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QUANTIFICATION AND CHARACTERIZATION OF NATIVE MICROORGANISMS UNDER CONTRASTING RAINFOREST ENVIRONMENT IN ECUADORIAN AMAZON

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Abstract

Ecuadorian Amazon Region is known as one of the richest biodiversity environment worldwide. However, it is a fact that microorganisms biodiversity have been poorly studied. In order to contribute to unravel microbe biodiversity and applications, this research aimed to quantify and characterize native microorganisms associated with cocoa (*Theobroma cacao*) plantations under high cadmium levels in two contrasting Ecuadorian Amazon conditions (CIPCA and Ahuano). Soils samples were collected from two depth levels (0-10 cm and 10-30 cm) to compare the number of bacteria and fungi in both environments assessed. For microorganisms quantification, decimal dilution methods was performed and most probable number was calculated. Bacteria biodiversity was assessed by isolation of every different single colony and morpho-cultural characterization was performed measuring: colour, growth, shape, elevation, edges, Gram stains and morphology. Fungi biodiversity was evaluated by mycelia shape, colour and radial growth. Results showed the increase in bacteria and fungi under CIPCA environment, where the rainy range was not so high. However, in both ecosystems from 10-30 cm depth, the number of microorganisms were remarkable as compared with 0-10 cm depth. Bacteria characterization highlighted a huge diversity, with 22 different isolates in CIPCA and 16 isolates in Ahuano. For fungi, the differences in morpho-cultural characteristics within both ecosystems were not wide, but also CIPCA had a high diversity with 25 different isolates as compared with 22 from Ahuano.

These results are the base for further researches related with microbe applications, such as cadmium bioremediation.

Keywords: bacteria, fungi, isolation, identification, bioremediation

1. Introduction

Before the advent of the Omics era, microorganisms were generally identified and characterized based on their morphological, physiological and cultural characteristics. Biotyping, serotyping, bacteriocin typing, phage typing, antimicrobial susceptibility patterns, and other protein-based methods are all examples of commonly employed phenotypic methods (Fakruddin et al., 2013). Although molecular techniques have revolutionized the microbiological studies, nowadays these traditional methods are still proper and used for microbe typing where the molecular analyses are unreachable.

Ecuadorian Amazon biodiversity is being increasingly investigated. New accession of plants, amphibians, birds, fishes, reptiles and other species are often reported (Lessmann et al., 2016). However, microorganism communities are still under limit knowledge.

Microbe identification is crucial for further application in any biotechnological process (Sanguinetti and Posteraro, 2016), much more in those related with environmental processes such as bioremediation. Unravel efficient microorganism upon specific environment to reduce contaminants, could become a cornerstone for restoring ecosystems (Liu et al., 2017). In this regards our research aims to quantify and characterize native bacteria and fungi associated with cocoa (*Theobroma cacao*) plantations under high cadmium levels in two contrasting Ecuadorian Amazon conditions.

2. Results and Discussions

The total bacteria and fungi were quantified at seven days after plating. Figure 1 shows the comparison between both depths levels measured (0-10 and 10-30 cm) for bacteria (panel A) and fungi (panel B) at the two sampled sites. In Ahuano, the bacteria colony-forming unit (CFU) were statistically higher at 10-30 cm depth (3.08E+07) as compared with 0-10 cm (6.73E+06); while in CIPCA no significant differences among depths levels were observed.

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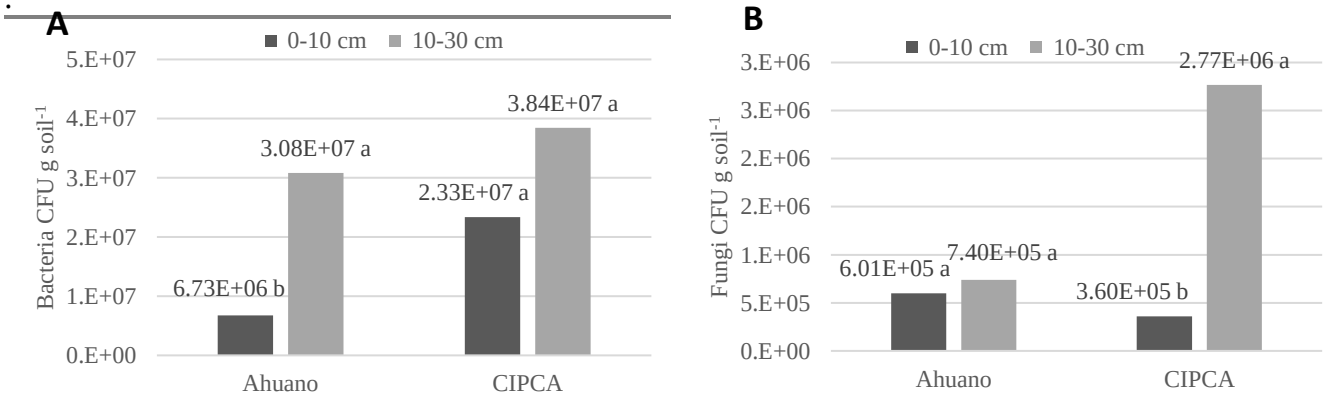

Figure 1. Quantification of bacteria (Panel A) and fungi (panel B) communities at two levels depth (0-10 and 10-30 cm) in Ahuano and CIPCA. Values are given in colony-forming unit (CFU) per each gram of soil. Different letters in columns differs statistically (p<0.05, Tukey HSD).

For fungi, the results were reverse than for bacteria regarding sampling sites, being the CIPCA the site with more fungal abundance. However, as for bacteria, the depth level 10-30 cm in CIPCA turned out the best significant values. In Ahuano no significant differences were shown.

Although for bacteria and fungi the assessed site varied the quantification of microbial communities (Figure 1 A and B), these results clarify the influence of depth levels in the abundance of each microbe group. As well as for Ahuano and for CIPCA, a distribution pattern of the microbial communities was presented, favouring bacteria

and fungi from 10 to 30 cm depth. It might be due to the edaphoclimatic conditions that are presented in this Amazon region, mainly the rainy regime, allowing the leaching of organic matter and the microbe community with it.

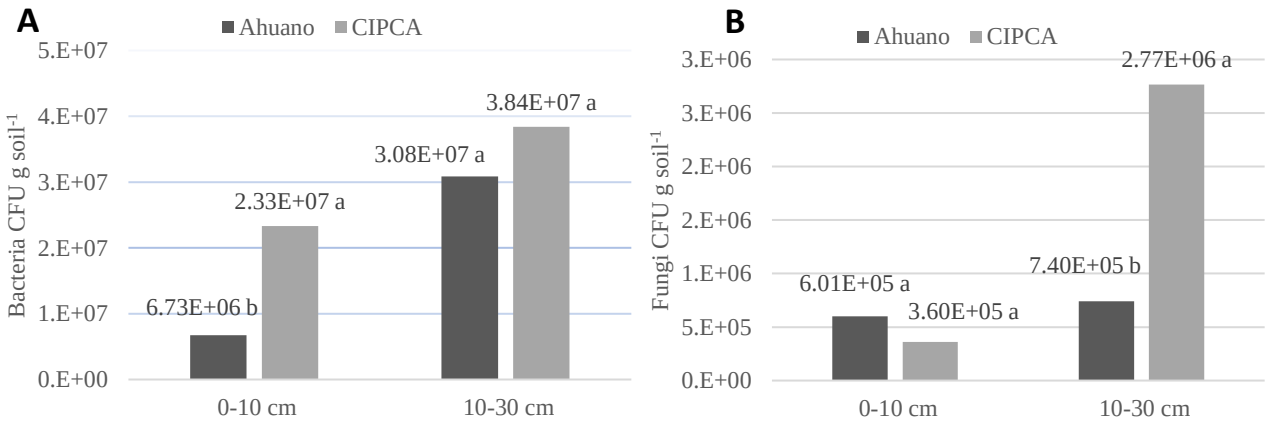

Figure 2. Comparison of bacteria (Panel A) and fungi (panel B) quantification in Ahuano and CIPCA according to depth levels. Values are given in colony-forming unit (CFU) per each gram of soil. Different letters in columns differs statistically (p<0.05, Tukey HSD).

The comparison of bacteria and fungi quantification among sampling sites can be seen clearly in Figure 2. In panel A is evident the increase of bacterial community in CIPCA at both depth levels, although only at 0-10 cm significant difference were observed. Despite the number of bacteria at this depth level was less than from 10-30 cm, there was a difference of 28.9% of CIPCA bacteria compared to the those found in Ahuano. As seen for previous results (Figure 1), the quantification of fungi colonies was remarkable in CIPCA as compared with Ahuano, having an increase of 26.7%.

Several studies have reported the close link of microbial communities with organic matter in soils. Schnecker et al. (2014) focus the effect of organic matter properties and microbial community composition even in Artic soils. Authors found microbial community composition (estimated by phospholipid fatty acid analysis), was similar in cryoturbated material and in surrounding subsoil, although carbon and nitrogen contents were similar in cryoturbated material and topsoils. Our work is concomitant with this finding, suggesting that as the organic matter progresses in the soil, the microbial communities move with it.

Table 1 represents the morpho-cultural characterization of all different bacteria isolates in both contrasting sampling sites. From all the colonies grown in nutrient agar media, a total of 22 different isolates for CIPCA and 16 for Ahuano were released. All colonies differed at least in one of the parameters assessed. Within the most significant results in this table, stands out the 20% of bacteria with moderate and abundant growth in CIPCA and 34% in Ahuano, as well as 32% of Gram negative stain bacteria in CIPCA against 42% in Ahuano. Similar results are reported by Sánchez et al. (2014), which highlighted the wide range of bacteria in tropical soils and its biodiversity.

Table 1. Morpho-cultural characterization of different bacteria isolates in contrasting sampling sites.

Location	Apar.	Shape	Elevation	Edges	Growth	Colour	Gram stain
CIPCA	70%- B 20%- C 10%- E	80%- D 10%- B 10%- E	90%- A 10%- B	90%- B 10%- E	80% + 10% ++ 10% +++	70% - B 30% - A	68% (+) 32% (-)
Ahuano	92%- B 8%- C	67%- D 33%- B	50%- B 42%- A 8%- C	67%- B 33%- A	66% + 17% ++ 17% +++	43%- A 33%- B 8%- C 8%- D	48% (+) 42% (-)

8%- E

Legend: Appearance: equinulade (B), barbade (C), arborescent (E). Shape: circular (B), irregular (D), rhizoid (E). Elevación: flat (A), elevated (B), convex (C). Edges: continuous (A), wavy (B), prickly(E). Growth: light (+), moderate (++), abundant (+++). Colour: white(A), beige (B), yellow(C), orange (D), red(E). Gram: negative (-), positive (+).

3. Conclusions

The amounts of bacteria in the Amazonian ecosystems evaluated are high, especially in CIPCA soils (6.2E + 07) compared to Ahuano (1.9E + 07), with a predominance of bacterial communities in the horizon of 10-30 cm in soil.

There is a broad biodiversity of bacterial communities, expressed in the differentiation of morpho-cultural characteristics. Twenty-two different isolates were determined in CIPCA and 16 in Ahuano.

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