). The stock solution was prepared in a test tube with 10 mL of sterile distilled water and 1.0 g of soil; a 10-2 dilution was obtained from this solution; subsequently, a 0.1-mL aliquot was taken and uniformly placed on Petri-dishes with Potato Dextrose Agar (PDA). The dishes were incubated at room temperature for 72 h and then the number of colonies formed was quantified. The quadrant streak method was used on solid culture medium (PDA) to purify the colonies (Córdoba-Bautista *et al.*, 2009).

Macroscopic and microscopic characterization of bacteriological colonies

The macroscopic morphological characterization of the bacterial colonies was carried out at 72 h of growth. The main morphological characteristics considered were: shape (FOR), surface (SUP), edge (BOR), color (COL), and mucus-forming (FDM) (Table 2). For the microscopic characterization, temporary preparations were made, the Gram stain test was performed (Ramírez-Gama *et al.*, 1998), and the samples were subsequently observed in a compound microscope at 40 and 100x (Leica, DM550®, Heerbrug, Switzerland). For this purpose, the following elements were considered: Cell type (TIC) and Gram (GRA).

The population distribution by altitude range was compared using Tukey's test. A multivariate analysis was performed using qualitative data with the SAS® (Statisticall Analysis System, version 9.4) statistical package. In addition, a Principal Component Analysis (ACP) was carried out to observe the distribution of the characterized strains

Table 2. Morphological characters of the different bacterial colonies evaluated in order to analyze the morphotypes diversity.

Character	Code	Scale
Shape	FOR	1: Punctiform, 2: Circular, 3: Irregular
Surface	SUP	1: Flat, 2: Acuminate, 3: Convex-flat, 4: Umbilicated, 5: Convex
Border	BOR	1: Rounded, 2: Speculated, 3: Undulated
Color	COL	1: White, 2: Yellow
Mucus forming	FDM	1: No, 2: Yes
Type	TIC	1: Cocus, 2: Bacillus
Gram	GRA	1: Positive, 2: Negative

and, subsequently, a Hierarchical Cluster Analysis (ACJ) was carried out to distinguish the groups that the strains had created (Balzarini *et al.*, 2015).

RESULTS AND DISCUSSION

Isolation of bacterial colonies

Forty-three bacterial colonies were isolated and purified from the six sampling sites, distributed in three altitude ranges. This population is similar to that reported by Alcarraz *et al.* (2019) for coffee soils in Peru (60 colonies); however, it is higher than the population reported by Granda-Mora *et al.* (2020) in Ecuador (8 colonies).

The largest number of colonies (23) was obtained in the low altitude range, where site 2 had the largest number of colonies (14); meanwhile, site 6 of the high-altitude range had the lowest number of strains (2) (Figure 1). Similar results were reported by Lyngwi *et al.* (2013), who found a larger number of bacteria in low gradients and a lower number in higher gradients, in tropical and subtropical forests.

Sites where organic management had been applied for a longer time showed a higher number of bacterial colonies. According to Mendes *et al.* (2013), the microbial diversity of soils is related to the type of agronomic management used, as well as its intensity and age (Caldwell *et al.*, 2015). Plots with organic management have greater microbial diversity (Paolini, 2017), because microorganisms have more useful nutrients available to them (Cristóbal *et al.*, 2012).

According to the Tukey's test (α=0.5), the population distribution of bacterial colonies in the altitude ranges showed a statistically significant difference; the low altitude range harbored the highest number of bacterial strains (Figure 2).

Morphological characterization of the colonies

Figure 3 shows the morphological and microscopic characteristics, indicating the frequency of each level of the characteristic of each descriptor.

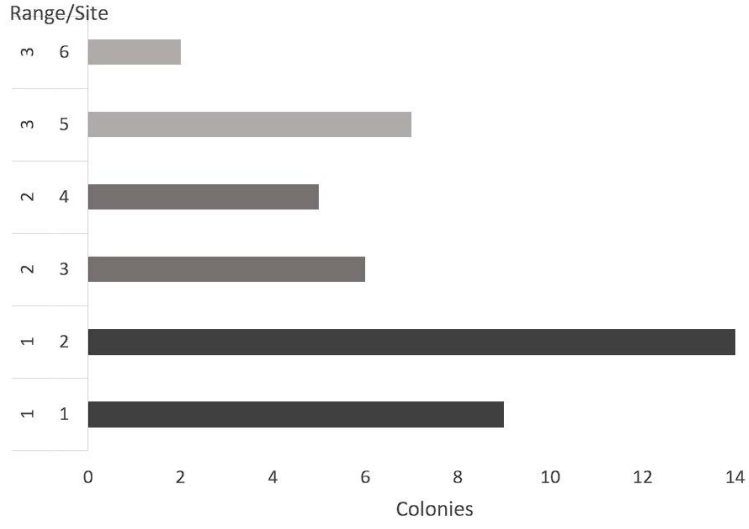

Figure 1. Bacterial colonies frequencies by sampling site. 1=altitudinal low, 2=altitudinal medium, 3=altitudinal hight.

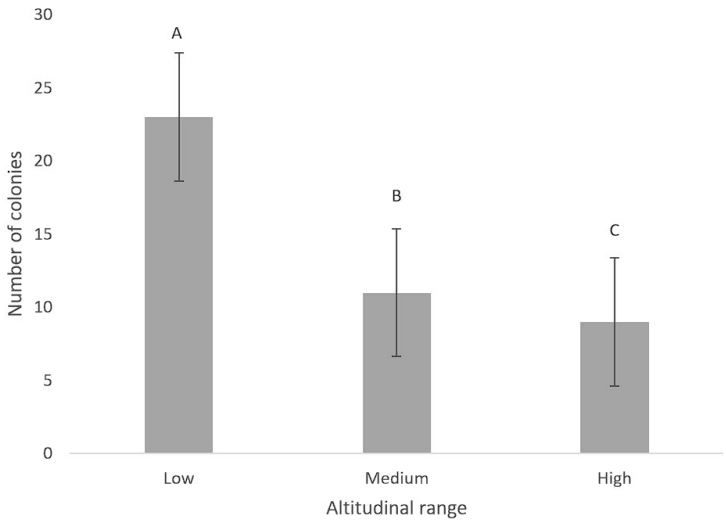

Figure 2. Bacterial colonies distribution according to altitudinal ranges.
Different letters (A, B, C) show significant differences al 95% confidence interval. Low=1200 m to 1400 m, Medium=1700 m to 1800 m and High ≥1900 m.

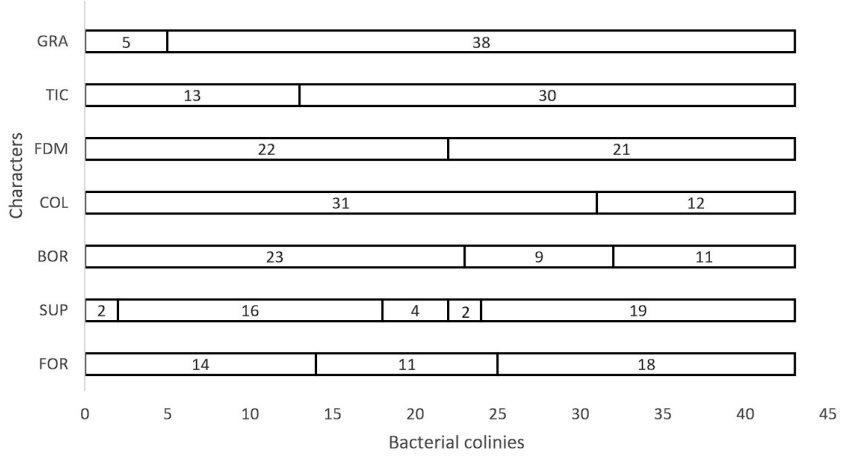

Figure 3. Frequency by interval class of morphological (5) and microscopic (2) character of 43-insolated bacterial colonies from coffee plantation soils in Oaxaca. FOR=shape, SUP=surface, SUP=border, COL=colour and FDM=mucus forming. TIC=cell type and GRA=gram.

The most frequent characteristics of the bacterial populations were: rounded edge (46%), irregular shape (36%), convex surface (38%), gram negative (76%), and rod cell shape (60%).

Principal Component Analysis (ACP)

The first three Principal Components (CP) accounted for 74.19% of the total variability in the 43 bacterial colonies. According to the ACP, the said percentage was divided among CP1, CP2, and CP3 by 33.63%, 21.66%, and 18.9%, respectively (Table 3).

Based on the analysis of the morphological characteristics, the distribution of the strains is wide. These results were obtained using CP1 and CP2 (Figure 4), as well as CP1 and CP3 (Figure 5).

Table 3. Principal Component, eingenvalues and proportion of the total variance explained by morphological characters of the bacterial strains.

CP	Eigenvalue	Difference	Proportion	Accumulated
1	2.35	0.83	0.33	0.33
2	1.51	0.19	0.21	0.55
3	1.323	0.58	0.18	0.74

CP=Principal Component.

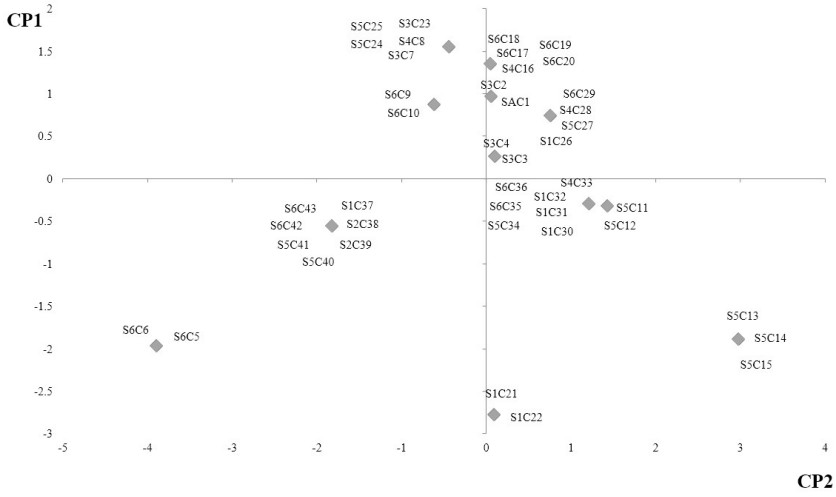

Figure 4. Distribution of 43-insolated bacterial colonies found coffee plantation soils in Oaxaca. Distribution according to the Principal Component 1 (CP1) y 2 (CP2).

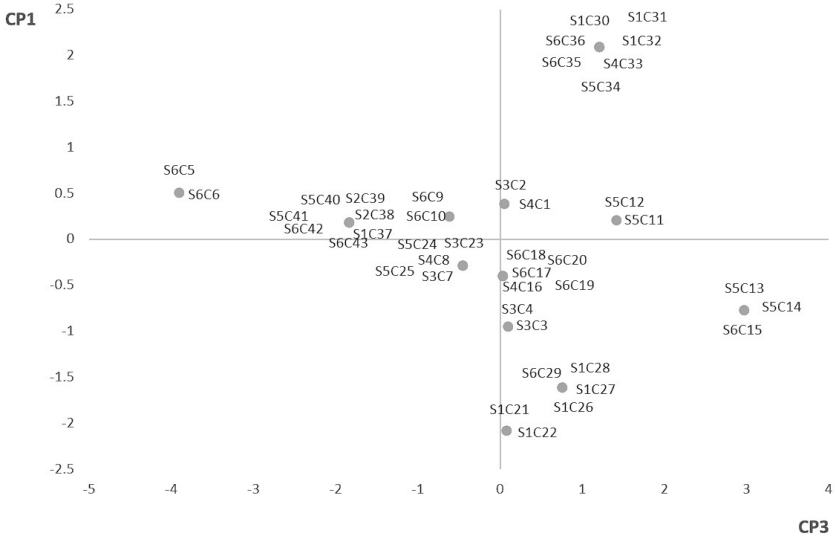

Figure 5. Distribution of 43 bacterial colonies found in coffee plantation soils in Oaxaca. Distribution according to Principal Components 1 and 3 (CP1=Principal Component 1 y CP3=Principal Component 3).

Groups formed from the ACJ

Eight groups were determined (GI, GII, GIII, GIV, GV, GVI, GVII, and GVIII) based on their morphological and microscopic variables; they were subsequently divided

into subgroups based on the semi partial correlation coefficient of 0.005 (Figure 6). The main characteristics of the bacterial colonies according to which the different groups were divided were: GI=rounded edge, white, mucus-forming, bacilli-type, and Gram negative; GII=circular shape; GIII=convex surface and speculated edge; GIV=yellow; GV=undulated edge; GVI=convex surface and irregular shape; GVII=speculated border and bacillary shape; and GVIII=wavy edge (Figure 6).

On the one hand, 100% of the colonies of group V belong to the high range (3), while 100% of groups IV and VII belong to the low range (1); on the other hand, 50% of group II and 45% of group I belong to the middle range (2) (Figure 7).

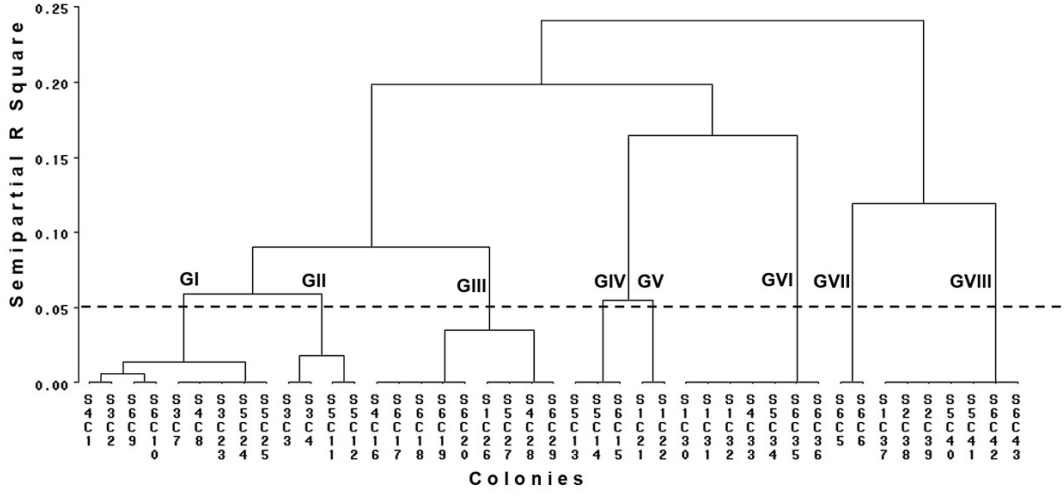

Figure 6. Dendrogram of qualitative characters for 43 isolated bacterial colonies of coffee plantation soils in Oaxaca. GI=Group one, GII=Group two, GIII=Group three, GIV=Group four, GV=Group five, GVI=Group six, VII=Group seven and GVIII=Group eight.

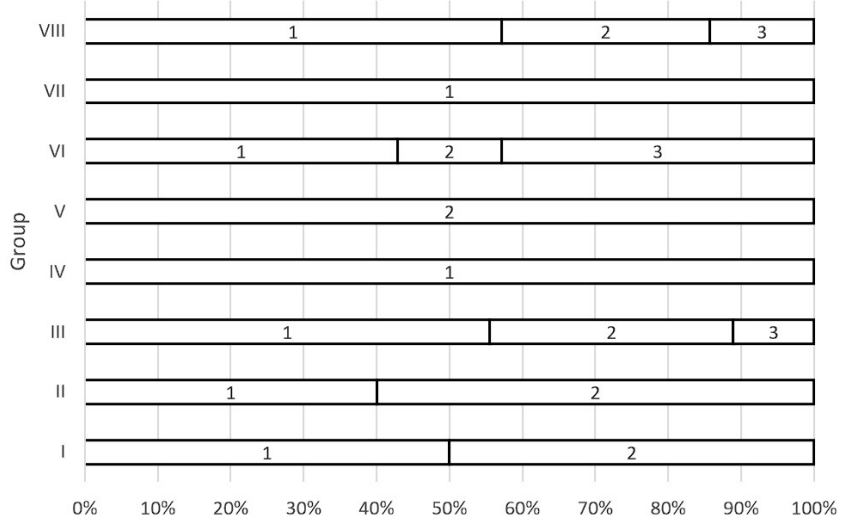

Figure 7. Frequency by Interval class of altitudinal range between groups (8). I=Group 1, II=Group 2, III=Group 3, IV=Group 4, V=Group 5, VI=Group 6, VII=Group 7, VIII=Group 8. 1=low altitude range, 2=medium altitude range and 3=high altitude range.

The low altitude range recorded the highest number of bacterial colonies (23) and morphotypes (7), while the high range had the lowest number of morphotypes (4).

CONCLUSIONS

The diversity of bacteria present in the rhizosphere of coffee plantations is directly related to the type of agronomic management and altitude range; therefore, they are extremely important components for the coffee agroecosystem. Organically managed coffee soils have a diversity of bacteria, which is demonstrated by the variety of various morphological and microscopic characteristics.

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