International Journal of Plant & Soil Science

21(2): 1-13, 2018; Article no.IJPSS.39052
ISSN: 2320-7035

Study of Microorganisms Associated with the Chemical Compounds in a Tropical Soil

M. Palma-Jiménez[1*], M. Blanco-Meneses[2] and J. R. Vega-Baudrit[1]

[1]National Laboratory of Nanotechnology, National High Technology Center, Rorhmoser, Pavas, San José, Costa Rica.
[2]Center for Research on Crop Protection, Laboratory of Molecular Techniques, Universidad de Costa Rica, San Pedro de Montes de Oca, San José, Postal Address: 11501-2060, Costa Rica.

Authors' contributions

This work was carried out in collaboration between all authors. Author MPJ designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MBM and JRVB managed the analyses of the study. Author MPJ managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2018/39052
Editor(s):
(1) R. Manikandan, Department of Plant Pathology, Tamil Nadu Agricultural University, Tamil Nadu, India.
Reviewers:
(1) Tariq Mukhtar, Pir Mehr Ali Shah Arid Agriculture University, Pakistan.
(2) V. Vasanthabharathi, Annamalai University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/22896

Original Research Article

Received 28th October 2017
Accepted 21st January 2018
Published 27th January 2018

ABSTRACT

Soil is the most complex of all microbial habitats. Tropical soils have high levels of biodiversity and microorganisms that display fascinating mechanisms for interaction and biotransformation, thus regulating the mobility of chemical compounds in the environment. This paper will provide an understanding the relationship between chemical compounds present in a tropical soil and the microorganisms isolated from different sampling points in the biologically diverse area of Lepanto, Costa Rica. Microbiological (bacterium and fungi culture media), biochemical (oxidation-fermentation and catalase test), molecular (ITS and 16S gene fragments), thermal (TGA), and spectroscopic (FTIR, SEM-X-ray) techniques were used for this purpose. Four bacterial species, *Klebsiella quasipneumoniae, Klebsiella pneumoniae*, *Micromonospora* sp. and *Paenibacillus* sp., and three fungal species, *Penicillium rubens*, *Penicillium stekii* and *Meyerozyma guilliermondii*, were identified. The soil chemical composition allowed the identified microorganisms to be associated with fatty acid groups, humic substances, aromatic compounds, conjugated ketones and

**Corresponding author: E-mail: melissapal@gmail.com;*

carboxylic groups. In addition, metals present in the soil (Al, Mg, C, O, Si and Fe) were correlated with the activity of these microorganisms. The present study highlights the potential utility of the combined use of inference tools to identify ecologically meaningful microorganismal associations in environmental samples and the chemical compounds in a tropical soil.

Keywords: Biodiversity; elemental composition; habitat heterogeneity; histosols; microcosm experimentation.

1. INTRODUCTION

Soils are among the most extensive and biodiverse microbial habitats on Earth [1]. Soil microbial ecology allows the determination of which natural forces affect the extremely high biodiversity observed even at a very small scale [2]. Soil-borne microorganisms play a pivotal role in many terrestrial ecosystem functions including nutrient cycling, the sustenance of plant growth, water purification, carbon storage, and the maintenance of the soil structure [3]. Microbial diversity is defined in a broad sense as the variety of microorganisms and their adaptation mechanisms. This is evident in terms of structural and functional variety, such as variations in cell size, morphology, cell division, or metabolic and adaptation capacity [4].

Studies of the abundance and species of microorganisms have been traditionally carried out with microbiological methods, biochemical techniques, taxonomic classification and molecular techniques. The benefit conferred by high genetic diversity is under debate because it is not always related to functional diversity [5,6].

Both the bacterial biomass and community structure have been shown to differ among different particle classes [7]. However, it is difficult to identify clear and consistent trends in the accumulated data because of methodological differences between studies, the differences in the soils examined and natural sources of variation, which highlights the influence on bacterial distribution of environmental factors such as plant growth and nutrient status that vary with time. Microhabitats also clearly vary positionally within an aggregate; in the same way, we still have only a rudimentary understanding of the processes and microbial populations that inhabit the inside versus the surface of such aggregates [8]. One study exploited the fact that bacteria on the surface can be washed from an aggregate surface more easily than bacteria in the interior, but no significant differences in community composition were found between easily dislodged and more closely bound communities [9].

Physical and chemical properties of soil aggregate fractions are assumed to vary with aggregate size [10]. Thus, a separate analysis of different soil size fractions has the potential to provide insight into the physical and chemical differences between different soil microenvironments [11].

Phenotypic identification systems have inherent problems since not all strains of the same species have homogeneous characteristics - the same strain can generate different patterns in repeated trials, and databases also have limitations. Molecular methods have been used as a complementary alternative or even as reference procedures to understand phenotypes [4,11].

The recent application of high-throughput DNA sequencing strategies in microbial ecology has been instrumental in rekindling our appreciation of soil-borne microbial diversity [12]. The analysis of the 16S rRNA gene fragment has been the most widely used in bacterial taxonomy. This marker is present in all bacteria, its function does not change with time, and it acts as an efficient marker of evolution. Fungi can be identified by their nuclear ribosomal internal transcribed spacer sequences as a universal DNA barcode marker, for example, with the ITS 4 and 5 genomic fragments [13]. High-throughput sequencing technologies continue to advance our appreciation of this remarkable phylogenetic and functional diversity, but we still have only a rudimentary understanding of the forces that allow diverse microbial populations to coexist in the soil [8]. To identify and relate the microorganisms with the chemical compounds in a tropical soil from the geographical region of Lepanto, Puntarenas, Costa Rica, microbiological, biochemical, molecular, thermal and spectroscopic analyses were used.

2. MATERIALS AND METHODS

2.1 Study Site

The samples were collected in the Lepanto district, Puntarenas, Costa Rica. The geographical coordinates are shown in Table 1. The isolation and characterization of the samples were carried out at the National Laboratory of Nanotechnology (LANOTEC) and the Laboratory of Molecular Techniques, a unit of the Center for Research in crop protection (CIPROC) of the University of Costa Rica.

2.2 Sample Collection

Soil samples were collected randomly from ten different sites in the mountains, and 10 g were kept in a sterilized falcon tube (Table 1). Samples were isolated from the rhizosphere soil (pH 7), which is a habitat for a variety of microorganisms.

2.3 Microorganism Conditions

Samples were serially diluted, and 100 mL aliquots were then spread on agar plates. Enrichment culture acid medium, PDA (to grow fungi), MacConkey agar (for gram-negatives) and mannitol salt agar (for gram-positives) were used for microbial isolation. Agar plates were incubated according to physiological conditions (Table 2). Microbial colonies were selected on the basis of colony morphology and color and were examined under increasing magnifications of 4x, 10x, 20x, 40x and 100x with a light microscope (model IX51, Olympus Optical Co., Japan). Isolated single colonies were then picked and re-streaked on new agar plates of the same medium until pure cultures were obtained. The plates were stored at 4°C for further use.

2.4 Biochemical Analysis

Strains were identified according to the presence of catalase activity, and oxidation-fermentation reactions were performed. The catalase test was performed by adding a drop of 3% hydrogen peroxide to a 72 h old bacterial colony on a clean glass slide and mixing with a sterile tooth-pick. Effervescence indicated catalase activity. Oxidation-fermentation was performed for 18 to 48 h.

2.5 Molecular Analysis

The protocol by Murray and Thompson [14] was used. The primers used were ITS 4 and ITS 5 for fungi and 16S for bacterium. A 1x (µl) solution was used for all PCR reactions and contained the following: 13.5 µL of H2O, 2.5 µL of buffer (10x), 2 µL of dNTPs (2 mM), 1.5 µL each for each pair of primers (10 µM), 0.3 µL of Dream Taq polymerase (5/µL) to 23 µL of master mix per Eppendorf tube, all Fermentas reagents, and 2 µL of DNA (10 µg/mL). The amplification reaction was performed using the following thermal profile: an initial predenaturation at 96°C for 2 min, followed by 35 cycles of denaturation at 96°C for 1 min, annealing at 56°C for 1 min, chain elongation at 72°C for 2 min, and a final extension at 72°C for 10 min. The reactions and cycling were carried out in an automated thermocycler Eppendorf Mastercycler pro. The PCR product was separated on an agarose gel (agar + 0.5X TBE buffer). The PCR product was digested with Exonuclease I (ExoI) from Fermentas. Sequencing was performed on the purified PCR product at a concentration of 50 ng/µL by Macrogen, Inc. (South Korea). Sequences in both directions were obtained. The quality of the sequences was confirmed in a bidirectional alignment and by comparison of the chromatograms using the BioEdit program v7.0.5 [15]. GenBank was used to determine the species according to the sequencing results [16].

2.6 Fourier Transform Infrared Spectroscopy (FTIR)

Eighty grams of a soil sample was maintained in 400 mL of type 3 water in constant agitation for 24h. The solution was filtered, first using a quantitative filter membrane with an 8-12 *µm* pore size (Boeco Germany), followed by filtration through a filter membrane with a 0.2 µm pore-size (Sartorius stedim, Minisart^{R}). The recovered solution was evaporated on a rotary evaporator. The final 5 mL aliquot obtained was used to identify the soil sample composition.

The infrared spectra of the extracted soil samples was determined with an FTIR spectrometer (Nicolet 6700, Thermo Fisher Scientific, USA) and scanned from 4000 to 400 cm–1. The spectra were baseline-corrected and normalized to 1.0 for comparison.

2.7 Thermogravimetric Analysis (TGA)

The organic, inorganic and volatile contents of the soil samples were analyzed using a TA

Instrument (TGA Q500, USA). The degradation of the soil was assessed in a temperature range from 180°C to 1200°C. The Universal V3.9A TA Instrument software was used to analyze the results.

2.8 Energy Dispersion of X-ray Spectroscopy (EDX)

To determine the metal composition, all samples were examined by Scanning Electron Microscopy (SEM X-ray). Samples were mounted on aluminum stubs using carbon tape. Imaging and a semi-quantitative chemical analysis were performed on a JEOL JSM-5900 LV-SEM (Japan) fitted with an EDX energy dispersive X-ray spectrometer. All samples were heat-dried and ground to a fine powder before the EDX analyses. EDX spectra were collected at a 20 kV accelerating potential.

3. RESULTS

3.1 Morphological Characterization

The bacterial isolates were evaluated according to their phenotypic characteristics, and revealed to be types of bacilli, cocci and structures similar to actinomycetes. Likewise, the presence of fungi (ascomycetes and yeasts) was morphologically identified, and these results were reinforced by the molecular results.

3.2 Molecular Characterization

The sequences obtained were compared with sequences from GenBank [16]. Four bacteria were identified from the inoculated samples: *Klebsiella quasipneumoniae, Klebsiella pneumoniae*, *Micromonospora* sp. and *Paenibacillus* sp. In a similar fashion, three fungi from the inoculated samples were identified: *Penicillium rubens* (formerly *P. chrysogenum*), *Penicillium stekii* and *Meyerozyma guilliermondii.*

3.3 Biochemical Characterization of Bacteria

Microorganisms isolated from the soil samples are listed in Table 3, which represents a summary of the analyzed tests from all isolates. A catalase test and an oxidation-fermentation analysis were done to biochemically characterize the bacteria.

Catalase activity was detected in all bacterial isolates. Bubbling occurred as a consequence of the presence of the enzyme catalase, which decomposed hydrogen peroxide into oxygen and water. In the oxidation-fermentation test, green tubes indicated that glucose was not metabolized. A negative reaction occurred in both tubes, indicating a lack of acid production from glucose. Nonsacchrolytic bacteria give a negative result. This result was indicated by the lack of color change in the oil-covered tube. *The samples were able to ferment* glucose; however, glycerol was shown to be a nonfermentable carbon: S5 and S6 (*Paenibacillus sp).* On the other hand, the yellow tubes indicated a fermentative response and acid production in both open and covered tubes.

The anaerobic (fermentative) and aerobic degradation of glucose was indicated. Nonfermenting bacteria that metabolize glucose oxidatively give an oxidative result. Both *Klebsiella quaineumonia*e and *Klebsiella pneumonia* samples were capable of fermenting glucose with acid production in open and oil-covered tubes. The oil-covered anaerobic tube showed an oxidative result, and acid production occurred in the open tube (aerobic). This result was seen in S6. The results of *Micromonospora* sp. were shown to be the same in both tubes (Table 3).

A positive mannitol test was indicated by a color change in the culture medium from red to yellow that indicated the presence of a gram-positive bacterium and was present in S1-S10. A positive MacConkey test for gram-positives occurred only in S6 and S7.

The Gram-positive bacteria *Micronomospora* sp. was identified in samples S1-S5 (wet and dry) and S7-S10 (from different points around the lagoon), and *Paenibacillus* sp. was seen in samples S5 and S6 (at the foot of a tree and in the shade, respectively). The bacteria *Klebsiella quasineumoniae* and *Klebsiella pneumoniae*, both gram-negative, were identified in samples 6 and 7 (in the shade and near a lagoon, respectively) (Table 3).

The pH values were very homogeneous in the evaluated sites and were between 7 and 8. The optimal pH for the growth of these bacteria is between 6.5 and 8.

Table 1. Sample geographical coordinates. All soil samples were collected in 2016

Sample collected number	Characteristic zone	Geographical coordinates	
		Latitude	Longitude
Sample1 (S1)	Wet	N 09° 58' 40.3"	O 084° 50' 55.3"
Sample2 (S2)	Dries	N 09° 56' 57.7"	O 085° 01' 51.1"
Sample3 (S3)	Very humid	N 09° 56' 58.2"	O 085° 01' 51.0"
Sample4 (S4)	A lot of shade	N 09° 56' 58.5"	O 085° 01' 50.5"
Sample5 (S5)	At the foot of a tree	N 09° 56' 59.3"	O 085° 01' 50.2"
Sample6 (S6)	With shadow	N 09° 56' 59.8"	O 085° 01' 49.6"
Sample7 (S7)	Close to a lagoon	N 09° 57' 00.6"	O 085° 01' 49.3"
Sample8 (S8)	Closer to the lagoon	N 09° 57' 00.3"	O 085° 01' 48.8"
Sample9 (S9)	Away from the forest (grass)	N 09° 56' 56.2"	O 085° 01' 51.3"
Sample10 (S10)	Lagoon outlet	N 09° 56' 56.5"	O 085° 01' 50.6"

Table 2. Culture medium to identify the biological microorganism

Culture medium	Microorganism growth	Conditions	Time
Acid medium PDA	Fungi	28°C, 80% RH	12 h light
MacConkey	Gram-negativebacterium	37°C, 12 h luz	12 h light
Mannitol salt	Gram-positive bacterium	35°C, 80% RH	24 h Incubation

3.4 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were recorded over a range of 4000–400 cm^{-1}. The chemical characteristics of the extracted samples were determined from the FTIR spectrum (Fig. 1). All of the spectra featured common and distinctive absorption bands, with some differences in their relative intensity. The following spectral regions were identified: 3273.49 cm^{-1}, 2131.18 cm^{-1}, 1635.26 cm^{-1}, and 579.69-533.28 cm^{-1}. These results indicate the recovered chemical compounds that had similar characteristic to fatty acid groups, humic substances, aromatic compounds, H-bonded conjugated ketones and carboxylic groups of peptides [17-19].

3.5 Thermogravimetric Analysis (TGA)

The thermal stability was studied by means of a thermogravimetric analysis (TGA). The TGA curves and differential thermal analysis of the dry soil samples are shown in Fig. 2. All of the samples showed very similar thermal behavior; however, they were characterized by weight losses in three distinct temperature ranges. The first sharp weight loss, according to the derivatives of the curves S2, S4 and S7, was detected at a temperature of 61°C for sample 443. Then, two large weight losses according to the derivatives of the curves S6, S8, S9 and S10 were detected at the temperatures of 53°C for sample 445 and 66°C for sample 645. At the end, the derivatives of the curves S1, S3 and S5 indicated weight losses at temperatures of 06°C for sample 345, and 37°C for sample 496 and 78°C for sample 636.

According to the analysis, the percentages of water and volatile, organic and inorganic compounds in the soil samples are shown in Table 4. The highest percentage of humid compounds was shown in S10 (12.7%) and S5 (9.3%). This respectively corresponds to a point at the lagoon outlet and at the foot of a tree. The other samples came from a variety of habitats, wet, humid and dry. On the other hand, the organic compounds were high in S5 (19.8%), which came from the foot of a tree, S3 (16.1) which came from a very humid area, and S1 (12.3%), which came from a wet area. A high percentage of inorganic compounds was found in S6-S9, close to 90% for shaded areas, close to the lagoon and in the grass.

Organic species become relevant (approximately 19.8%) to the loss at 333.36°C, while a lower contribution (approximately 4.6% and 5%) was observed at the heating steps at 322.07 and 436.65°C. The first step in the curves is associated with the loss of water and volatile compounds, and its exact height depends on the initial degree of hydration of the material. All samples show a continuous weight loss (4.9-12,7%) up to a temperature of 180°C.

Table 3. Summary of bacterial test data. Results of Catalase, Oxidation-Fermentation and Molecular tests according to Mannitol and MacConkey media

Test	Sample collected									
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
Catalase[a]	+	+	+	+	+	+	+	+	+	+
Oxidation-Fermentation[a-d]	+/-	-/-	+/+	-/-	+/+	-/+ (-Mc)	- /- (+Mc)	+/+	+/+	+/+
Molecular test to Mannitol (gram- positive bacterium)	*Micromonospora* sp.				*Micromonospora* sp. *Paenibacillus* sp.	*Paenibacillus* sp.	*Micromonospora* sp.			
Molecular test to MacConkey (gram-negative bacterium)	-	-	-	-	-	*Klebsiella quasineumoniae* *Klebsiella pneumoniae*		-	-	-

([a]Mannitol test yellow: +; [b]Mannitol test red: -; [c]MacConkey test: Mc+; [d]MacConkey test:Mc-)

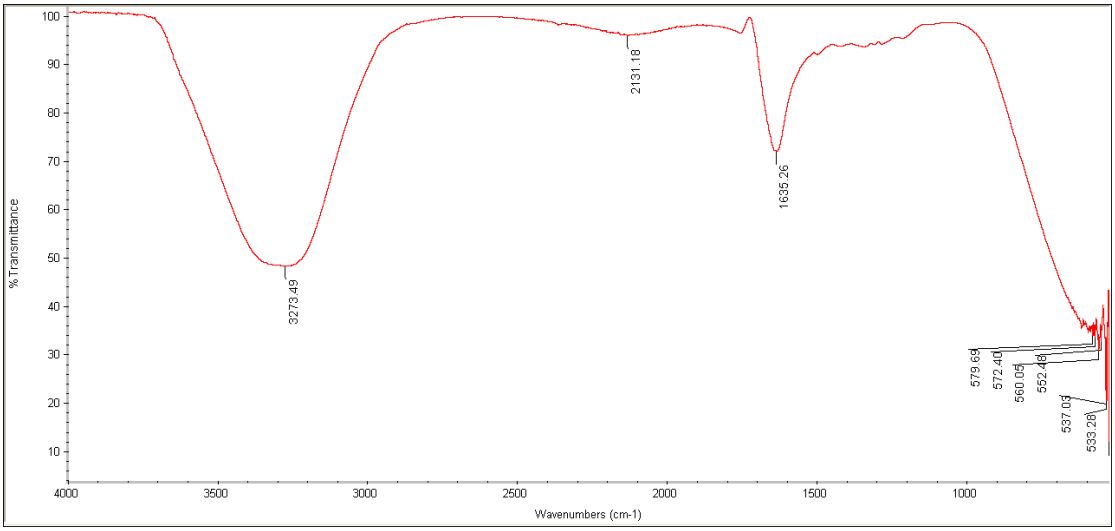

Fig. 1. Chemical characteristics of the soil samples determined by Fourier transform infrared spectroscopy recorded over the range of 4000–400 cm^{-1}

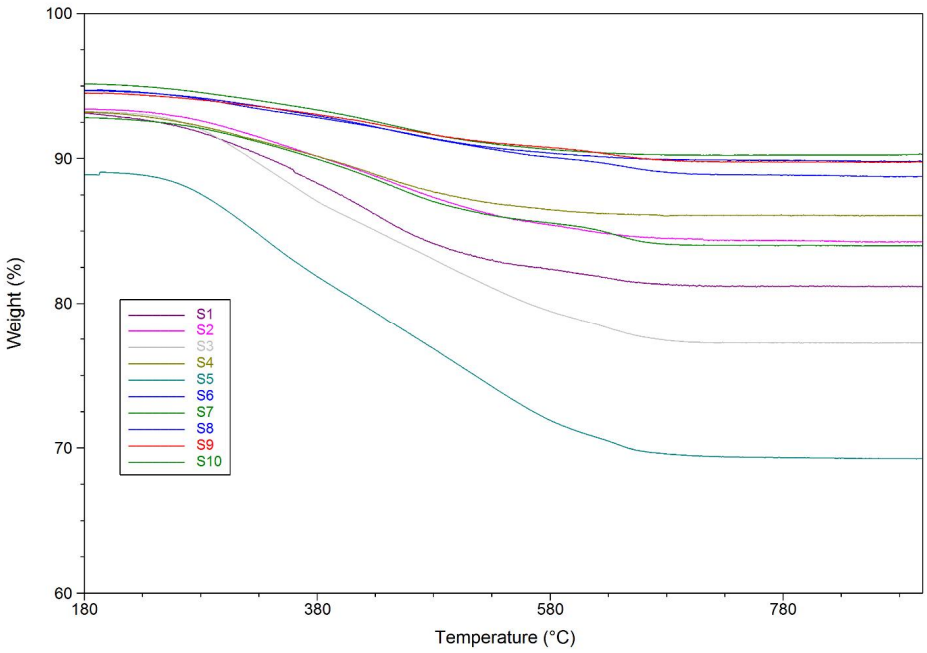

Fig. 2. The temperature dependence of the soil samples. A single sigmoidal shape was evident, and the shift in the temperature at which the speed of thermal degradation is maximal as the loading with soil is increased is emphasized by the dotted line. The inset shows the Thermal Gravimetric Analysis data for the entire temperature range (180°C - 900°C)

3.6 Energy Dispersion of X-ray Spectroscopy Analysis (EDX)

An EDX analysis coupled to SEM (SEM X-ray) measured the atomic composition of all of the samples collected. A chemical composition analysis showed peaks and weight percentages of 7.8% Al, 3% Mg, 3.6% C, 43.9% O, 22.6% Si, and 19.1% Fe (Fig. 3).

Table 4. Summary of the percentage of water and volatile compounds, organics and inorganics in the soil samples

Sample	Water and volatile compound (%)	Organic compound (%)	Inorganics compound (%)
S1	6.3	12.3	82.0
S2	6.4	8.5	86.0
S3	6.6	16.1	77.6
S4	6.0	6.8	87.5
S5	9.3	19.8	70.1
S6	4.9	5.3	90.0
S7	6.0	5.0	89.4
S8	6.2	4.6	89.5
S9	5.4	5.0	90.0
S10	12.7	9.0	83.5

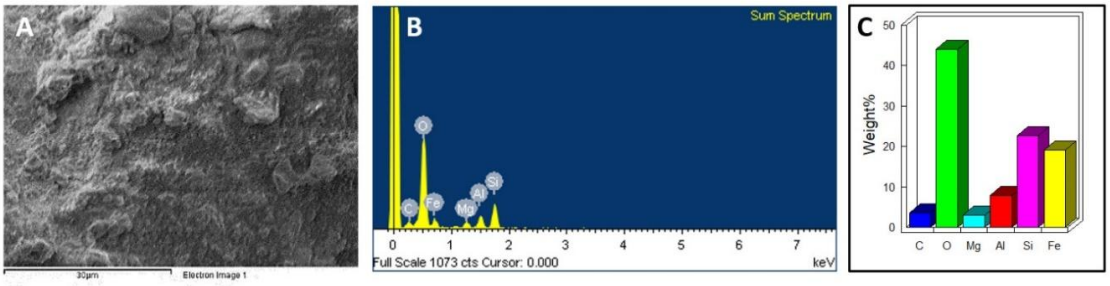

Fig. 3. Chemical composition of the soil samples analyzed by SEM-X-ray spectroscopy for elemental soil composition. (A) Morphology of the soil samples. (B) Elemental chemical composition. (C) Weight percentage of chemical elements

4. DISCUSSION

According to Vos et al. [8], it is clear that typical soil sampling strategies are not well suited for studies that seek to examine factors that drive and maintain microbial diversity. The authors mention that a large proportion of the processes that shape microbial diversity in soil occur at scales that we have generally failed to address to date.

Merroun [20] emphasizes the metal absorption capacity and activity of different gram-positive and negative bacteria and notes the morphological structures of the cell wall as a factor that allows understanding and relating microbial interactions to the chemical compounds in a particular soil.

Early research by Beveridge and Murray [21] related bacterial metal interactions to the anionic character of specific functional groups situated on membrane components. These authors considered the major cell wall components (e.g., teichoic acids, peptidoglycan, phospholipids) to be principally responsible for the overall bacterial reactivity.

The presence of certain soil metals (such as carbon, oxygen, magnesium, aluminum, iron and silicon) involved in important physiological pathways of microorganisms has been noted, and this interaction has been related to the type of habitat. Certini et al. [22] mention that microorganisms in the environment have a significant effect on the release of nutritional elements from minerals into the soil environment through the bio-weathering process. The mineral composition of the soil has been shown to affect the structure and physiological activities of the associated microbial communities [2].

Minerals, organic matter and microorganisms are intimately associated in the soil and closely interact in environmental processes. These interactions are especially important in the rhizosphere and the sediment-water interface, where low-molecular weight biochemical compounds are abundant and microbial activity is intense. The transformation of chemical compounds is governed by abiotic and biotic

processes in the soil and related environments. Abiotic processes include solution complexation, adsorption, desorption, precipitation, dissolution, redox reactions, and catalysis. In addition, microbial activity has a very important role in influencing the dynamics of metals in the terrestrial ecosystem [5].

It has been determined that aluminum is the most abundant metal in the earth's crust. Magnesium (Mg) is an essential component of chlorophyll. Oxidation of carbohydrates liberates energy that is stored in high-energy phosphate bonds. It is believed that the process of oxidative phosphorylation occurs in the mitochondria of cells and that magnesium is necessary for this reaction [23]. Plants release up to 40% of their photosynthetically fixed carbon through the roots into the surrounding area. The availability of molecular oxygen (O_2) is closely connected to soil aeration, and it is one of the most crucial factors for aerobic activity in the soil [24].

It has been determined that redox reactions are important in controlling the chemical speciation of a number of metals. Redox reactions are also important in controlling the transformation and reactivity of iron (Fe) oxides in the soil, which have an enormous capacity to adsorb metal pollutants [5].

Iron is an important element for the growth of almost all living microorganisms. Many of the oxidation-reduction reactions linked to Fe and manganese (Mn) are mediated by microorganisms. Fe is necessary as a cofactor in various enzymatic processes, oxygen metabolism, electron transfer, DNA and RNA synthesis, cytochromes and proteins involved in electron transport [25], due to their ability to exist in two stable oxidation states, soluble (Fe^{2+}) and insoluble (Fe^{3+}) [26]. Aerobic oxidation of Fe from a soluble to an insoluble state is an energy producing reaction for some bacteria. The fermentative facultative anaerobe *Klebsiella pneumonia* isolated from subterranean sediments can reduce Fe^{3+} oxides and carbon tetrachloride (CT). The fermentative facultative anaerobe *K. pneumoniae* can not only reduce various crystalline Fe^{3+} oxides but also CT. *K. pneumoniae* has two types of reductive capabilities under anaerobic conditions [27].

Studies have indicated that Klebsiella sp. isolates are of environmental origin and nearly identical to clinical isolates with respect to several phenotypic properties and could have important microbiological consequences [28]. However, the pathogenic potential of environmental *K. pneumonia* isolates is essentially unknown. It has been found in a tropical soil [29]. *K. quasipneumoniae* fixes N2 at an intermediate frequency, while *K. pneumoniae* fixes N2 at a low frequency to [30].

Members of *Micromonospora* sp. are widely distributed in a variety of habitats, notably soils rich in humus, and they play an important role in the decomposition of organic matter [31]. This was the most common bacteria identified at almost all sample points analyzed in the present investigation.

Paenibacillus was recognized as a new genus distinct from *Bacillus* according to differences in the 16S rRNA sequences [32]. In the present investigation, the bacterium *Paenibacillus* sp. was found close to the lagoon. The taxonomic diversity of *Bacillus* spp. and *Paenibacillus* spp. was shown by their capability of reducing plant disease, which indicates that much work remains to be done on the mechanisms by which these bacteria promote plant health. Numerous *Bacillus* sp. and *Paenibacillus* sp. strains express activities that suppress pests and pathogens or otherwise promote plant growth. Greater knowledge of the diversity, distribution, and activities of *Paenibacillus* spp. will be useful for the identification of new inoculant strains and cropping systems into which they can be most profitably applied [33,34].

Ma et al. [35] noted that some beneficial microbes can help plants acquire sufficient mineral nutrients. Ahmed and Holmström [36] correlated a high content of iron and magnesium (4.5% Fe and 7.8% Mg), the main essential elements for fungal growth, on the biotic surface with a high concentration of ferrichrome siderophores (i.e., quelatin agents).

The genus *Penicillium* is cosmopolitan and is found virtually everywhere from the tropics to the poles. *Penicillium* species are major decomposers and represent an important element of the terrestrial nutrient cycle. *The Penicillium* populations isolated may be useful as biological indicators of anthropogenic contamination because they are common contaminants of human foods [37]. *Penicillium rubens* was formerly known as Penicillium *chrysogenum* but was reclassified in 2011 [38]. It possesses antiviral, antibacterial, antifungal, herbicidal and antiprotozoal activities [37].

The catalase analysis in the present study determined that all bacterial isolates were catalase-positive. According to Kumar et al. [39] bacterial strains showing catalase activity must be highly resistant to environmental, mechanical and chemical stress. Previous studies indicate that this microfauna is capable of assimilating metals and influences environmental regulation.

According to the FTIR spectra, the results allowed to determinate soil samples of specific chemical compounds. The main peak showed a wavenumber of 3273.49 cm^{-1}, following by a small peak of 2131.18 cm^{-1}. According to Pon-On et al. [18] about 3000–2800 cm^{-1} spectrum is commonly assigned to fatty acid related compounds. The band located around 3200 cm^{-1} is generally attributed to OH groups [40]. The FTIR absorption band and assignment for sodium humates and commercial lignohumates appearing around 1640-1600 cm^{-1} (present in our results as the second main peak). It is assigned to aromatic C=C skeletal vibrations, C=O stretching of amide group (amide I band), C=O of quinone and/or H-bonded conjugated ketones [17,9,41], or as Pon-On et al. [18] mention, carboxylic groups of peptide. The fingerprinting absorptions 500-1000 cm^{-1} (minor compounds identified in the spectrum) are significantly different, which implies that the general structure of mobile humic acid is similar to that of humic acid but the substituent groups attached in the general structure significantly varied. There numerous differences hidden in the spectra among different humic substances [41]. Further, results of FTIR, suggested that samples of humates isolated from soil, were a more polycondensed, oxidized, unsaturated, humified, and aromatic structure [19].

The thermal behavior of atmospheric particulate matter was investigated by a TGA analysis to explore their value in environmental studies [42]. This analysis showed sharp weight losses at three temperature points higher than 345,6°C. Previous studies showed that the release of organics occurs at temperatures of 200°C lower, while at temperatures above 500°C, dust pyrolysis arises [43]. We could determine that compounds such as carbohydrates, proteins, lipids, and inorganic compounds were degraded in the first steps of heating. Thermal decomposition of carbohydrates is initiated at temperatures as low as 100°C for cellulose. Acetal and acetone were found, though in relatively small amounts, in cellulose heated to 170°C. As the temperature was increased, volatile products increased in number and concentration [44]. Protein degradation occurred above 41°C to 80°C [45]. Polar compounds in oil were degraded at 180°C, but more significantly at 220 and 250°C [46].

5. CONCLUSIONS

Elucidating the presence of microorganisms and chemical compounds was helpful to determine the forces that shape the diversity at the micro-scale in the tropical soil studied. It was possible in this investigation to associate all organic and inorganic compounds identified in the soil samples with fungi and bacteria at all of the sample points analyzed by determining different percentages of chemical elements. Research into the interactions between these and other microorganisms, humic materials and minerals in the soil continues to influence the field of environmental regulation. The application of new techniques to the relationships between microorganisms and soil particles will allow such studies to continue and promote an understanding of this microhabitat. In the future, the microorganisms will be placed in media containing controlled concentrations of metal ions, and their reproduction will be followed to assess any changes. Such phenomena will be compared with another soil to determine if the soils absorb metals differently.

Additionally, the application of the isolated microorganisms in the field of nanotechnology is an option. Thus, the surface layer protein of an isolated bacterium could be used as a template for the interaction of metallic nanoparticles for industrial applications.

ACKNOWLEDGEMENTS

This investigation was supported by the National Laboratory of Nanotechnology (LANOTEC, acronyms in spanish) of National High Technology Center (CENAT, acronyms in spanish) and the Center for Research on Crop Protection, Laboratory of Molecular Techniques (CIPROC, acronyms in spanish), Universidad de Costa Rica. We thanks the trips to collect the samples according to the project financed by the committee of vicerectors of extension and social action (FEES 2015-2016).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Quince C, Curtis TP, Sloan WT. The rational exploration of microbial diversity. ISME J. 2008;2:997-1006. DOI:10.1038/ismej.2008.69.
2. Carson JK, Gonzalez-Quinones V, Murphy DV, Hinz C, Shaw JA, Gleeson DB. Low pore connectivity increases bacterial diversity in soil. Appl Environ Microbiol. 2010;76:3936–3942. DOI:10.1128/AEM.03085-09.
3. Black HIJ, Hornung M, Bruneau PMC, Gordon JE, Hopkins JJ, Weighell AJ, et al. Soil Biodiversity Indicators for Agricultural Land: Nature Conservation Perspectives. In: Francaviglia R, editors. Agricultural Impacts on Soil Erosion and Soil Biodiversity: Developing indicators for analysis. 1st ed. Rome: OECD; 2006.
4. Montaño AN, Sandoval PAL, Camargo RSL, Sánchez YJM. Los microorganismos: pequeños gigantes. Elementos. 2010;17: 15-23. Spanish.
5. Bollag JM. Interactions of Soil Components and Microorganisms and their Effects on Soil Remediation. 5th International Symposium ISMOM, Pucón, Chile. 24-29 November 2008.
6. Neilsen MN, Winding A, Binnerup S, Hansen BM, Hendriksen NB, Kroer N. Microorganisms as indicators of soil health. Tech Rep. 2002;388:1-82.
7. Sessitsch A, Weilharter A, Gerzabek MH, Kirchmann H, Kandeler E. Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. Appl Environ Microbiol. 2001;67:4215-4224. DOI:10.1128/AEM.67.9.4215-4224.2001.
8. Vos M, Wolf AB, Jennings SJ, Kowalchuk GA. Micro-scale determinants of bacterial diversity in soil. FEMS Microbiol Rev. 2013;37:936–954. DOI:10.1111/1574-6976.12023.
9. Kim JS, Dungan RS, Crowley D. Microarray analysis of bacterial diversity and distribution in aggregates from a desert agricultural soil. Biol Fertil Soils. 2008;44:1003-1011. DOI:http://dx.doi.org/10.1007/s00374-008-0291-5.
10. Bronick CJ, Lal R. Soil structure and management: a review. Geoderma. 2005;124:3-22. DOI:10.1016/j.geoderma.2004.03.005.
11. Fernández AO, García de la Fuente C, Nieto JAS, Valdezate SR. Métodos de identificación bacteriana en el laboratorio de microbiología. 1st ed. Seimc: España; 2010. Spanish.
12. Delmont TO, Prestat E, Keegan KP, Faubladier M, Robe P, Clark IM, et al. Structure, fluctuation and magnitude of a natural grassland soil metagenome. ISME Journal 2012;6:1677-1687. DOI:10.1038/ismej.2011.197.
13. Barghouthi SA. A Universal Method for the Identification of Bacteria Based on General PCR Primers. Indian J Microbiol. 2011;51:430–444. DOI:10.1007/s12088-011-0122-5.
14. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 1980;8:4321-4325. PMCID:PMC324241.
15. Hall TA. BioEdit; 2009, version 7.2.5th ed. for Windows 95/98/NT. Carlsbad, California.
16. NCBI. National Center for Biotechnology Information. Rockville Pike, Bethesda, USA. 2017. Accessed on 1 July 2017. Available: http://www.ncbi.nlm.nih.gov.
17. Maity JP, Kar S, Lin CM, Chen CY, Chang YF, Jean JS. Identification and discrimination of bacteria using Fourier transform infrared spectroscopy. Spectrochim Acta A Mol Biomol Spectrosc. 2013;116:478–484. DOI:10.1016/j.saa.2013.07.062.
18. Pon-On W, Charoenphandhu N, Teerapornpuntakit J, Thongbunchoo J, Krishnamra N, Tang IM. Physicochemical and biochemical properties of iron-loaded silicon substituted hydroxyapatite (FeSiHAp). Mater Chem Phys. 2013;141:850–860. DOI:https://doi.org/10.1016/j.matchemphys.2013.06.014.
19. Enev V, Pospíšilová L, Klučáková M, Liptaj T, Doskočil L. Spectral Characterization of Selected Humic Substances. Soil & Water Res. 2014;9:9–17.
20. Merroun ML, Méndez-Vilas A. Interactions between Metals and Bacteria: Fundamental and Applied Research. 1st ed. FORMATEX: Zurbarán, Spain; 2007.
21. Beveridge TJ, Murray R. Sites of metal deposition in the cell wall of *Bacillus subtilis*. J Bacteriol. 1980;141:876-887. PMCID:PMC293699.
22. Certini G, Campbell CD, Edwards AC. Rock fragments in soil support a

different microbial community from the fine earth. Soil Biol Biochem. 2004;36:1119–1128.
DOI:10.1016/j.soilbio.2004.02.022.

23. Nordberg G. Enciclopedia de salud y seguridad en el trabajo. Metales: propiedades químicas y toxicidad. 2013. Accessed 28 May 2017. Available:http://www.insht.es/InshtWeb/Contenidos/Documentacion/TextosOnline/EnciclopediaOIT/tomo2/63.pdf. Spanish
24. Kai M, Effmert U, Piechulla B. Bacterial-Plant-Interactions: Approaches to Unravel the Biological Function of Bacterial Volatiles in the Rhizosphere. Front Microbiol. 2016;7:1-14. DOI:https://doi.org/10.3389/fmicb.2016.00108.
25. Verkhovtseva YV, Filina YY, Pukhov DE. Evolutionary role of iron in metabolism of prokaryotes and in biogeochemical processes. J Evol Biochem Physiol. 2001;37:444–450. DOI:https://doi.org/10.1023/A:1012987214946.
26. Arcos YA, Betancur JU, Peñuela G Aguirre NJ. Relationship between soluble forms of iron and manganese and the presence of oxidizing bacteria of both elements in the dam Riogrande II-Don Matías (Antioquia, Colombia). Rev Fac Ing Univ Antioquia. 2010; 55:45-54.
27. Li XM, Zhou SG, Li FB, Wu CY, Zhuang L, Xu W, et al. Fe (III) oxide reduction and carbon tetrachloride dechlorination by a newly isolated Klebsiella pneumonia strain L17. J Appl Microbiol. 2009;106:130-139. DOI:10.1111/j.1365-2672.2008.03985.x
28. Brown C, Seidler RJ. Potential pathogens in the environment: *Klebsiella pneumoniae*, a Taxonomic and Ecological Enigma. Appl Microbiol. 1973;25:900-904. PMCID:PMC380937.
29. Struve C, Angeliki KK. Pathogenic potential of environmental *Klebsiella pneumoniae* isolates. Environ Microbiol. 2004;6:584–590. DOI:10.1111/j.1462-2920.2004.00590.x.
30. Chen M, Li Y, Li S, Tang L, Zheng, J, An Q. Genomic identification of nitrogen-fixing *Klebsiella variicola, K. pneumoniae* and *K. quasipneumoniae*. J Basic Microbiol. 2015; 56:78–84. DOI:10.1002/jobm.201500415.
31. Qiu D, Ruan J, Huang Y. Selective isolation and rapid identification of members of the genus micromonospora. Appl Environ Microbiol. 2008:74:5593-5597. DOI:10.1128/AEM.00303-08.
32. Guardabassi L, Perichon B, van Heijenoort J, Blanot D, Courvalin P. Glycopeptide Resistance vanA Operons in Paenibacillus Strains Isolated from Soil. Antimicrob Agents Chemother. 2005;49:4227-33. DOI:10.1128/AAC.49.10.4227-4233.2005.
33. McSpadden GBB. Ecology of *Bacillus* and *Paenibacillus* spp. in agricultural systems. Phytopathology. 2004;94:1252-1258. DOI: 10.1094/PHYTO.2004.94.11.1252.
34. Sheng XF, He LY, Wang QY, Ye HS, Jiang C. Effects of inoculation of biosurfactant producing *Bacillus* sp. J119 on plant growth and cadmium uptake in a cadmium amended soil. J Hazard Mater. 2008;155:17–22. DOI:10.1016/j.jhazmat.2007.10.107.
35. Ma Y, Oliveira RS, Freitas H, Zhang C. Biochemical and Molecular Mechanisms of Plant-Microbe-Metal Interactions: Relevance for Phytoremediation. Front Plant Sci. 2016;23:918. DOI:10.3389/fpls.2016.00918.
36. Ahmed E, Holmström SJM. The effect of soil horizon and mineral type on the distribution of siderophores in soil. Geochim Cosmochim Acta. 2014;131:184–195. DOI:10.1016/j.gca.2014.01.031.
37. Godinho VM, Gonçalves VN, Santiago IF, Figueredo HM, Vitoreli GA, Schaefer CE, et al. Diversity and bioprospection of fungal community present in oligotrophic soil of continental Antarctica. Extremophiles. 2015;19:585-96. DOI:10.1007/s00792-015-0741-6.
38. Houbraken J, Frisvad JC, Samson RA. Fleming's penicillin producing strain is not Penicillium chrysogenum but *P. rubens*. IMA Fungus. 2011;2:87–95. DOI:10.5598/imafungus.2011.02.01.12.
39. Kumar A, Kumar A, Devi S, Patil S, Payal Ch, Negi S. Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: An in vitro study. Recent Res Sci Technol. 2012;4:01-05.
40. Wei L, Wang K, Kong X, Liu G, Cui S, Zhao Q, et al. Application of ultra-sonication, acid precipitation and membrane filtration for co-recovery of

protein and humic acid from sewage sludge. Front Env Sci Eng; 2016. DOI:10.1007/s11783-014-0763-9.

41. Xu J, Wu J, He Y. Functions of natural organic matter in changing environment. 1st ed. Springer Science & Business Media: Dordrecht, Netherland; 2013.
42. Perrino C, Marconi E, Tofful L, Farao C, Materazzi S, Canepari S. Thermal stability of inorganic and organic compounds in atmospheric particulate matter. Atmos Environ. 2012;54:36-43. DOI:https://doi.org/10.1016/j.atmosenv.2012.02.078.
43. Fushimi A, Tanabe K, Hasegawa S, Kobayashi S. Investigation of characterization method for nanoparticles in roadside atmosphere by thermal desorption gas chromatography/mass spectrometry using a pyrolyzer. Sci Total Environ. 2007;386:83-92. DOI:10.1016/j.scitotenv.2007.05.025.
44. Fagerson IS. Thermal degradation of carbohydrates; a review. J Agric Food Chem. 1969;17:747–750. DOI:10.1021/jf60164a019.
45. Matsuura Y, Joti Y, Ogasahara K, Tanaka T, Ono N, Kunishima N, et al. Thermodynamics of protein denaturation at temperatures over 100 °C: CutA1 mutant proteins substituted with hydrophobic and charged residues. Sci Rep. 2015;5:15545. DOI:10.1038/srep15545.
46. Fournier V, Destaillats F, Juanéda P, Dionisi F, Lambelet P, Sébédio J-L, et al. Thermal degradation of long-chain polyunsaturated fatty acids during deodorization of fish oil. Eur J Lipid Sci Technol. 2006;108:33-42. DOI:10.1002/ejlt.200500290.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/22896