Fatty acid profile and metagenomic analysis of a cocoa soil from western Venezuela

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Abstract

Natural forests represent the most important biome; however, the introduction of agriculture has modified the structure of the vegetation in the forest, causing changes in energy balances and biodiversity. Agroforestry systems such as cacao cultivation are an opportunity for the development of new research that allows us to infer the behaviour of soils and the microorganisms that inhabit there and, in this way, contribute to the factors associated with soil quality. The objective of this research is to apply an analytical methodology that allows analysing the microbial communities and the behaviour of a cocoa soil in western Venezuela, in order to obtain an approximation of the population of bacteria and fungi based on a metagenomic analysis, through the extraction of genetic material and quantify the concentration of said microorganisms and evaluate the chemical and biochemical properties associated with soil functions and the profile of fatty acids present under cocoa cultivation. The results indicate that the use of metagenomic analysis accompanied by physicochemical and biochemical variables has multiple applications, from the molecular to the environmental in order to jointly assess their activities and functions.

Introduction

There is a great discrepancy between the evaluations of the microbial diversity present in natural environments carried out with different techniques. These discrepancies reflect the physiological state of the bacteria, which can be cultured under certain conditions and develop into dormant forms and probably not cultivable under other environmental conditions. Soil appears to be dominated by nine phyla belonging to the *Eubacteria* domain, representing on average 92 % of soil libraries, but there are in total about 52 bacterial phyla. Members of the phylum *Proteobacteria* represented on average 40 % of the libraries derived from soil bacterial communities (Janssen, 2006).

Figure 1. Cocoa Porcelana. Venezuela.

Methods for measuring microbial diversity in soil can be classified into two groups: biochemical and molecular. The first ones include plate counts, the physiological profile of the community by use of substrates and the analysis of Fatty Acid Methyl Esters (FAME). To overcome the problems associated with non-culturable bacteria, various methods have been developed to identify and study these microorganisms including fatty acid, DNA and RNA analysis (Kirk *et* al., 2004).

Material and methods

Obtaining soil samples

Due to the importance that cocoa represents in the economy of the Mérida state and considering the potential of the Sucre municipality as a cocoa producer, the selected study area was the San Juan de Lagunillas Experimental Field, geographically located in the Venezuelan Andes, forming part of the lower middle basin of the Chama river, in the town of San Juan de Lagunillas, Sucre municipality of Mérida state. Its location is: Latitude 08º30'55.7" N, Longitude 71º20'24.8" W at an altitude of 1077 m.a.s.l.

Figure 2. Relative location of the San Juan de Lagunillas Experimental Field of INIA-Mérida.

Isolation and cultivation of bacteria from soil samples in selective and enriched media.

Culturable microorganisms: the quantification of culturable bacteria isolated from the soil with cocoa cultivation from the San Juan de Lagunillas Experimental Field of INIA-Mérida was performed, using classical microbiology techniques. The samples were collected in sterile containers with lids and then stored at 4 °C. To 1 g of each soil sample, 9 mL of sterile distillate was added and serial dilutions were made up to 10^{-6} from this suspension. Subsequently, 100 µL of the dilutions from 0,001 to 10^{-6} were taken for seeding in Petri dishes with trypticase soy agar (TSA) medium, as represented in Figure 3.

Extraction of total DNA from the soil: the extraction was carried out according to the method the Yeates *et al*. (1998), in which 2.5 mL of Tris-EDTA-NaCl (pH 8) is added to 1 g of soil. It was shaken for 2 min and 1 mL of solution was added. 20 % SDS. The suspension was incubated at 65 °C for 1 h, centrifuged for 20 min at 3,000 g. The supernatants was added 0.5 volumes of polyethylene glycol in 1.6 M NaCl. It incubated for 2 h 25 °C. It centrifuged at 3000 g for 30 min. The pellet precipitate was resuspended in 2 mL of Tris-EDTA by adding 140 µL of 7.5 M potassium acetate. The samples were placed in an ice bath for 5 min and centrifuged for 1 hour at 4 °C at 6,000 g. Next, extractions were carried out with a chloroform/isoamyl alcohol (24:1) solution and then with the chloroform/phenol/isoamyl alcohol (25:24:1) solution. It was precipitated with 2.5 volumes of ethanol and incubated for 1 hour at -20 °C. It was centrifuged for 30 min at 3,000 g, discarding the supernatant, the pellet was resuspended in 100 µL of TE buffer.

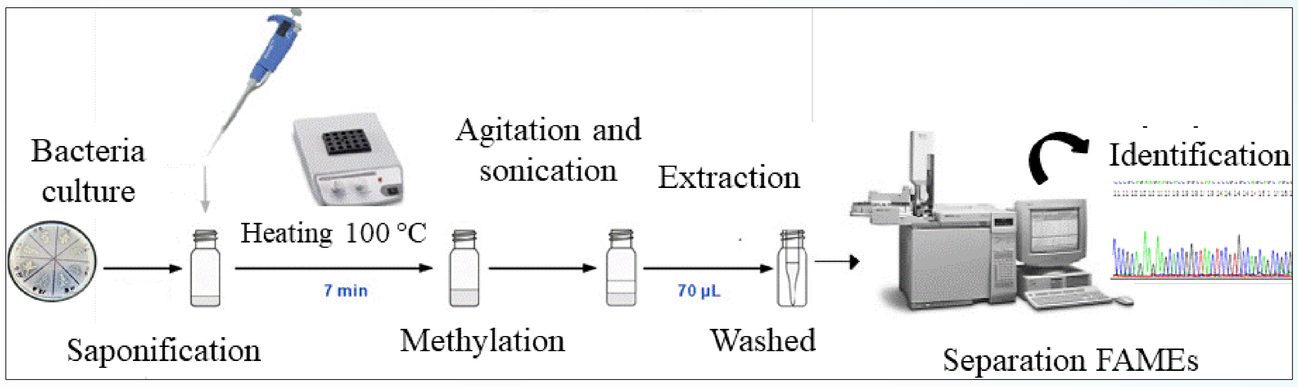

Figure 3. Extraction, methylation and identification of FAMEs in soil.

Results

Identification of bacteria through cellular fatty acids by gas chromatography (GC).

Taking advantage of the cultures carried out for the extraction of DNA in the phosphorus solubilizing bacteria in the San Juan de Lagunillas Experimental Field, the FAMEs of the fatty acids of said bacteria, product of their metabolism under culture conditions, were determined. This procedure allowed the identification of said fatty acids for each identified bacterial colony. In this case the MIDI (1990) methodology was followed, incorporating the application of ultrasound in the esterification as previously indicated and in the last stage instead of performing the identification with the Microbial Identification System (MIS) database.

Tables 1 and 2. Bacteria and FAMEs obtained in the soil evalued.

Bacteria (Strain code)	FAMEs	Concentration (ppb)*
Uncultured *Escherichia sp*. clone K53-9 16S ribosomal RNA gene, partial sequence (1BP1)	C12	4,7279
	C14	2,0738
	C16	13,2936
	C18	8,8782
	C18:1	14,5634
	C18:3	7,6137
	C22	11,4076
Escherichia coli strain NCIM2674 16S ribosomal RNA gene, partial sequence (1CP1)	C16	33,2027
Acinetobacter soli strain Se6 16S ribosomal RNA gene, partial sequence (2AP2)	C12	4,6807
	C16	14,4812
	C18	5,89
	C18:3	37,1488
	C22	97,1077
Uncultured *Escherichia sp*. clone K53-9 16S ribosomal RNA gene, partial sequence (2CP2)	C12	10,8895
	C14	19,7646
	C16	19,6019
	C16:1	25,4638
	C18	4,5737
	C18:1	38,2128
	C18:2	7,2542
	C18:3	39,5862
	C22	16,5728
Uncultured bacterium isolate DGGE gel band B3 16S ribosomal RNA gene, partial sequence (2IP2)	C12	9,9793
	C14	13,5657
	C16	32,0531
	C16:1	18,9977
	C18	9,9048
	C18:1	37,5254
	C18:3	48,1113
	C22	38,4482
Enterobacteriaceae bacterium strain M2/2 16S ribosomal RNA gene, partial sequence (5AP3)	C14	7,1587
	C16	8,8382
Enterobacter sp. strain B73A-2 16S ribosomal RNA gene, partial sequence (5DP3)	C16	4,1703
	C18	8,1752
Uncultured bacterium clone TA2FL_6 small subunit ribosomal RNA gene, partial sequence (5EP3)	C12	19,9821
	C14	23,8980
	C16	29,0869
	C16:1	103,5698
	C18	21,2875
	C18:1	25,5549

Bacteria (Strain code)	FAMEs	Concentration (ppb)*
Pseudomonas aeruginosa strain ACC7 16S ribosomal RNA gene, partial sequence (7BP4)	C16	8,2152
	C16:1	11,2695
	C18	11,2390
	C18:1	12,7451
	C18:3	7,4170
	C22	38,2594
	C22:1	3,7294
	C24	35,2515
Uncultured bacterium clone Adhufec88ml 16S ribosomal RNA gene, partial sequence (8AP4)	C14	4,3778
	C16	10,7376
	C16:1	7,2525
	C18:1	55,2769
	C18:3	41,9445
	C22	11,6664
Uncultured bacterium clone PCF17-65 16S ribosomal RNA gene, partial sequence (8BP4)	Unidentified	
Pseudomonas aeruginosa strain AMAAS57 16S ribosomal RNA gene, partial sequence (9BP4)	C16	2,7563
	C16:1	4,7886
	C18	2,6041
	C18:1	9,3152
	C18:3	9,9005
Uncultured bacterium clone BBD-Aug08-4BB-8 16S ribosomal RNA gene, partial sequence (9CP4)	C16	23,6187
	C18	2,5383
	C18:1	11,7617
	C18:2	5,9107
	C18:3	28,4474
	C22	105,7607
Uncultured bacterium gene for 16S rRNA, partial sequence, clone: N2_65L19f (9DP4)	C18:3	16,2909
	C22	31,7495
Uncultured bacterium clone V4nest_TXEOR_56day_10H 16S ribosomal RNA gene, partial sequence (11BP5)	Unidentified	
Klebsiella oxytoca gene for 16S ribosomal RNA, partial sequence, strain: NGB-FR21 (11CP5)	C16	6,0853
	C16:1	2,4832
	C18	2,3010
	C18:1	5,2101
	C18:2	8,4201
	C22:1	7,7999
	C24	7,8761
Uncultured bacterium clone LNH_9_9_11_Pumice.252423 16S ribosomal RNA gene, partial sequence (11DP5)	C16	6,6408
	C16:1	5,2896
	C18:1	12,3363
	C18:2	13,8171
Uncultured Escherichia sp. clone RL-9 16S ribosomal RNA gene, partial sequence (11GP5)	C16	2,4060
	C18	5,7821

Conclusions

The analysis of the metagenome of bacteria and fungi by molecular techniques has made it possible to determine some possibly non-culturable bacterial groups, as well as the study of the metabolic bacterial and fungal diversity of the San Juan de Lagunillas Experimental Field. The prevalence of the genus *Glomus* with a high number of spores in the soil studied, demonstrates the facility that fungi of this genus have to colonize cocoa roots, which improves soil quality and fertility.

References

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