

Study of bioaerosols and site influence in the La Plata area (Argentina) using conventional and DNA (fingerprint) based methods

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Abstract The present study approaches the characterization of seasonal samples of bioaerosols taken during 2005–2006 in rural, urban, industrial, coastal and residential sites within La Plata area, Buenos Aires, Argentina. Culturable airborne fungal and bacterial communities were collected on DG18 agar and R2 Agar plates respectively, using a single-stage SKC sampling device. Fungal genera were identified based on their micro- and macro-morphological characteristics. Bacterial populations were analyzed by denaturing gradient gel electrophoresis (DGGE) of PCR 16S rDNA bacterial amplification. Geometric mean concentration of bacteria and fungi ranged from 10 to 10³ CFU m⁻³ and were comparable with those from other reports. No differences were observed among sites for fungi and *Cladosporium* sp. was predominant, with 85.7% of total concentration counts; secondary genera that contributed were *Alternaria* (6.7%), *Penicillium* (1.8%), *Aspergillus* (1.3%), *Epicoccum* (1.0%) and the group of yeasts

(1.9%). The dominance of *Cladosporium* in all sites suggests that the most abundant fungal aerosol was neither significantly affected nor primarily generated by any anthropogenic area source more than the natural ambience present in the La Plata area. With regard to bacteria, the results showed on several occasions differences in concentration among sites during sampling events, but these differences were not observed when the community structure was analyzed by means of DGGE. Bacterial DGGE banding profiles from all sites revealed the existence of a relatively diversified, culture-based airborne community. Construction of similarity dendrograms exposed a distribution of site samples in which replicates intra-site equalled those encountered among sites, rendering substantial inference of site distinction unfeasible.

Keywords Atmosphere · Bacterial community · DGGE · Fungi · Outdoor environment

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1 Introduction

Although physical and chemical processes occurring in the atmosphere have been studied for a long time and are taken to be a major issue for the better understanding of ecosystem functioning and life, the presence of bioaerosols in the atmosphere has on the contrary been largely omitted and only in recent years more thoroughly considered. Moreover, in classical

model environment studies dealing with characterizations of bacterial diversity, microbiologists have not taken the atmosphere into account sufficiently (Maron et al. 2005). On the contrary, major attention has been paid to bioaerosols with regard to their related adverse health effects (ACGIH 1999; WHO 1990), and currently there are cases of well-established associations between airborne fungi and symptoms of respiratory allergy and asthma (Garrett et al. 1998; Adhikari et al. 2004). Literature on this topic is promissory and has been carried out in indoor environments such as hospitals (Bauer et al. 1990), homes and offices (Dales et al. 1991; Pastuszka et al. 2000; Górny and Dutkiewicz 2002), and farms and processing plants (Wilson et al. 2002a). Surveillance studies of outdoor bioaerosols have been carried out in natural or disrupted rural areas, and urban and industrial sites (Jones and Cookson 1983; Kramer and Eversmeyer 1984; Shaffer and Lighthart 1997). Nevertheless, there are still severe gaps in the historical records and characterizations of bioaerosols for some parts of the world that make diagnosis of relationships between health effects and bioaerosol presence hardly possible in those regions, except by means of extrapolation to areas with a similar climate. Another major issue regarding the bioaerosol records measured is their great variability depending on sampling method, collection equipment, type of micro-organism, physiological status, etc., and it has become imperative to pay special attention to the guidelines proposed in order to make possible comparisons with other related studies (WHO 1990; Henningson and Alhberg 1994; Jones and Harrison 2004). Even though the most relevant topic in recent times has been the election of culture- or non-culture-based methods, the latter has acquired an enormous impetus in response to the development of several effective diagnostic, PCR-based methods (Peccia and Hernandez 2006). Comparative studies have shown that culturable micro-organisms grown in frequently used media represent only a small proportion of the total (0.1–10%) in an oligotrophic environment such as the atmosphere (Shaffer and Lighthart 1997; Peccia and Hernandez 2006). Nonetheless, culturing permits differences in cellular activity to be taken into account, enabling the measurement of ecologically relevant organisms, excluding inactive cells that do not contribute to ecosystem functioning, and this selectivity has

proven to be useful for determining the impact of anthropogenic activity (Ellis et al. 2003).

In this context, the present study approaches the characterization of culturable airborne fungal and bacterial communities in rural, urban, industrial, coastal and residential sites located near the La Plata area, Province of Buenos Aires, Argentina during 2005–2006, utilizing a culture-dependent method of fungus identification and a PCR-DGGE (denaturing gradient gel electrophoresis) method (Muyzer et al. 1993, 1998) to characterize the genetic structure of indigenous bacterial communities from culturable airborne samples. In this sense, the main goal of this study was to assess whether sites under urban and industrial burdens, in which past studies revealed an anthropogenic influence on air quality (Massolo et al. 2002; Rehwagen et al. 2005), had modified microbial community structure in comparison with the less modified environments. Regarding the PCR-DGGE-based method, the aim was to assess its capacity to characterize the airborne bacterial community structure when short-term samples were collected on Petri plates with a conventional bioaerosol sampling device. In order to overcome low DNA recovery (for effective PCR) inherent in short-term air sampling, the selection of a culture-based collection method followed by surface washing allowed the study of culturable bacterial communities.

2 Materials and methods

2.1 Site description and air sample collection

The La Plata area includes several well-defined sectors characterized as urban, industrial, rural, coastal and residential, covering a total surface of 25 km², and a population of approximately three quarters of a million inhabitants. Its climate is that of a humid temperate zone, with an average annual temperature of 16°C and an average annual rainfall of 1,000 mm, with fairly homogenous distribution among seasons. A sampling site for each of the five different sectors was selected. A brief description providing essential data on the sites is shown in Table 1.

Sampling of viable airborne bacteria and fungi was carried out on five sampling sites distributed among four seasonal times (winter 2005, spring 2005,

Table 1 Characteristic features of sampling sites

Sites	Primary air pollution factor	Local ground cover	Description
1. Rural	None	Vegetation	Presence of agricultural fields and grasslands; occurrence of flooding. Sparse vegetation of trees. Scarcely populated. 15 km away from urban and industrial area
2. Urban	Vehicle emission	Hard surface	Densely populated. Vegetation of trees, mainly foreign species. Number of mobile sources (light and heavy) over 200 thousand
3. Industrial	Oil refinery	Vegetation/hard surface	Prevalence of air pollution of industrial origin. Site characterized by presence of country's main oil refinery (total crude oil distillation capacity: 32,000 m ³ day ⁻¹), and six petrochemical plants. Low to medium density population. Presence of grasslands and <i>Eucalyptus</i> sp.
4. Coastal	None	Vegetation	At margin of Río de La Plata river. Vegetation dominated by the presence of pasture fields and relicts of tropical jungle. Scarcely populated. Residential area
5. Residential	Low vehicle emission	Hard surface/vegetation	Green areas dominated by introduced vegetation. Highway located nearby with heavy traffic

autumn 2006 and winter 2006), using a single-stage SKC sampler (Anderson N6 equivalent) with 400 0.25-mm holes ($D_{ae50} = 0.68 \mu\text{m}$), drawing air at a flow rate of 28.3 l min^{-1} (velocity = 24 m s^{-1}) (Jones et al. 1985). Calibration was performed prior to each measurement, ensuring desirable flow rates. Samples were collected for 1.5–3 min (depending on previous charge estimation, and assurance of colony counts between 30 and 300 per plate) on 20 ml of the supplied nutrient media in Petri dishes placed within the impactor. In all cases samples were drawn at 1.5 m above ground (critical human health-related height) and at between 09.00 and 13.00 h in order to minimize variations between sites attributable to shifting daily atmospheric conditions (Jones and Harrison 2004). Sampling was done on relatively stable days with low wind velocities and no precipitation events having taken place immediately prior to sampling. Temperature, wind velocity and relative humidity conditions were obtained from a local meteorological station (35.0° S 57.9° W, Servicio Meteorológico Nacional) and recorded in order to ensure that no pronounced shifting of conditions had occurred during retrieval of samples taken on different sites.

2.2 Fungal and bacterial counts

At least three replicate counts for fungi and bacteria were made at each site on any of the seasonal

sampling dates, except for winter 2006, when no replicates were taken for coastal or residential areas. DG-18 (Dichloran-Glycerol 18%) agar plates were used for fungi count, with the addition of streptomycin (0.025%; wt vol⁻¹) to inhibit bacterial growth. R2 Agar plates were used for the bacteria count, with the addition of cycloheximide (0.025%; wt vol⁻¹) to inhibit fungal growth. These culture media are frequently used as nutrient media in oligotrophic environments and had produced satisfactory results in preliminary samples. From all sampling dates, a total of 87 samples of culturable bacteria and 68 samples of culturable fungi were collected. The R2 Agar and DG-18 agar plates were incubated at 28–30°C for 5 and 7 days respectively. After the incubation period, the plate counts were recorded and reported as colony-forming units per cubic meter of air (CFU m⁻³).

2.3 Fungal classification

Fungal genera were identified based on their micro- and macro-morphological characteristics, using standard taxonomic keys (Carrillo 2003; Samson et al. 2004). Colonies that did not generate spores were isolated and incubated for 21 days in DG18 agar plates. Less than 1% of all colonies were not identified and were not considered for further analysis.

2.4 Bacterial DNA extraction

The bacterial biomass from R2 agar plates was obtained by a wash of the agar surface with 5 ml of sterile physiological solution (NaCl 0.85% [wt vol⁻¹]). Genomic bacterial DNA was extracted following the protocol described by Kuske et al. (1997). Due to the presence of well-grown bacterial colonies on agar plates after incubation, no major optimization steps (i.e., incubation to induce spore germination of non-culture-based air samples as proposed by Maron et al. 2005) were applied for a better recovery of low bacterial densities. The DNA pellets obtained were resuspended in 25 µl of TE buffer (Tris-EDTA). Of the total bacterial samples taken on the four seasonal dates, 47 were extracted and treated for analysis by means of DGGE (10, 20 and 17 samples in winter 2005, spring 2005 and autumn 2006 respectively; no DGGE profiles were constructed for the winter 2006 samples). Replicates of samples extracted from each site varied from a minimum of two in winter 2005 to a maximum of four in spring 2005. Those replicate samples with invasive morphotypes masking the presence of other colonies were discarded.

2.5 PCR amplification and DGGE

Polymer chain reaction amplification of fragment 626-pb of the 16S-rRNA gene was carried out with eubacterial primers 341fGC and 907r, as described by Muyzer et al. (1993, 1998). PCR reactions contained: 1 µl of a 1/25 dilution of total extracted community DNA, 1 U GoldTaq DNA polymerase, buffer (manufacturers recommended buffer as supplied with the polymerase enzyme), 200 mM of BSA, 0.4 mM dNTPs and 20 pM of each primer at a total volume of 50 µl. Amplification was performed on Progene FPROGO5Y (Techne, Burlington, NY, USA). The procedure included an initial denaturing step of 4 min at 95°C, and a first cycle step for 30 s at 94°C, 45 s at 62°C and 1 min at 72°C (10 cycles), followed by a step-down of 30 s at 94°C, 40 s at 57°C, and 1 min at 72°C (25 cycles). Amplification was completed by a final extension step at 72°C for 10 min. Agarose gel electrophoresis was performed to verify the presence of PCR products.

Denaturing gradient gel electrophoresis was performed using the BioRad D GENE System (BioRad, Munich, Germany). The purified PCR amplicons were directly applied onto 6% (wt vol⁻¹) polyacrylamide gels, which were formed with acrylamide stock solution (acrylamide-*N,N'*-methylenebisacrylamide, 37; 5:1). The gel contained a linear gradient of 30–65% denaturant (100% denaturant corresponds to 7 M urea and 40% [vol vol⁻¹] formamide). Polymerization of gels was achieved by adding 0.26% (vol vol⁻¹) ammonium persulfate (10% solution) and 0.15% (vol vol⁻¹) *N,N,N',N'*-tetramethylethylenediamine (TEMED). Electrophoresis was performed in 1× TAE buffer (20 mM Tris acetate (pH 7.4), 10 mM sodium acetate, 0.5 mM Na₂EDTA). Thirty microliters of the PCR product of each sample was applied and gels were run at 60°C at a constant voltage of 100 V for 16 h. After the run gels were stained in ethidium bromide for 1 h and de-stained in pure water for 45 min, banding patterns were visualized under UV light and digitalized as a tif.file using a CCD camera.

2.6 Statistical analysis

The statistical analyses of microbial counts were performed using the StatSoft (STATISTICA version 6) software program. The Shapiro-Wilk statistical test was employed to evaluate normality of the data, and then the data were analyzed using ANOVA or the nonparametric Kruskal–Wallis test when normality was not met. The geometric mean (GM) and geometric standard deviation (GSD) were used to express the log-normal UFC m⁻³ concentrations. The criterion for significance in the procedure was $P < 0.05$. The Shannon Diversity index (H'), evenness (E) and richness were calculated for the data of fungal genera and sets of data were compared by means of a t test ($P < 0.05$).

Bacterial DGGE profiles were analyzed using the Gelcompar II program (Applied Maths, Kortrijk, Belgium). Normalized positions of detected bands in all lanes were used for cluster analysis and band matching. Dendrograms for comparison of DGGE banding patterns were constructed using the unweighted pair group method (UPGMA) with arithmetic averages following the pairwise calculation of Dice coefficients according to the presence

and absence of matching bands (Sokal and Michener 1958).

3 Results and discussion

3.1 Fungal populations

The density of airborne fungi populations at each site during seasonal sampling dates are presented in Table 2. GM values ranged from 10 to 10^3 CFU m⁻³ and were comparable with those from other reports (Pastuszka et al. 2000; Lee and Jo 2006). Maximum concentration values observed in single plates or replicates occurred during autumn 2006 and corresponded to the urban site (9,434 CFU m⁻³) and the industrial site (8,303 CFU m⁻³). A descriptive approach to a comparison of sites during seasonal sampling dates shows that there is a prevalence of concentration patterns due to seasonal conditions disregarding sampling sites. Comparative site analysis shows that, despite differences observed upon fungal geometric mean concentrations among some sites (i.e. during spring, rural GM: 2,035 CFU m⁻³ almost four-fold urban GM: 462 CFU m⁻³ and during winter 2005 rural GM: 227 CFU m⁻³ three-fold urban GM: 69 CFU m⁻³), these results were of no significance when statistically analyzed as part of the total data sampled. The seasonal total fungi count was highest during autumn 2006 (GM: $2,652 \pm 1.9$ CFU m⁻³), and lowest during the sampling date

in winter 2005 (GM: 137 ± 1.6 CFU m⁻³). Statistical analysis by means of the Kruskal–Wallis nonparametric test reveals significant differences among all seasonal sampling dates except for spring 2005 and winter 2006, which showed no differences between them. The fungal genera observed, their corresponding overall contribution percentages, H' index, the richness and evenness for each sampling site, and each seasonal sampling event are presented in Table 3. An overview of the overall genera contributions in Table 3 shows *Cladosporium* to be the major contributor, responsible for roughly 86% of the total counts, so any significant difference in statistical analysis of the total count between seasons is largely due to *Cladosporium* oscillation during the sampling dates. Secondary genera were *Alternaria* (6.7%), *Penicillium* (1.8%), *Aspergillus* (1.3%), *Epicoecum* (1.0%) and the group of yeasts (1.9%). Only during the winter 2005 seasonal sampling was a more uniform distribution of genera observed, as demonstrated by its comparatively higher diversity index (H' : 2.0), higher E (0.76), and the overall percentage contribution of *Cladosporium* (14%). Although the principal genera found in this study were coincident with previous studies conducted in India (Adhikari et al. 2004), Saudi Arabia (Al-Suwaine et al. 1999), and Korea (Lee and Jo 2006) among others, showing that these are major components typically present in outdoor environments, discrepancies were observed in the overall contribution of *Cladosporium*, being less dominant in the latter studies and more uniformly

Table 2 Bioaerosol concentrations (CFU m⁻³) expressed as GM $\pm$ GSD, according to season

Micro-organism	Site	Winter 2005	Spring 2005	Autumn 2006	Winter 2006
Fungi	Rural	227 ± 1.2	$2,035 \pm 1.2$	$1,545 \pm 1.3$	$1,927 \pm 1.3$
	Urban	69 ± 1.3	462 ± 1.1	$4,773 \pm 1.9$	830 ± 1.4
	Industrial	138 ± 1.3	$1,426 \pm 1.3$	$3,449 \pm 2.2$	699 ± 1.5
	Coastal	167 ± 1.5	$1,298 \pm 1.5$	$2,295 \pm 1.8$	–
	Residential	134 ± 1.5	$1,180 \pm 1.3$	$2,480 \pm 1.5$	–
	Seasonal GM	137 ± 1.6	$1,155 \pm 1.7$	$2,652 \pm 1.9$	847 ± 1.7
Bacteria	Rural	$2,781 \pm 1.8$	$2,987 \pm 1.7$	$1,031 \pm 1.4$	$2,463 \pm 1.5$
	Urban	644 ± 1.4	$1,873 \pm 1.4$	$1,207 \pm 1.9$	$2,131 \pm 1.6$
	Industrial	$1,474 \pm 1.3$	$1,998 \pm 1.1$	$1,260 \pm 1.5$	752 ± 1.7
	Coastal	$1,611 \pm 1.3$	957 ± 1.1	672 ± 2.2	–
	Residential	$1,925 \pm 1.2$	$1,643 \pm 1.1$	581 ± 1.9	–
	Seasonal GM	$1,448 \pm 1.8$	$1,774 \pm 1.6$	906 ± 1.9	$1,484 \pm 2.0$

–: no samples taken at this location during the seasonal sampling event

Table 3 Overall percentage contributions of fungi genera as a total for each seasonal sampling moment and for each site

Genera	Season				Site					Mean by fungal type
	Winter 2005	Spring 2005	Autumn 2006	Winter 2006	Rural	Urban	Industrial	Coastal	Residential	
<i>Cladosporium</i>	14.0	89.8	89.5	72.0	87.2	82.5	83.0	89.1	90.3	85.7
<i>Alternaria</i>	35.5	5.4	7.0	1.8	3.9	11.4	5.1	3.5	5.9	6.7
Yeasts	3.2	–	2.0	7.0	1.6	1.7	5.9	–	0.2	1.9
<i>Penicillium</i>	15.0	0.9	0.9	5.5	0.6	1.9	2.5	2.0	2.4	1.8
<i>Aspergillus</i>	4.3	–	0.3	8.0	2.1	1.4	0.6	0.9	0.6	1.3
<i>Epicoccum</i>	3.2	3.0	–	2.2	2.5	–	1.4	1.2	0.5	1.0
<i>Wallemia</i>	–	–	0.2	2.0	–	0.7	0.5	0.4	–	0.4
<i>Geotrichum</i>	14.0	–	–	–	0.7	–	–	1.1	0.2	0.3
<i>Eurotium</i>	–	0.1	0.2	0.4	–	0.1	0.5	0.4	–	0.2
<i>Arthrimum</i>	3.2	–	–	–	0.3	–	–	–	–	0.1
<i>Pithomyces</i>	–	–	0.1	–	0.2	–	–	–	–	0.1
<i>Amblyosporium</i>	–	–	0.1	–	–	–	–	0.4	–	0.1
<i>Scopulariopsis</i>	–	–	–	0.5	0.3	–	–	–	–	0.1
<i>Stemphylium</i>	2.0	–	–	–	–	–	–	0.4	–	0.1
<i>Bipolaris</i>	1.0	–	–	–	–	–	–	0.2	–	<0.1
<i>Sphaerulina</i>	–	0.1	–	–	–	0.1	–	–	–	<0.1
<i>Curvularia</i>	–	0.1	–	–	–	0.1	–	–	–	<0.1
<i>Acremonium</i>	–	0.3	–	–	0.3	–	–	–	–	0.1
<i>Nigrospora</i>	1.0	0.1	–	–	0.1	–	0.2	–	–	0.1
<i>Trichothecium</i>	1.0	–	–	–	0.1	–	–	–	–	<0.1
<i>Fusarium</i>	1.0	0.1	0.1	–	–	–	0.2	0.6	–	0.1
<i>Beauveria</i>	1.0	–	–	–	–	–	–	0.2	–	<0.1
<i>Botrytis</i>	–	–	–	0.4	–	0.1	–	–	–	<0.1
Richness	14	10	10	10	13	10	10	13	7	
H'	2.00	0.46	0.46	1.10	0.63	0.67	0.74	0.58	0.43	
Evenness	0.76	0.20	0.20	0.48	0.24	0.29	0.32	0.22	0.22	

Richness, Shannon diversity index (H'), and evenness (E) values for sampling events and sampling sites

–: genera not detected

distributed with other genera (values in the order of 24–43%). In this sense, in the present study, the dominance of *Cladosporium* in all sites suggests that the most abundant fungal aerosol is neither significantly affected nor primarily generated by any anthropogenic area source more than the natural ambience present in the La Plata area. When individual genera among different sampling sites were compared using two-way ANOVA, statistical differences ($P < 0.05$) were observed only for *Epicoccum* during spring 2005 between rural and urban sites, but not during other seasonal sampling events (data not shown). Comparison of H' index values by means of a t test revealed the existence of significant

differences between the winter 2005 and 2006 sampling events and spring 2005 and autumn 2006, also showing that both winter sampling events have a relatively higher index value, demonstrating a more even distribution among genera. Comparison of H' index values for each site by means of a t test reveals significant differences for all sites with regard to the residential site, which we infer is primarily due to the relatively low richness shown at this site. Since the contributions of most important genera were almost identical to the other sites and lower richness was due to the absence of the relatively minor genera, this could hardly be attributed to residential site influences.

3.2 Bacterial cell density and community genetic structure

The bacteria cell densities at each site during sampling events are presented in Table 2. These results show densities ranging from 10^2 to 10^3 CFU m^{-3} , which are slightly higher but in general accordance with previous atmospheric studies accomplished by means of culture-based methods (Bovallius et al. 1978; Shaffer and Lighthart 1997; Pastuszka et al. 2000). Comparative analysis of sampling sites showed significant differences between among during winter 2005, spring 2005 and winter 2006; a Tukey test revealed that differences observed during winter 2005 were attributable to low bacterial density at the urban site and higher counts at the rural and residential sites. During spring 2005, differences existed between coastal and all other sites due to the lower average concentration value of the former. Finally, the differences observed during winter 2006 were attributable to low bacterial concentration at the industrial sampling site, which differed significantly from the higher counts of rural and urban sites. When all seasonal samples of each site were statistically compared, differences among sites were not observed.

Denaturing gradient gel electrophoresis schematic banding profiles, dendrograms and band matching lines of 16SrDNA fragments amplified from bacterial DNA during winter 2005, spring 2005 and autumn 2006 are shown on Figs. 1, 2 and 3 respectively. The dendrograms show that the fingerprinting profiles of

replicates of each sampling site did not belong to the same cluster, demonstrating that differences observed between samples taken in each location were not necessarily greater than those observed among successive replicate samples per site. However, a tendency in the distribution of replicates was observed during autumn 2006 (Fig. 3). This figure shows that urban and industrial sites clustered closely and relatively separate from coastal, residential and rural sites, which produced poor similarity values. These results may indicate that a less heterogeneous and stable culturable bacteria community ruled at that moment in the more polluted sites in contrast to the more heterogeneous and variable distribution of the less polluted sites. Dendrograms showed relatively broad similarity percentages ranging from 50 to 87%; 32 to 80%, and 19 to 92% for winter, spring and autumn profiles respectively. Comparison of bacterial concentrations for each sampling site and their respective number of DNA bands demonstrated that higher CFU m^{-3} counts did not necessarily correspond to higher numbers of bands (e.g., winter 2005 urban site with one of the highest sum number of different bands: 22, showed one of the lowest average GMs: 644 CFU m^{-3}). Previous studies conducted on outdoor environments have exhibited the variable nature of airborne bacteria communities; highly diversified groups of airborne bacteria were detected by Radosevich et al. (2002) in samples collected with culture-independent methods and analyzed by means of cloning and sequencing and Maron et al. (2005) identified a total of 97 sequences by A-RISA DNA

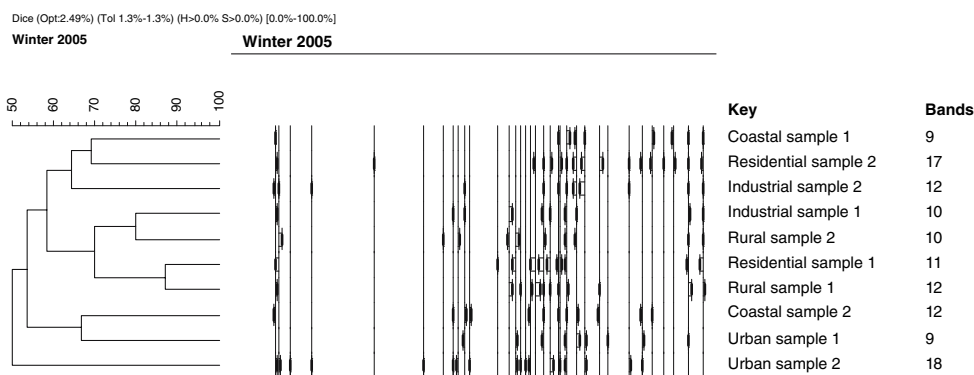


Fig. 1 Dendrogram of the cluster analysis of the denaturing gradient gel electrophoresis (DGGE) schematic banding profiles as calculated using the Dice coefficients and the unweighted pair group method (UPGMA). The banding

profiles represent the different bacterial communities from each sampling site in winter 2005. The column on the right corresponds to the number of bands detected from each sampling site

Dice (Opt:0.25%) (Tol 0.4%-0.4%) (H>0.0% S>0.0%) [0.0%-100.0%]

Spring 2005

Spring 2005

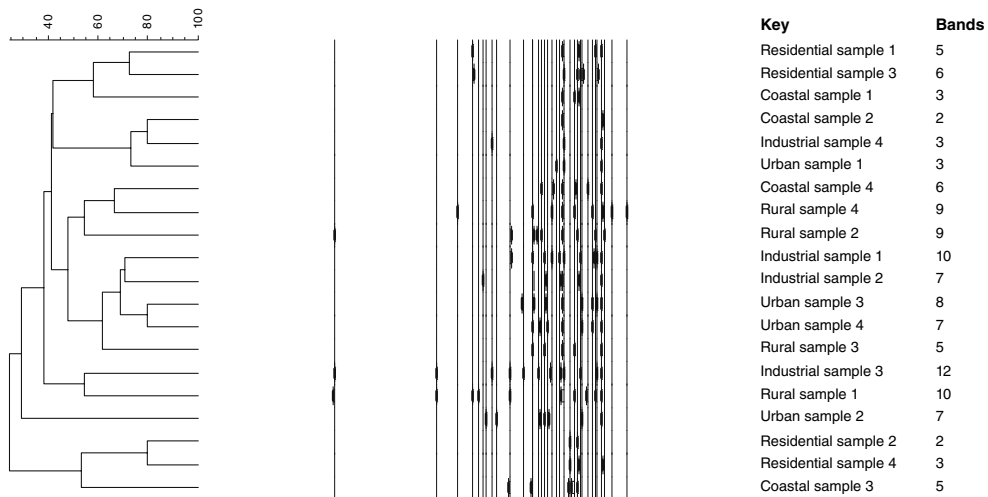


Fig. 2 Dendrogram of the cluster analysis of the DGGE schematic banding profiles as calculated using the Dice coefficients and UPGMA. The banding profiles represent the

different bacterial communities from each sampling site in spring 2005. The column on the *right* corresponds to the number of bands detected from each sampling site

Dice (Opt:0.60%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-100.0%]

Autumn 2006

Autumn 2006

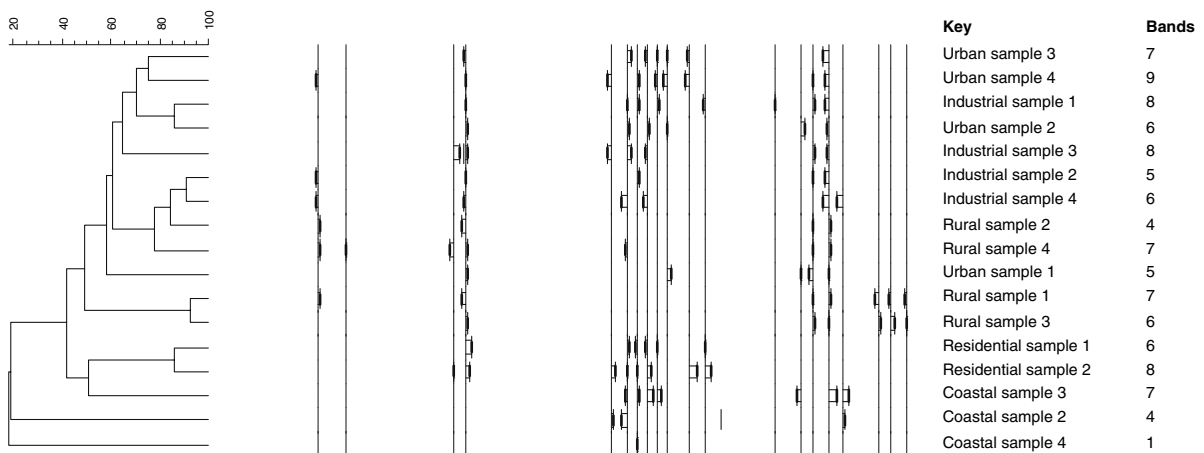


Fig. 3 Dendrogram of the cluster analysis of the DGGE schematic banding profiles as calculated using the Dice coefficients and UPGMA. The banding profiles represent the

different bacterial communities from each sampling site in autumn 2006. The column on the *right* corresponds to the number of bands detected from each sampling site

fingerprinting. In contrast, Wilson et al. (2002b) showed a weakly diversified community and stated that more than 95% of the organisms identified were *Bacillus* sp. In the present study, the account for total DNA banding patterns of fingerprints from all sites in

each sampling event (33, 33 and 20 different bands detected in winter 2005, spring 2005 and autumn 2006 respectively) reveals the existence of a relatively diversified culture-based airborne community; moreover, further site comparison of banding

demonstrated that all sites had similar band numbers during all seasons with only slight differences. On the other hand, the contribution of unique bands (not shared with other sites, for all seasonal samples) was considerable in rural, urban and industrial sites (8, 7 and 5 respectively), but almost absent for residential (2 bands) and coastal (1 band) ambiances, suggesting that the first sites might have unique sources of bioaerosols that are absent in the latter.

4 Conclusions

The results obtained provide evidence that local source influences at the sites did not significantly affect the fungal airborne population during sampling events. In contrast, bacterial results showed, on several occasions, differences in concentration among sites during sampling events, but these differences were not observed when the community structure was analyzed by means of DGGE. It could be assumed that effective spore release and dispersal mechanisms may evenly distribute airborne fungi in the 25-km² study area. Since bacteria in many cases are attached to larger airborne particles (soil and other organic matter), and taking into account that previous studies have shown an uneven particulate matter size distribution pattern (Rehwagen et al. 2005), it is most likely that the occasional shifting bacterial counts among sites during sampling may be reflecting differences in the pattern of airborne particles in each site sampled. Regarding fungi, the prevalence of the genera *Cladosporium* in outdoor environments should be taken into account by local health care organizations due to its well-known association with allergies, hypersensitivity, pneumonitis and dermatitis. PCR-based genetic profiling techniques such as DGGE have proven to be promising techniques for assessing differences in microbial environments (McCaig et al. 2001; Smit et al. 2001; Ellis et al. 2003); nonetheless, in the present study, the variability of replicates intra-site equalled those encountered among sites, discouraging the authors from making the substantial inference of site distinction. Difficulties in identifying differences among sites by means of 16SrDNA banding patterns from culturable bacterial communities could be associated with the state of the art of present methodologies. The authors acknowledge that

reduced sampling time, or constraints in PCR (such as masking of the less abundant colonies by the more abundant) and DGGE (such as resolution and detection limits of staining, smears and comigration) are likely to be relevant restrictions that should be overcome. Future studies identifying unique bands detected on each site, may demonstrate whether the tendencies observed (i.e., during autumn 2006) were due to the biases mentioned or to polluted site influences.

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