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Characterization of Bacterial and Fungal Soil Communities by Automated Ribosomal Intergenic Spacer Analysis Fingerprints: Biological and Methodological Variability

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Automated rRNA intergenic spacer analysis (ARISA) was used to characterise bacterial (B-ARISA) and fungal (F-ARISA) communities from different soil types. The 16S-23S intergenic spacer region from the bacterial rRNA operon was amplified from total soil community DNA for B-ARISA. Similarly, the two internal transcribed spacers and the 5.8S rRNA gene (ITS1-5.8S-ITS2) from the fungal rRNA operon were amplified from total soil community DNA for F-ARISA. Universal fluorescence-labeled primers were used for the PCRs, and fragments of between 200 and 1,200 bp were resolved on denaturing polyacrylamide gels by use of an automated sequencer with laser detection. Methodological (DNA extraction and PCR amplification) and biological (inter- and intrasite) variations were evaluated by comparing the number and intensity of peaks (bands) between electrophoregrams (profiles) and by multivariate analysis. Our results showed that ARISA is a high-resolution, highly reproducible technique and is a robust method for discriminating between microbial communities. To evaluate the potential biases in community description provided by ARISA, we also examined databases on length distribution of ribosomal intergenic spacers among bacteria (L. Ranjard, E. Brothier, and S. Nazaret, Appl. Environ. Microbiol. 66:5334–5339, 2000) and fungi.

Advances in molecular biology led to the development of culture-independent approaches for describing bacterial communities without bias, i.e., the selectivity and unrepresentativity of the total community due to cultivation. DNA fingerprinting allows the rapid assessment of the genetic structure of complex communities in diverse environments (17) and of the extent of changes caused by environmental disturbances (5, 15, 25, 27). DNA fingerprinting analyzes part of the genetic information, mostly the ribosomal operon, contained in nucleic acids directly extracted from environmental samples. The target genes are amplified by PCR, and the amplified fragments are subsequently differentiated by their size or sequence variability. More recently, attempts have been made to use these approaches to characterize fungal communities because culture-dependent methods have similar limitations as for bacteria (8). The denaturing gradient gel electrophoresis and single-strand conformation polymorphism fingerprinting methods have been applied successfully to roots (10), soil (3, 9, 28), and compost (19).

A simple and reliable method is rRNA intergenic spacer analysis (RISA), which exploits the variability in the length of the intergenic spacer (IGS) between the small (16S) and large (23S) subunit rRNA genes in the *rrn* operon. This approach has been used successfully to assess the structure of soil bacterial communities (21) and to evaluate the changes that follow antibiotic treatment (24), mercury stress (20), and deforestation (2). An automated RISA (ARISA) method has been developed to improve both resolution and analysis (6) and efficiently compared freshwater communities and differentiated these communities on a fine spatial scale. Like terminal restriction fragment length polymorphism (RFLP) (4, 12, 13) and length heterogeneity RFLP (23), ARISA involves the use of a fluorescence-tagged oligonucleotide primer for PCR amplification and for subsequent electrophoresis in an automated system. This allows the bacterial community structure to be rapidly investigated even when there are a large number of samples. Due to the high resolution of the gels and the high sensitivity of fluorescence detection, the number of peaks detected is much higher on ARISA profiles than on RISA profiles. Similarly, differences in the intensity of the bands can be estimated precisely, which allows a finer comparison of the profiles. However, this level of sensitivity might have some drawbacks because it may introduce a variability within profiles that has no biological origin.

We standardized the ARISA conditions for the characterization of soil bacterial communities (B-ARISA) and evaluated the methodological (DNA extraction and PCR amplification) and biological (geographical and spatial heterogeneity) variability. We studied communities from various soils from different geographical origins with different vegetation cover and physicochemical characteristics. In addition, we investigated the potential use of ARISA for the characterization of fungal communities. Fungal ARISA (F-ARISA) exploits the length polymorphism of the nuclear ribosomal DNA (rDNA) region that contains the two internal transcribed spacers (ITS) and the 5.8S rRNA gene (ITS1-5.8S-ITS2). We evaluated the information on the length heterogeneity of the ITS1-5.8S-ITS2

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TABLE 1. Soil characteristics

Soil	Origin	Vegetation cover	Soil type	Texture	% Organic matter	pH (H_2O)
LCSA	France	Maize culture	Granular	Silty-loam	2.0	7.0
Montrond	France	Permanent pasture	Crumbly	Silty-clay	6.7	6.2
Saint-Elie	French Guyana	Forest	Dispersed	Clayey	14.4	4.4
Ô Mon	Vietnam	Paddy field	Dispersed	Silty-clay	2.7	5.3
Lamto	Ivory Coast	Savanna	Gravelly	Sandy (ferrugineous)	1.5	6.6

region by searching the GenBank database to assess the extent of the variability within the main fungal taxonomic groups.

MATERIALS AND METHODS

Collection of soils. The soils used in this study were chosen because of their different geographic origins, their differing vegetation cover, and their contrasting physicochemical properties (Table 1). Two soils were from temperate ecosystems (France), one from a permanent pasture of perennial grasses at Montrond and one from a maize culture at La Côte Saint André (LCSA). One tropical ecosystem sample was taken from the Lamto Reserve (Ivory Coast), which is a perennial grass savanna. The remaining soil samples were taken from the equatorial forest in Saint-Elie, French Guyana, and from paddy fields in Ô Mon, Vietnam. All soils were collected from the upper layer (5 to 20 cm), sieved (2 mm), and stored at −20°C until they were used.

DNA extraction and amplification. A direct lysis method was used to extract DNA from bacteria and fungi in the soil samples (21). The bacterial IGS located between the small- and large-subunit rRNA genes were amplified with the following primers: S-D-Bact-1522-b-S-20 (eubacterial rRNA small subunit, 5′-TGCGGCTGGATCCCCTCCTT-3′) and L-D-Bact-132-a-A-18 (eubacterial rRNA large subunit, 5′-CCGGGTTTCCCCATTCGG-3′) (18). Amplified sequences contained the IGS plus approximately 150 bp corresponding to the 20 nucleotides of the S-D-Bact-1522-b-S-20 primer and about 130 bp of the 23S rRNA gene. PCR conditions were as previously described (21). The length polymorphism of the ITS1-5.8S-ITS2 region was exploited to characterize the fungal community. The primers used to amplify this region represent consensus sequences found at the 3′ end of the 18S genes in various organisms (primer 2234C, 5′-GTTTCCGTAGGTGAACCTGC-3′) and at the 5′ end of the 28S genes (primer 3126T, 5′-ATATGCTTAAGTTCAGCGGGT-3′) (26). PCR conditions were as for B-ARISA.

Standardization of ARISA. ARISA involves the use of a fluorescently labeled primer for PCR. The ABI 373 DNA sequencer allows the use of different phosphoramidite dye fluorochromes such as FAM (6-carboxyfluorescein), HEX (6-carboxyhexafluorescein), and ROX (6-carboxyrhodamine) (Perkin-Elmer, Courtaboeuf, France). The ROX dye is used as the size standard. To evaluate the influence of the labeled primer and the fluorochrome on PCR efficiency and profile resolution, we labeled the forward or the reverse primer with either FAM or HEX dye. Although FAM provided the best PCR efficiency, as estimated by the total intensity of the profiles, a background of nonspecific fluorescence hampered the detection of some bands. We also noted that the PCR efficiency was better when S-D-Bact-1522-b-S-20 and 2234C were the labeled primers in the B-ARISA and F-ARISA amplification reactions, respectively. This highlights the influence of the fluorochrome on the annealing of the primer. Subsequent PCRs were performed using the S-D-Bact-1522-b-S-20 and 2234C primers labeled at their 5′ end with the HEX fluorochrome.

Reproducibility of ARISA. Reproducibility was tested on the soil samples collected from Saint-Elie. Three different levels of variability were examined: plot (within field), soil DNA extraction, and PCR amplification. To determine the plot variability, DNA was extracted from composite samples obtained from each of three replicate plots located 50 m away from each other. To determine the soil DNA extraction or within-plot variability, we performed three separate DNA extractions from the composite soil sample from each plot. To determine the variability at the PCR amplification level, we compared the profiles from three separate PCR amplifications per DNA extract.

ARISA fingerprinting of soil samples. ARISA fragments were resolved on 6% polyacrylamide gels and run under denaturing conditions for 12 h at 1,500 V on an ABI 373. The data were analyzed by the GeneScan 3.1 software program (Perkin-Elmer). The concentration of labeled PCR product was estimated, and between 1 and 3 μl of the product plus 0.5 μl of internal size standard (GeneScan 1,000-bp ROX; Perkin Elmer) were added to deionized formamide, and the mixture was denatured at 95°C for 5 min. The software converted fluorescence data into electrophoregrams; the peaks represented fragments of different sizes, and the heights of the peaks were the relative proportion of the fragments in the total product. Lengths (in base pairs) were calculated by using the size standards in conjunction with the no-smoothing option and the Local Southern method in GeneScan. Up to 900 bp, we observed that peaks differing in size by 1 bp were easily separated. Above 900 bp, profiles were usually less complex, and contiguous bands differing in size by less than 3 bp were never observed. The relative peak heights within a profile were calculated by dividing the height of an individual peak by the total peak height (sum of the heights of all peaks).

Fungal IGS length database. The sizes of the ITS1-5.8S-ITS2 region within the main true fungal groups (Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota, Oomycota, and Plasmodiophoromycota) were obtained from GenBank in December 2000. These data were for 104 genera and 251 species, and only complete sequences were recorded.

Statistical analysis. Principal component analysis (PCA), which requires that profiles be encoded on the basis of the presence or absence of bands and their intensity, was used to evaluate similarities between communities. To convert data obtained from the GeneScan software rapidly into a table summarizing band presence (i.e., peak) and intensity (i.e., height or area of peak), a computer program was developed (PrepRISA). This software allowed us to choose the number of peaks (i.e., all detected populations versus most dominant populations), the profile resolution (between 1 and 10 bp), and the method of evaluating peak intensity (height or area). Various combinations of parameters were tested to evaluate the influence of using some or all of the data derived from the profiles. With these data sets, no difference was observed in subsequent analysis whether we used the height or the area of the peaks. Similarly, no difference was observed whether the profile resolution was 1, 3, or 10 bp and whether all, 50, or 100 peaks were considered in the analysis. When similar results are observed whatever the parameters used, the ordination of profiles is considered robust. In this study we present the results obtained with a 1-bp resolution over the entire gel length, all peaks, and the height of peak used to determine the peak intensity.

PCA on covariance matrix was performed on the data matrix (bacterial communities as rows and bands as columns). This method provided an ordination of bacterial or fungal communities and of the encoded bands, which were plotted in two dimensions based on the scores in the first two principal components. PCA was performed using the ADE-4 software (29).

RESULTS

B-ARISA community profiles. Complex electrophoregrams (profiles) with peaks (bands) ranging from 200 bp (i.e., a 50-bp IGS) to 1,200 bp (a 1,050-bp IGS) as extrapolated by the GeneScan 1,000-bp ROX standard (Fig. 1), were obtained. Due to the high sensitivity of the automated sequencer, the GeneScan software allowed us to detect a high number of peaks per ARISA profile. Considering optimal (1 bp) and similar resolution power throughout the entire gel and a low fluorescence threshold (50 U of fluorescence intensity), between 182 and 232 bands were detected per profile, depending on the DNA sample.

The bacterial communities from the five sites were easily differentiated because the number and intensity of bands detected on the electrophoregrams were totally distinct (Fig. 1). The structure of the profiles, characterized by the number and length distribution of major bands (peaks of highest relative fluorescence intensity), varied between soil types. The major bands in the Lamto and Montrond profiles were of small fragment sizes (from 300 to 450 bp), whereas the major bands in

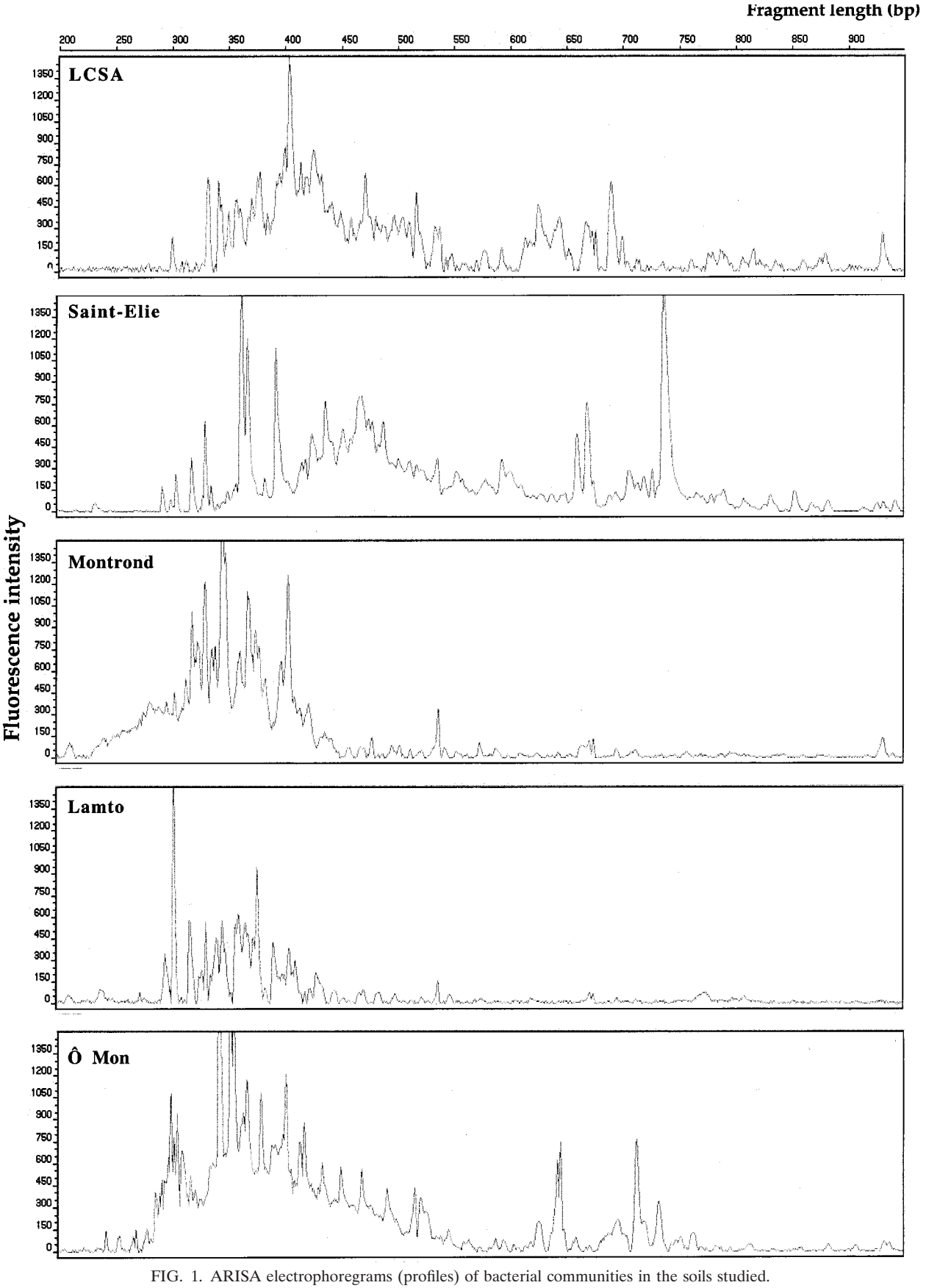

FIG. 1. ARISA electrophoregrams (profiles) of bacterial communities in the soils studied.

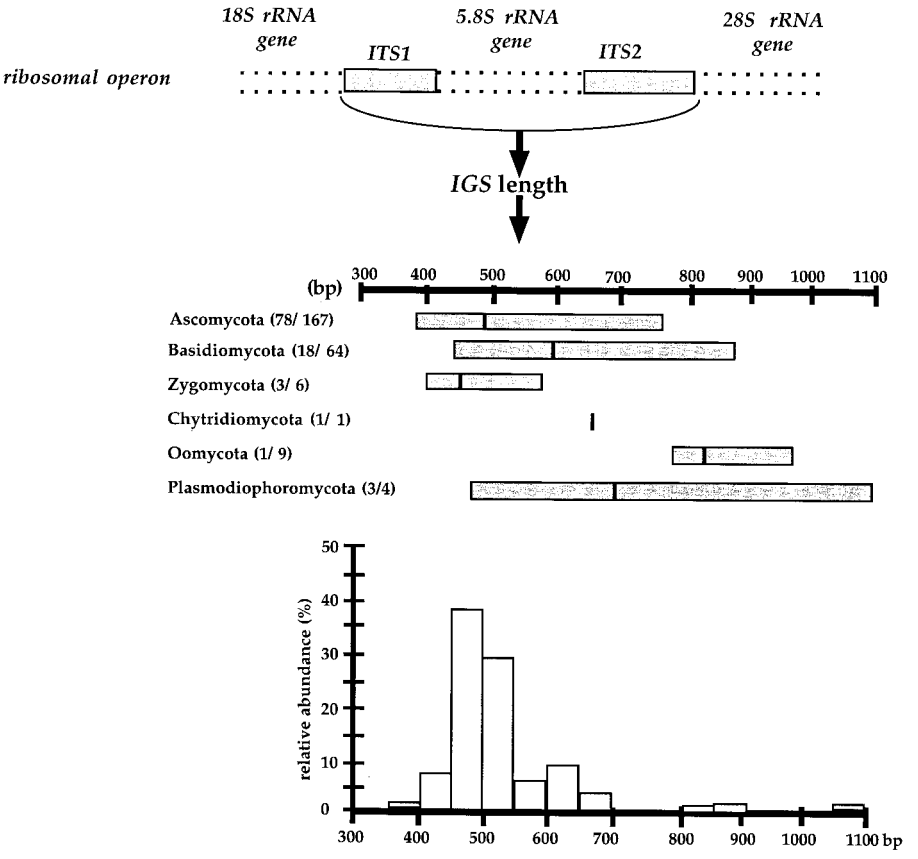

FIG. 2. Length distribution of ITS1-5.8S-ITS2 regions between *rrs* and *rrl* genes among the groups Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota, Oomycota, and Plasmodiophoromycota, represented by approximately 104 genera and 251 species. The data were compiled from the GenBank database (December 2000). The numbers in parentheses indicate the number of genera/species in each group. The vertical lines within boxes indicate the median IGS size for each group.

the LCSA, Saint-Elie, and Ô Mon soil profiles were between 300 and 750 bp. However, regardless of the soil type, no major bands of over 800 bp were observed.

Fungal database examination. We determined the size of the ITS1-5.8S-ITS2 region of 104 genera and 251 species belonging to Ascomycota (78 genera, 167 species), Basidiomycota (18 genera, 64 species), Zygomycota (3 genera, 6 species), Chytridiomycota (1 genus, 1 species), Oomycota (1 genus, 9 species), and Plasmodiophoromycota (3 genera, 4 species) (Fig. 2). The average genus size was 481 bp (range, 390 to 766 bp) among Ascomycota, 591 bp (434 to 882 bp) among Basidiomycota, 442 bp (401 to 543 bp) among Zygomycota, 808 bp (771 to 936 bp) among Oomycota, 675 bp (434 to 1,065 bp) for the Plasmodiophoromycota, and 641 bp for the only species of Chytridiomycota. When only the true fungi (Ascomycota, Basidiomycota, Zygomycota, and Chytridiomycota) were considered, the length of the region ranged from 390 bp to 882 bp (Fig. 2). The smallest spacer (390 bp) was found in an Ascomycota genus (*Eneoelia*), and the longest (882 bp) was found in a Basidiomycota genus (*Armillaria*). When the Oomycota and the Plasmodiophoromycota genera were included, the spacers were longer (up to 1,065 bp). Sixty-eight percent of the reported genus spacers were between 450 and 550 bp long, and only 24% were longer than 550 bp (Fig. 2).

F-ARISA community profiles. F-ARISA on soil samples generated complex profiles with bands of between approximately 300 and 1,100 bp (Fig. 3). With an optimal resolution power of 1 bp and a medium fluorescence threshold detection of 20, the number of bands per profile varied from 82 to 115, depending on the soil sample. As for B-ARISA, the number and intensity of bands and of the dominance structure in the F-ARISA profiles were distinct for each site (Fig. 3). The major bands in the Lamto F-ARISA profile were restricted to about 550 bp, whereas the other soil profiles contained major bands of between 400 and 800 bp. No major fragments of over 850 bp were observed in any of the profiles.

Reproducibility of ARISA. PCA was performed on ARISA profiles to evaluate the between-site variation. Each site could be differentiated on the basis of B-ARISA fingerprinting (Fig. 4A). There were more differences between sites than between independent repetitions for the same site. LCSA, Montrond, and Ô Mon were easily separated on the first axis, which explained 20% of the total variability, and Montrond, Lamto, and Saint-Elie were separated on the second axis, which explained 18% of the variability. PCA of the F-ARISA data revealed a weaker discrimination between sites than with B-ARISA (Fig. 4B). The Ô Mon soil was strongly separated from the other sites on the second axis, which explained 16% of the

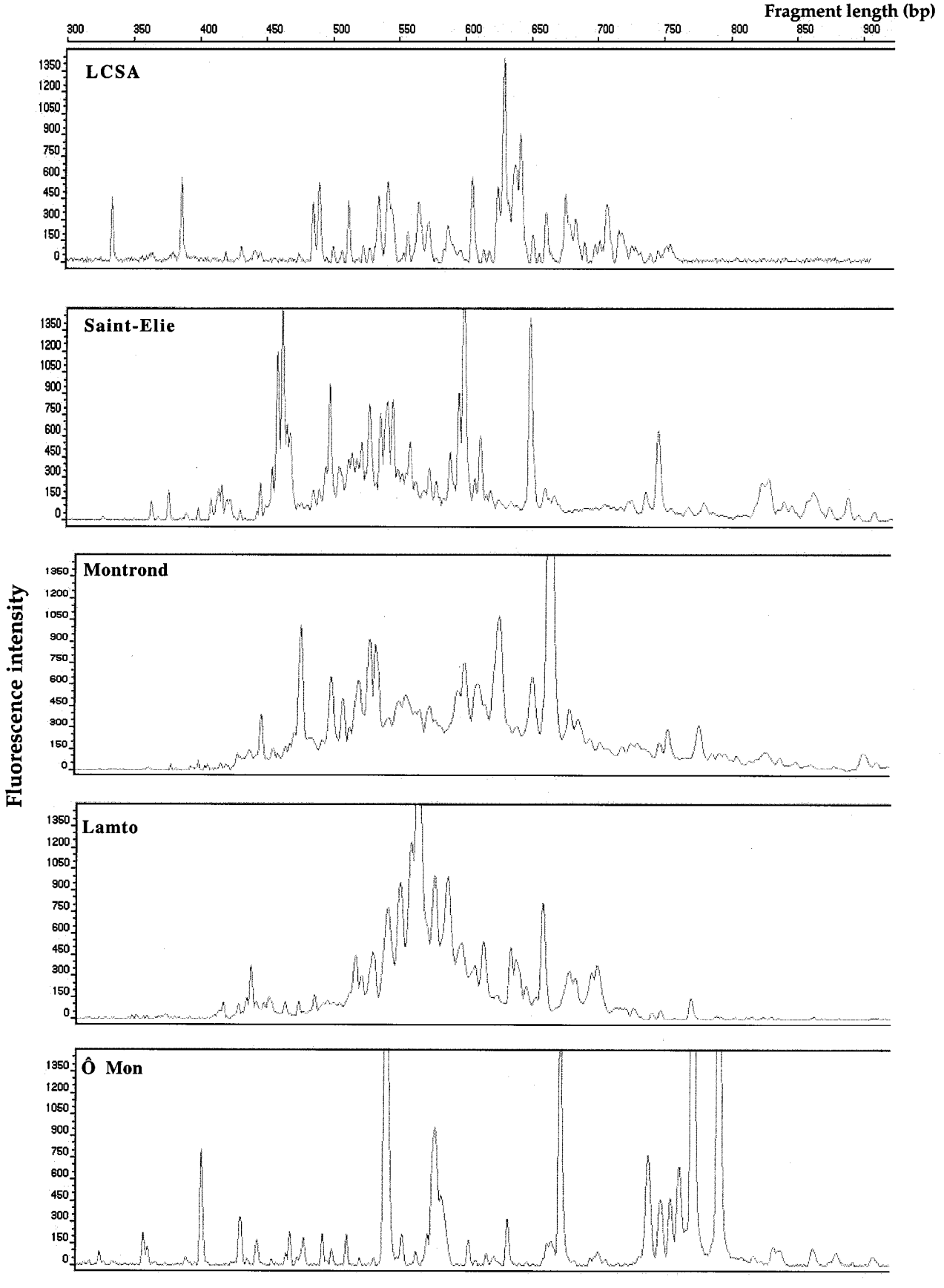

FIG. 3. ARISA electrophoregrams (profiles) of the fungal communities in the soils studied.

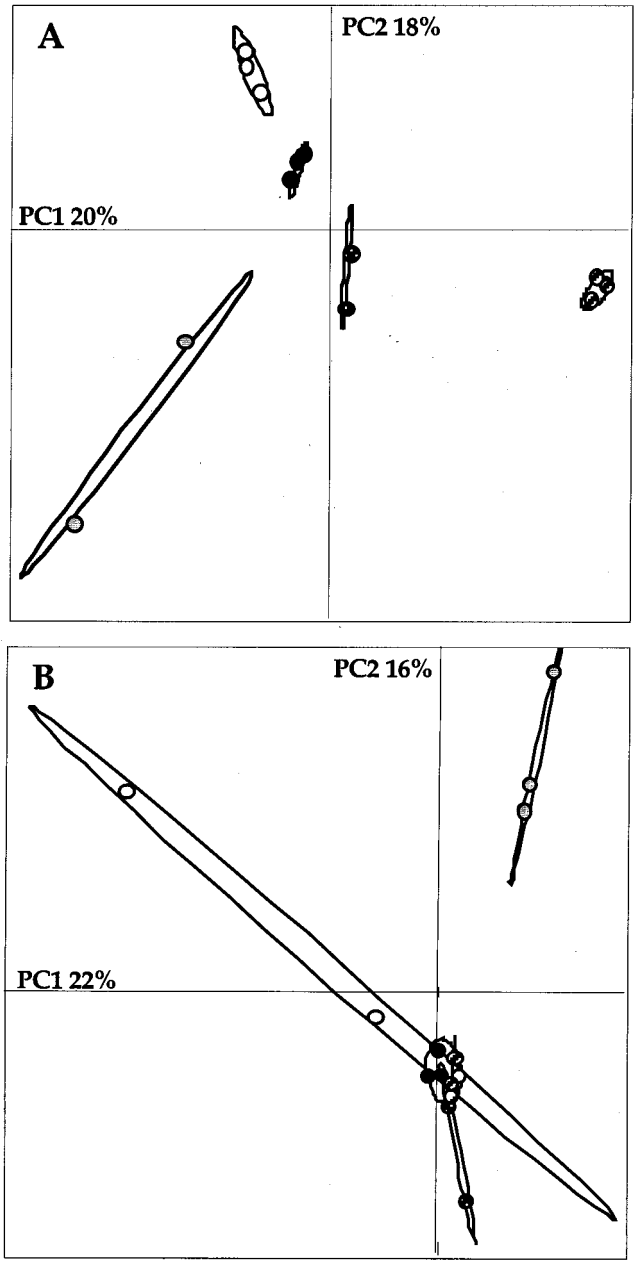

FIG. 4. Principal component (PC1 × PC2) plots generated from ARISA profiles of bacterial (A) and fungal (B) communities of five different soils. Symbols: ○, independent duplicate of Ô Mon soil; ▨, independent triplicate of LCSA soil; ▩, independent duplicate of Montrond soil; ○, independent triplicate of Saint-Elie soil; ●, independent triplicate of Lamto soil. Ellipses represent 90% confidence limits.

variability. However, there were more differences between sites than within sites except for the Saint-Elie site, where there was much variability between repetitions.

We also examined the variability of B-ARISA fingerprinting between plots, between DNA extractions, and between PCR amplifications. Three plots (St-Elie1, St-Elie2, and St-Elie3) 50 m away from each other at the Saint-Elie site were sampled. The high resolution of the sequencing gel and the complex profiles obtained from soil samples detected slight differences between profiles derived from replicate amplifications performed on the same DNA extract from St-Elie3, as evaluated by PCA analysis (Fig. 5A). However, these differences were smaller than those detected between the profiles derived from PCR performed on different DNA extracts from the plot St-Elie3. These differences led to separation of profiles on the first axis, which explained 24% of the variability. Similarly, differences between profiles derived from PCR performed on different DNA extracts were smaller than those between soil samples collected from different plots on the same site (Fig. 5A) or from different geographical sites (Fig. 4A).

We evaluated the variability of F-ARISA profiles between plots and between DNA samples at the St-Elie1, St-Elie2, and St-Elie3 plots. The variability between F-ARISA profiles derived from replicate DNA extracts of different plots was greater than that between samples derived from replicate DNA extracts of the same plot (Fig 5B). As for B-ARISA, there was more variation between the profiles obtained from different DNA extracts within the St-Elie3 plot than between those from the St-Elie1 and St-Elie2 plots. The three plots were separated on the second axis, which explained 25% of the total variability.

DISCUSSION

Fisher and Triplett (6) developed a method for ARISA involving a capillary electrophoresis system. This system provides a rapid and reproducible method for estimating the diversity and composition of indigenous bacterial communities. We used a polyacrylamide gel-based system to study bacterial communities from different soils and, as previously reported by these authors for natural freshwater communities, found that the ARISA method is sensitive and reproducible. We studied soil samples from different geographic locations with contrasting vegetation cover and pedoclimatic characteristics that were expected to have selected different indigenous microbial populations. The comparison of the B-ARISA profiles obtained from these soil samples showed that each was characterized by a specific pattern (Fig. 1), which suggests a particular genetic structure of the bacterial communities. These results confirmed the potential of ARISA for characterizing and differentiating the genetic structure of soil bacterial communities. Manual RISA (21) and ARISA (data not shown) approaches also detected differences between the bacterial subcommunities associated with various microenvironments of a cultivated soil on a fine spatial scale (aggregate size).

A large number of peaks per profile (about 200) were detected for the various soil bacterial communities (Fig. 1). Fisher and Triplett (6) observed fewer than 50 peaks within the profiles of the freshwater communities. Such discrepancies could be biological, since soil communities are considered more diverse than freshwater ones, or methodological. We used electrophoresis on polyacrylamide gels whereas they employed a capillary electrophoresis system. The use of different technologies may limit comparison of results. One can also mention that distinction of true peaks from background cannot be ascertained. This evidences the need for repetitions at various steps of the experiment (several DNA extractions or several PCRs) in order to obtain more accurate information. However, background information is common to all gel pro-

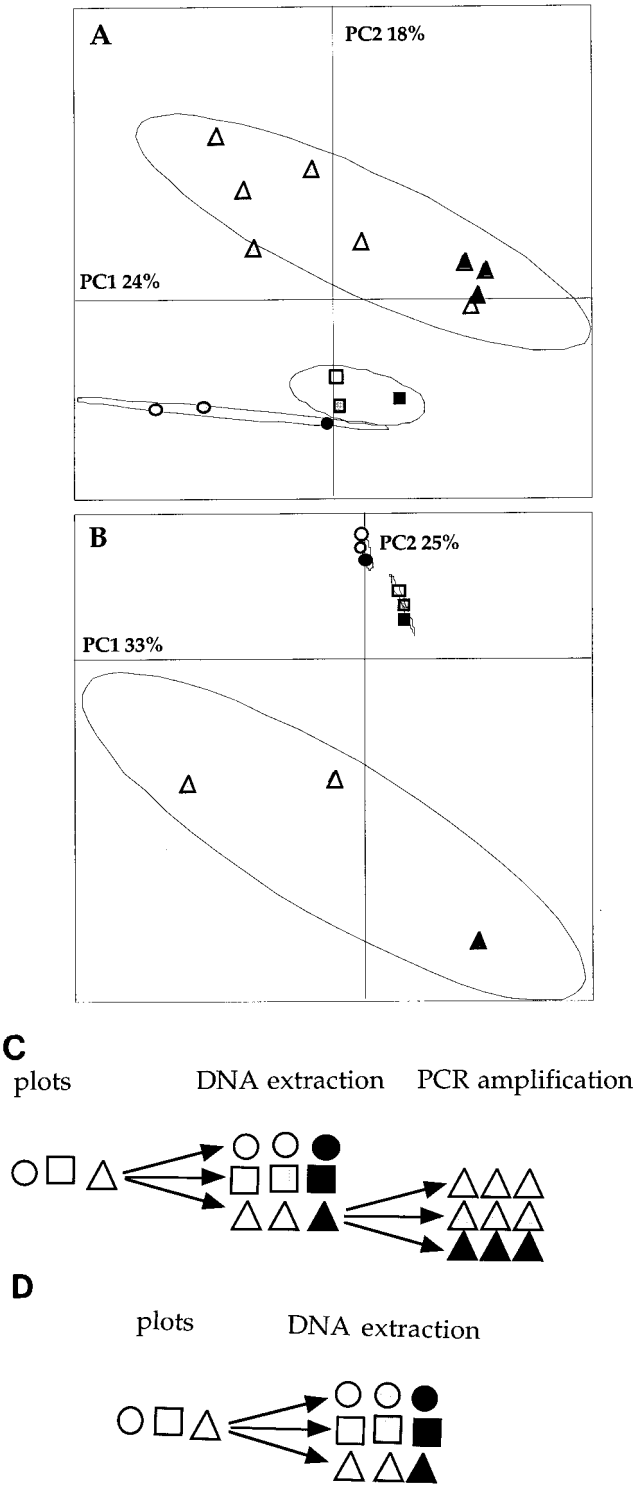

FIG. 5. Comparison of B-ARISA (A) and F-ARISA (B) variability performed by PCA of profiles obtained from the Guyana site. Three different reproducibility criteria were analyzed for B-ARISA, plots, DNA extraction, and PCR amplification (C). Two different reproducibility criteria were analyzed for F-ARISA, plots and DNA extraction (D). Ellipses represent 90% confidence limits.

files and should not influence the further comparison of communities based on multivariate analysis.

Differences between the B-ARISA profiles at different sites are due to differences in the number and intensity of bands and in the dominance structure of the patterns. The latter corresponds to the number and length distribution of the relative major bands of a profile that can be related to the dominant populations of a community. Although the RISA patterns cannot reveal the taxonomic composition of the community due to the overlapping of size classes among unrelated populations (22), some conclusions can be drawn about the distribution of the genera within the main phylum. For instance, the profiles from the soils originating from the Montrond and Lamto sites did not contain any IGS fragments of over 550 bp (i.e., a 400-bp IGS). Together with the data on the length distribution of IGS among the bacterial phyla (22), these results suggested that both soil communities are characterized by the dominance of low- and high-G+C Firmicutes and by the low abundance of alpha proteobacteria. Several authors used the 16S rDNA gene cloning strategy to evaluate the phylogenetic diversity in natural soil communities, and differences between soil communities in terms of the abundance of the main phylum are evidenced. For instance, whereas the alpha proteobacteria predominated in some grassland soils (16), the low abundance of genera belonging to alpha proteobacteria within bulk soil (1, 11) or the rhizosphere (14) was shown.

When all the bands were considered, some B-ARISA patterns clearly exhibited bands of less than 300 bp. This corresponds to an IGS of less than 150 bp, which has not been described previously in bacteria (22). We propose to determine whether these bands are artifactual or whether they originated from uncultured microorganisms by cloning and sequencing these fragments. The B-ARISA profiles were characterized by the small number of bands (minor or major) found above the largest band of the size standard (928 bp). A similar result was reported by Triplett and coworkers, who used RISA and ARISA on water (6) and soil (2, 24) samples. A previous study found few bands of over 900 bp in RISA profiles of soil (20). This may be due to the low representivity and diversity of the indexed populations harboring large IGS (longer than 750 bp), which all belong to the alpha subdivision of the proteobacteria (22). The large number of bands of between 300 and 700 bp and their high intensity may be explained by the wide diversity of organisms with an IGS of between 150 and 600 bp (22). PCR amplification might also increase bias to a small number of large bands by preferentially amplifying shorter templates.

We adapted ARISA for the characterization of fungal communities by using a pair of primers that targeted the 3′ end of the 18S rDNA sequence and the 5′ end of the 28S rDNA sequence. As a preliminary step, we screened the GenBank database to determine the range of reported ITS1-5.8S-ITS2 sizes. The length polymorphism of this region was found to be compatible with the separation of amplified fragments by use of an automated electrophoresis system. Data showed that each main group falls within a size class but that the groups overlap, particularly in the size range of 450 to 550 bp (Fig. 2). As for bacteria (22), there is no correlation between genus or species and the length of the spacer except for the largest IGS, which is found only in Oomycota and Plasmodiophoromycota (Fig. 2). Comparison of the F-ARISA profiles obtained from

the various soil samples showed that each site is characterized by a specific pattern (Fig. 1 and 3), suggesting a particular genetic structure of the fungal communities. Complex profiles with bands of between ~300 bp and over 1,100 bp (Fig. 2), which correspond to spacers of 230 to 1,030 bp, respectively, were observed. Viaud et al. (30) used primers located at equivalent positions at the 3′ end of the 18S subunit and at the 5′ end of the 28S subunit to amplify soil DNA extracts and observed a continuum of bands from 350 to 1,000 bp in size. Fragments of less than 390 bp were not expected based on the GenBank data. It remains to be determined whether the bands below this value originated from undescribed species (fungi from culture collections that have not yet been sequenced or fungi that have not yet been isolated). Similarly, bands of between 770 and 870 bp were detected in the Saint-Elie and Ô Mon profiles, whereas no genera with IGS of this size were found in any data bank (Fig. 2). However, the specificity of the primers may account for this anomaly. Although amplification tests on several bacterial genera (*Pseudomonas, Escherichia, Agrobacterium, Arthrobacter*, and *Streptomyces*) (data not shown) and Blast search showed that the selected primers do not amplify bacteria, the Blast search also showed that the primers were totally homologous to some ribosomal sequences from eukaryotic species that are unrelated to fungi. Although most of these species and consequently their DNAs are unlikely to be encountered in soil, we cannot totally exclude that some close organisms could harbor amplifiable sequences. These observations suggest that more appropriate primers should be designed to ensure a narrower amplification range. Specific phylogenetic groups could also be analyzed by using specific primers, such as those designed by Gardes and Bruns (7) for the study of mycorrhizal Basidiomycota.

PCA analysis showed that the differences between the F-ARISA profiles of each site were less pronounced than those of B-ARISA profiles (Fig. 4) because only the Ô Mon and some of the Saint-Elie soil samples could be clearly separated from the others. This could be due to low variability in the population structure between the various soil fungal communities and/or to lower variability in IGS size between fungal populations than bacterial ones. It can be seen that 69% of the genera belonging mainly to Ascomycota, Basidiomycota, and Zygomycota have an IGS of 450 to 550 bp (Fig. 2). However, technical biases such as a nonoptimal soil DNA extraction or the low specificity of the primers may be responsible for such an observation.

The tests to evaluate the resolution and the reproducibility of ARISA showed that the method can differentiate communities from soils with contrasting characteristics and can differentiate communities from different plots within a given site. Repetitions of PCR on different DNA extracts from the soil sampled at the St-Elie1 and repetitions of DNA extractions on each of the three plots showed that a slight variability exists, as shown by the PCA analysis, which separated both PCR or DNA extraction repetitions on the factorial map (Fig. 5A). Although the differences between or within sites are due to differences between the detected bands and their intensities, the variability between profiles generated by repetitions of DNA extractions or PCR amplification was mainly explained by differences in the relative fluorescence intensity of similar peaks. Consequently, the methodological variability was never high enough to mask the biological one. Fisher and Triplett (6) also found that the PCR step of this method was highly reproducible on profiles from lake bacterial communities. As previously reported for the length heterogeneity PCR approach (23), the most variability was observed between plots followed by between DNA extracts, and the least variability was between different PCR amplifications. For F-ARISA, the profiles were highly reproducible, and as before, there was greatest variability between plots (Fig. 5B). These results demonstrate that ARISA is a very effective and sensitive method for detecting differences between complex bacterial or fungal communities at various spatial scales (between- and within-site variability). The automation and the use of multivariate analysis allow many samples to be handled at once and allows experiments to be designed to test the reproducibility at various steps.

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