ETUDE *IN SITU* DE LA DIVERSITE MICROBIENNE AEROPORTEE

Dans la partie précédente, l'objectif principal de la recherche était de proposer un protocole fiable et efficace pour la caractérisation de la diversité microbienne en aérosols dans les environnements intérieurs par l'utilisation d'outils moléculaires. Cette étude a permis de développer une méthodologie intégrant à la fois l'échantillonnage, l'évaluation de la diversité bactérienne et eucaryote en suspension dans l'air intérieur et les analyses moléculaire ultérieures.

Le système de collecte cyclonique est apparu comme étant le plus efficace en se basant sur les critères définis dans notre étude pour l'analyse des microorganismes aéroportés (aspects quantitatifs et qualitatifs, le coût et la facilité de mise en œuvre).

Cette étude a également fait apparaitre la nécessité de recourir à des collecteurs fonctionnant à des débits importants afin de parvenir à la réalisation d'analyses moléculaires dans des conditions optimales.

Afin de maximiser la quantité de microorganismes collectée, et en raison des contraintes (évaporation de l'eau, notamment) de notre biocollecteur, la durée de prélèvement a été fixée à 40 minutes (contre 30, précédemment), soit un volume de 40 m^3 d'air échantillonné. Dans l'hypothèse d'un environnement investigué faiblement contaminé avec une fraction cultivable de 10 UFC/m^3, 4.10^5 unités génomiques par échantillon seraient alors collectées, quantité suffisante compte tenu de la sensibilité analytique des techniques moléculaires employées (CE-SSCP et PCRq).

II.1 ARTICLE 2 : STABILITE TEMPORELLE DES AEROSOLS MICROBIENS DANS LE MUSEE DU LOUVRE

Le manque de données moléculaires environnementales sur les microorganismes aéroportés dans les espaces clos a souligné la nécessité de planifier une surveillance de la diversité microbienne. Dans ce contexte, cette étude vise à donner une représentation globale de la diversité et de la dynamique des microorganismes aéroportés d'un ERP (le musée du Louvre) sur une période de six mois.

Notre approche s'est divisée en deux parties. La première, devait permettre d'évaluer la charge microbienne et d'identifier la stabilité ou les variations temporelles des communautés bactériennes et fongiques. La seconde partie a été consacrée à l'étude de la diversité bactérienne et a été appliquée de la même manière à des échantillons choisis au terme de l'analyse préliminaire.

Ce travail devait conduire à une meilleure description des communautés microbiennes rencontrées dans l'air du musée du Louvre.

STABILITY OF AIRBORNE MICROBES IN THE LOUVRE MUSEUM OVER TIME.

Carole Gaüzère[a*], Marina Moletta-Denat[a],Hélène Blanquart[b], Stéphanie Ferreira[b], Stéphane Moularat[a], Jean-Jacques Godon[c], Enric Robine[a]

[a]Université Paris-Est, Centre Scientifique et Technique du Bâtiment (CSTB), Laboratoire de Recherche et d'Innovation pour l'Hygiène des Bâtiments, 84, avenue Jean Jaurès - Champs-sur-Marne, 77447 Marne-la-Vallée Cedex 2, France.

[b]Genoscreen, Genomic Platform and R&D, Campus de l'Institut Pasteur, 1 rue du Professeur Calmette, 59000 Lille, France.

[c]INRA, UR50, Laboratoire de Biotechnologie de l'Environnement (LBE), Avenue des Etangs, 11100 Narbonne, France.

[*]Corresponding author: carole.gauzere@gmail.com

Telephone number: +33(0).1.64.68.85.17

Fax number: +33(0).1.64.68.88.23

Bioaerosols, indoor air quality, pyrosequencing, Q-PCR, CE-SSCP, Louvre Museum

Abstract

The microbial content of air has as yet been little described, despite its public health implications, and there remains a lack of environmental microbial data on airborne microflora in enclosed spaces. In this context, the aim of this study was to characterise the diversity and dynamics of airborne micro-organisms in the Louvre Museum using high-throughput molecular tools. Thus the microbial community was monitored for a six month period (autumn and winter). The total bacterial and fungal loads as well as and the dynamics of the structure of airborne micro-organisms were assessed using Quantitative Real Time Polymerase Chain Reaction, fingerprinting methods and phylogenetic analysis. The quantitative results revealed variations in the concentrations of less than one logarithm, with average values of 10^3 and 10^4 genome equivalent per m^3 for bacteria and fungi, respectively. Our observations highlighted the stability of the indoor airborne community over time, while the corresponding eukaryote community was less stable. Bacterial dynamics seemed to be driven mainly by stable indoor parameters and, more specifically, by the existence of the main source arising from human occupants. On the other hand, eukaryote dynamics seemed to be partially influenced by variable outdoor parameters.

1. Introduction

The proliferation of epidemic alerts due to microbial aerosols and anthropogenic changes of use (urbanization, intensive agriculture, waste treatment, travel, air-conditioning, etc.) has led governments to reconsider the health risks associated with air quality (Angenent et al., 2005). Modern lifestyles mean that populations throughout the world spend increasingly more time indoors, a level reaching about 90% in Western countries. Air is a natural vector of biological materials (pollen, different organic fragments, micro-organisms, etc.). The transmission of infectious agents cannot always be avoided because controlling the dissemination of airborne pathogens is far more difficult than preventing the pathogenic contamination of surfaces, water or food. However, data on the diversity of microbial aerosols in indoor environments, and their spatial or temporal variations still remains scanty. Much more information is needed on this diversity, including fluctuations in its quality and quantity, in order to better understand the role of air in microbes disseminating in indoor environments.

Although the diversity of the cultivable bacteria found in confined spaces is relatively well documented, this field of study has not benefited from recent technological advances such as the use of metagenomic tools and from potential improvements in the description of microbial diversity. Only three studies have investigated the microbial diversity of air in indoor environments since the first inventory of air was compiled by Radosevich et al. in 2002 (Angenent et al., 2005; Osman et al., 2008; Tringe et al., 2008).

This new data concerning the microbial diversity of indoor air has demonstrated the presence of all microbial domains (*Bacteria*, *Eukarya* and *Archaea*) and revealed bacterial diversity which differs from that observed using cultivation techniques.

Because airborne micro-organisms are present at low concentrations, it is a challenge to collect them, particularly when using molecular tools (Tringe et al., 2008). Collection devices with high flow rates or high collection efficiency may be necessary to achieve molecular analysis under optimum conditions (Kejun, 2011; C. Gaüzère, unpublished).

The measurement of total airborne bacteria using Real-time Quantitative Polymerase Chain Reaction (Q-PCR) is now widely applied in medical research as well as in industrial working environments (Nehme et al., 2008; Oppliger et al., 2008; Rinsoz et al., 2008; Moletta-Denat et al., 2010; Just et al., 2011), and, also, in some indoor environments such as commercial airline cabins (La Duc et al., 2007; Osman et al., 2008). It is now accepted that non-viable or as yet-uncultivable micro-organisms can induce allergic, toxic and inflammatory responses (Gorny et al., 2002; Rinsoz et al., 2008) and must be taken into account.

Additionally, little data is available regarding temporal variations in enclosed spaces determined using molecular techniques though indoor environments such as buildings, public places or transport systems, which are currently not studied, are where we spend most of our time in these settings.

The dynamics of the structure of bioaerosol communities have been assessed using molecular fingerprinting techniques and these have already been applied to outdoor air (Maron et al., 2006; Després et al., 2007), biogases (Moletta et al., 2007), composting sites (Bru-Adan et al., 2009) and, recently, to indoor environments such as offices (Kejun, 2011), poultry facilities (Just et al., 2011) and piggery buildings (Nehme et al., 2008).

Only one study on enclosed spaces has monitored bacterial load and diversity using a culture-independent approach; this focused on eight swine confinement buildings by means of visits throughout the year. During this study, total bacterial concentrations were found to be higher in winter than in summer. Despite this result, DGGE profiles and phylogenetic analyses revealed a similar biodiversity in each swine unit during both seasons. These results also suggested that pig slurry might be a potentially important source of the bioaerosol diversity encountered at such sites (Nehme et al., 2008).

Cultivation techniques have been used mainly when the objective has been to monitor variations in bacterial and fungal concentrations or diversity in enclosed spaces. All such studies came to different conclusions. Those by Augustowska and Dutkiewicz (2006) and Aydogdu et al. (2005), focusing on a hospital ward and primary schools respectively, highlighted seasonal variations: the concentration of fungi and bacteria varied as a function of different months. In the same way, Wang et al. (2010) demonstrated different concentrations of fungi and bacteria in commuter trains according to the seasons. In 2006, Cho et al. (2006) measured significantly higher levels of airborne fungi in five subways stations in Seoul when the number of passengers and the frequency of passing trains were the highest. Measurements performed in a Chinese museum also linked the results obtained to human activity (Chen et al., 2010). This study also suggested daily variations in the concentrations of micro-organism. These findings were consistent with those obtained by LeBouf et al. (2008).

In primary schools, Aygdodu et al. (2005) observed a predominance of certain bacterial genera such as *Staphylococcus*, *Corynebacterium* and *Bacillus* and of fungal genera such as *Penicillium*, *Cladosporium* and *Alternaria*. Furthermore, *Staphylococcus*, *Acinetobacter*, *Corynebacterium*, *Propionibacterium* and *Pseudomonas* genera were found during every month of the year.

The lack of environmental data on airborne micro-organisms in enclosed spaces has highlighted the need to develop the monitoring of microbial diversity. In this context, the present study was designed to characterize the diversity and dynamics of airborne micro-organisms in the Louvre Museum using high-throughput molecular tools.

Our study was divided into two parts. The first involved an assessment of the microbial load using molecular tools and the identification of overall variations through time i.e. the stability of bacterial and eukaryote communities. The second part sought to characterise the bacterial diversity of samples of interest as a function of the recorded variations over time, and to identify 'core-species' bacteria.

This objective was to achieve a clearer description of the microbial communities encountered in the air of the Louvre Museum, including both qualitative and quantitative data.

2. Results

Over a six-month period, 12 aerosol samples were collected in the Louvre Museum in order to determine the quantitative and qualitative dynamics of airborne micro-organisms over time.

2.1 Temporal stability of environmental parameters

During the measurement campaign (autumn and winter), the indoor temperature was 21.2°C ± 2.8°C and relative humidity was 41.6% ± 9.8%. The highest temperature values were recorded in samples D3, D4 and D5, while the highest relative humidity levels occurred in samples D2, D5 and D87. Microclimatic monitoring highlighted homogenous values. During the period analysed (at all sampling times), fluctuations in temperature averaged between about 0.8% and 7.4% and about 1.0% to 9.0% for relative humidity. Outdoor temperatures during the same period were 7.2°C ± 6.1°C, with higher values observed for samples D164 and D178 and a lower value for sample D87.

Between 89.7% and 92.5% of indoor particles were measured by the first two OPC channels (particle optical diameter between 0.3 and 0.5 µm). Between 6.5% and 7.8% of indoor particles were measured in OPC channels 3 and 4 (particle optical diameter between 0.5 and 0.8 µm). Less than 1% of the particles observed by the OPC had dimensions between 2 and 5 µm. The total airborne particle concentration in this indoor environment ranged from 2.2×10^7 to $1.1\text{x}10^8$ particles/m^3.

To provide a clearer representation, the particle size distributions were presented on a differential plot between $\Delta n/\Delta \log d_p$ and d_i, which showed the modes of particle size distribution as well as the amount of sample found in each size range along a continuous spectrum (Baron and Willeke, 2005). The corresponding mass and number concentrations of the aerosol particles in different size ranges are shown on Figure 35.

Mean matched comparisons of size distribution measurements did not reveal any significant differences between the five profiles. Indeed, all *p values* obtained using Wilcoxon tests were higher than 0.05 ($p > 0.65$). The number size distributions produced by the Grimm OPC displayed similar profiles for each week of the sampling campaign, containing a peak at an optical diameter of 2.5 µm which corresponded to 'coarse particles' (Figure 35).

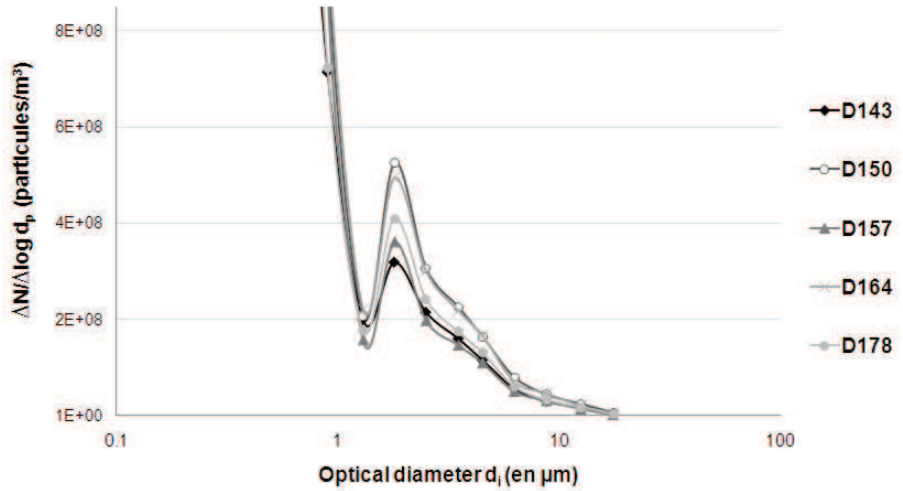


Figure 35 : Particle concentrations measured by the Grimm OPC.

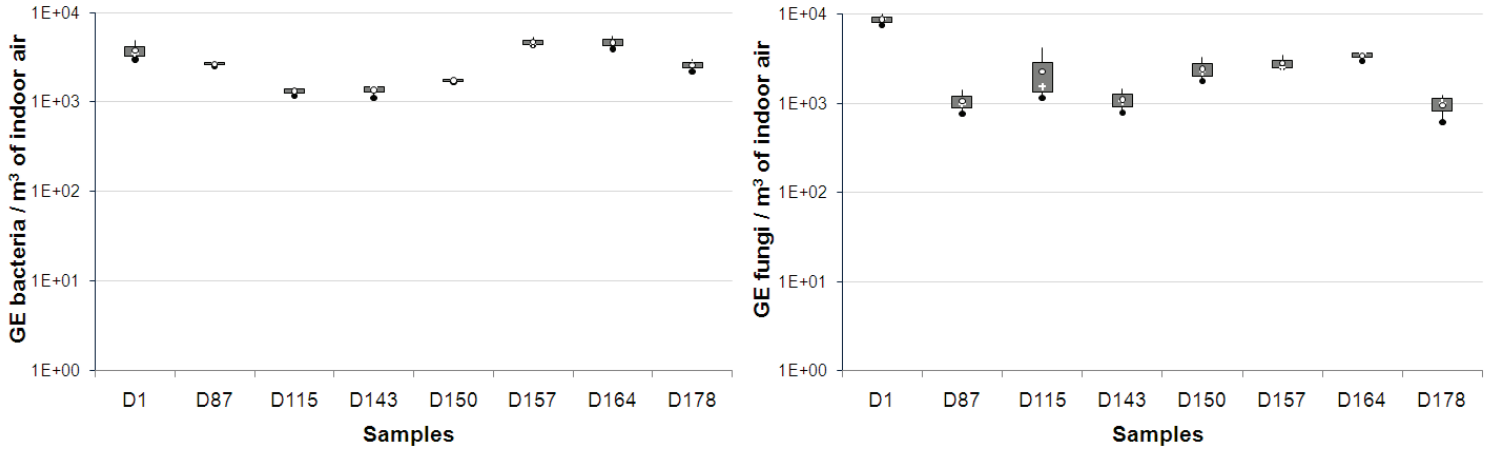


Figure 36 : Temporal monitoring of bacterial (left graphic) and fungal (right graphic) concentrations in the Louvre Museum.

2.2 Temporal stability of bacterial and fungal bioaerosol communities in terms of abundance

Quantitative values for bacterial and fungal concentrations were determined on 12 of the samples collected. Quantitative PCR results are presented herewith, together with the concentration of GE per cubic metre of air as a function of the sample collected.

The microbial concentrations in the samples from the Louvre Museum ranged from $1.1x10^3$ to $5.4x10^3$ GE bacteria/m^3 air and from $1.2x10^3$ to $9.9x10^3$ GE fungi/m^3 air (Figure 36).

The quantitative results revealed variations in the concentration that were smaller than one logarithm with respect to both bacterial and fungal loads.

A Friedman test was performed on all the samples for both bacterial and fungal quantification and did not reveal any significant differences between the values found (p=0.96 and 0.2 for bacteria and fungi, respectively). Nor were any significant differences observed between bacterial and fungal values during either weekly and monthly monitoring.

2.3 Temporal stability of bacterial and eukaryal bioaerosols communities at the structure level

The dynamics of the structure of microbial communities were assessed using CE-SSCP applied to the 12 samples collected. As shown in Figure 37, CE-SSCP bacterial profiles were represented in terms of the mean CE-SSCP profile and the standard deviation (+ 2SD).

The presence of similarly-migrating peaks in bacterial profiles suggested that some species were present in all samples. But although each sample collected had a similar bacterial profile, some differences could be observed (relative abundance, missing peaks). All samples appeared to be very similar even when they were collected at short intervals (several days) or longer intervals (several months). A comparison of all profiles revealed 97% similarity, while 98% similarity was observed between two replicates of two independent extraction patterns (data not shown).

By contrast, the structure of eukaryote communities displayed more pronounced differences, a comparison between all profiles producing 93% similarity (Figure 38).

The profiles revealed considerable bacterial richness (more than 20 common peaks in almost all profiles) and a high degree of diversity as shown by the Simpson index values (Table 16). By contrast, with respect to eukaryote richness there were only a limited number of common peaks (Figure 38) and, furthermore, diversity was less complex than that observed for bacteria (a smaller number of peaks and a lower Simpson index) (Table 16).

The common fraction of CE-SSCP profiles, based on the minimum value of each scan, produced values of 55% and 30% respectively for bacteria and eukaryotes.

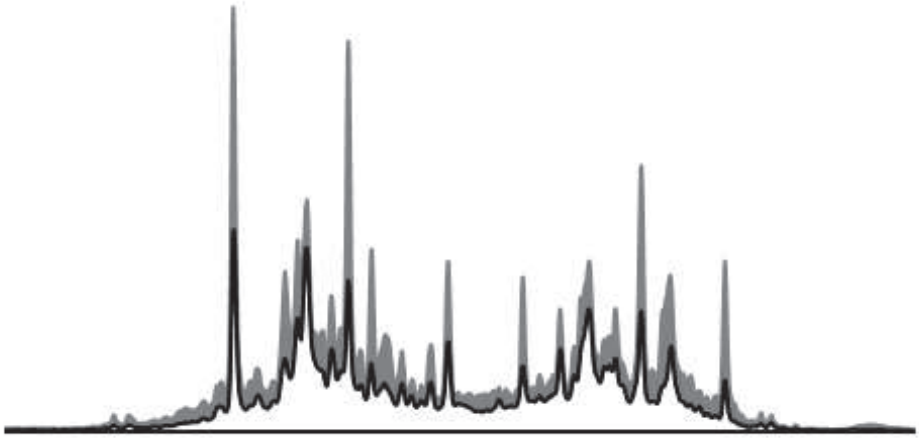


Figure 37 : Representation of the mean bacterial CE-SSCP profile and it standard deviation (+ 2SD).

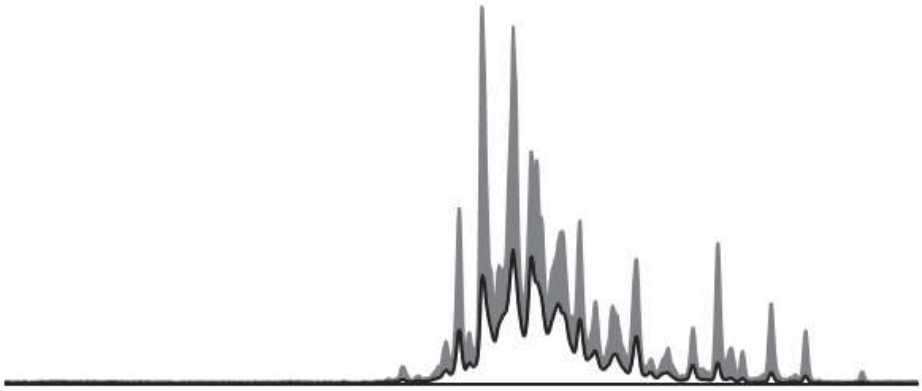


Figure 38 : Representation of the mean eukaryote CE-SSCP profile and it standard deviation (+ 2SD).

Table 16 : Analysis of bacterial diversity in indoor air samples.

	CE-CCSP		Pyrosequencing				
Sample	**Peak number (Bacteria / Eukarya)**	**Simpson index (Bacteria / Eukarya)**	**Number of sequences**	**Number of phylotypes**	**Coverage C (%)**	**S_{chao1} index**	**Simpson index**
Day 1	26 / 11	6.2 / 2.2	6053	1036	62	1257	5.8
Day 2	26 / 12	5.1 / 4.1					
Day 3	27 / 18	5.6 / 4.0					
Day 4	28 / 16	5.6 / 4.5					
Day 5	25 / 12	5.4 / 3.7					
Day 87	29 / 17	5.2 / 4.8					
Day 115	22 / 14	5.0 / 2.5					
Day 143	21 / 16	5.0 / 5.9					
Day 150	26 / 16	5.3 / 4.1					
Day 157	31 / 15	6.0 / 3.3	6880	1261	63	1532	6.0
Day 164	32 / 20	6.0 / 3.9	6558	1374	59	1775	6.1
Day 178	28 / 17	5.7 / 4.7					

Table 17 : Phylogenetic positioning and abundance of bacterial phylotypes in the three indoor bioaerosols. Only phylotypes with an abundance of more than 0.5% in one bioaerosol or more are presented. Phylotypes found in three, two or one samples are presented respectively as white-on-black shading, dark grey shading and light grey shading.

	Percentage of sequences (%)						
Phylotypes	**Day 1**	**Day 157**	**Day 164**	**Total**	**Closest relatives**	**Accession number**	**Similarity (%)**
Actinobacteria (535 phylotypes, 5157 sequences)							
MILIA 11	1.3	< 0.1	< 0.1	0.4	*bacterium Ellin6023*	100	AY234675
MILIA 15	0.9	0.8	1.6	1.1	*uncultured bacterium*	100	FM874763
MILIA 50	2.1	2.6	3.7	2.8	*uncultured bacterium*	100	FM874711

MILIA 55	1.1	2.7	2.5	2.1	*Micrococcus sp. TUT1210*	100	AB188213
MILIA 211	0.5	0.8	1.1	0.8	*unidentified bacterium*	100	AY345490
MILIA 10	0.7	0	0	0.2	*uncultured bacterium*	100	HM273602
MILIA 23	0.9	0.5	0.3	0.6	*Corynebacterium sp. IMMIB WACC-658*	100	FN295567
MILIA 33	0.8	0.1	< 0.1	0.3	*uncultured bacterium*	100	AY958846
MILIA 80	0.6	0.4	0.6	0.5	*uncultured bacterium*	100	HM308448
MILIA 128	0.3	0.8	0.7	0.6	*Kocuria carniphila (T)*	99	AJ622907
MILIA 139	0.6	0.2	0.3	0.4	*Arthrobacter agilis*	100	AF511518
MILIA 142	0.6	0.4	0.2	0.4	*Brevibacterium iodinum (T)*	100	X83813
MILIA 188	0.1	0.1	0.6	0.3	*Corynebacterium genitalium*	100	X84253
MILIA 386	0.1	0.6	0.1	0.3	*uncultured bacterium*	100	AY958846
Alphaproteobacteria (494 phylotypes, 3903 sequences)							
MILIA 43	1.4	1.9	0.7	1.3	*Paracoccus sp. mdw-1*	100	AY819696
MILIA 54	1.1	1.4	1.0	1.2	*Paracoccus marcusii (T)*	100	Y12703
MILIA 74	1.0	0.1	0.0	0.4	*Paracoccus sp. SA5*	100	AY864654
MILIA 78	1.0	0.9	1.4	1.1	*Sphingomonas sp. eh2*	100	AF548567
MILIA 115	1.8	0.2	0.0	0.7	*Methylobacterium podarium*	100	AY468363
MILIA 159	0.8	1.3	0.5	0.9	*Paracoccus marcusii (T)*	100	Y12703
MILIA 14	0.8	0.4	0.1	0.4	*Brevundimonas vesicularis (T)*	100	AJ227780
MILIA 77	0.6	0.0	< 0.1	0.2	*Methylobacterium podarium*	100	AY468363
MILIA 175	0.2	0.7	0.1	0.3	*bacterium str. 96446*	100	AF227855
MILIA 217	0.2	0.5	0.8	0.5	*uncultured alpha proteobacterium*	100	FM866280
MILIA 266	0.4	1.0	0.4	0.6	*uncultured bacterium*	100	DQ856552
Betaproteobacteria (145 phylotypes, 1065 sequences)							
MILIA 93	0.7	0.2	0.2	0.4	*uncultured Oxalobacteraceae bacterium*	100	AY395341

MILIA 99	0.9	0.5	0.4	0.6	*Burkholderia phenazinium (T)*	100	U96936
Gammaproteobacteria (268 phylotypes, 3496 sequences)							
MILIA 1	1.1	0.3	0.4	0.6	*unidentified bacterium*	100	Z93987
MILIA 9	3.2	3.8	1.4	2.8	*uncultured gamma proteobacterium*	100	AJ619051
MILIA 13	1.5	1.7	1.4	1.5	*Acinetobacter lwoffii*	100	AF188302
MILIA 98	1.2	0.6	0.4	0.7	*uncultured Pseudomonas sp.*	99	DQ234206
MILIA 292	0.8	1.3	0.3	0.8	*uncultured bacterium*	100	AY907718
MILIA 26	0.7	0.8	0.7	0.7	*uncultured bacterium*	100	HM822530
MILIA 35	0.6	0.6	0.4	0.5	*enrichment culture bacterium LB-P*	100	AF538773
MILIA 79	0.6	0.2	0.1	0.3	*uncultured Pseudomonas sp.*	100	AY082368
MILIA 101	0.7	0.1	0.1	0.3	*Acinetobacter sp. RD4355*	100	AJ633637
MILIA 112	1.0	0.6	0.6	0.7	*Acinetobacter schindleri (T)*	100	AJ278311
MILIA 148	0.8	0.2	0.2	0.4	*uncultured Aeromonas sp.*	100	HM159964
MILIA 198	0.6	0.2	0.2	0.3	*Pseudomonas sp.*	100	AJ387903
Firmicutes (439 phylotypes, 3052 sequences)							
MILIA 146	0.7	1.0	0.6	0.8	*uncultured bacterium*	100	AY958839
MILIA 174	0.8	1.2	1.3	1.1	*uncultured bacterium*	100	DQ532146
MILIA 7	0.9	0	0	0.3	*Bacillus massiliensis*	100	DQ350816
MILIA 8	0.6	0.2	0.9	0.6	*uncultured Streptococcus sp.*	100	AF408263
MILIA 108	0.6	0.1	0.0	0.2	*Planifilum yunnanense (T)*	100	DQ119659
MILIA 135	0.3	0.7	0.6	0.6	*uncultured Streptococcus sp.*	100	AM420131
MILIA 154	0.6	0.9	0.8	0.7	*Staphylococcus succinus (T)*	100	AJ320272
MILIA 222	0.6	0.9	0.7	0.7	*Staphylococcus epidermidis*	100	FJ357586
MILIA 243	0.8	0.0	0.0	0.3	*Bacillus sp. TAT105*	100	AB066342
MILIA 304	0.3	0.1	0.6	0.3	*uncultured bacterium*	100	FM873779

2.4 Temporal stability of bacterial bioaerosol communities at the phyla level

Because the CE-SSCP profiles were markedly similar, three environmental samples (D1 and D157/D164 separated by a period of five months and D157 and D164 separated by only one week) were chosen for the phylogenetic analysis.

A total of 52,752 raw 16S rRNA sequences were then sequenced and analyzed from three different air samples in order to characterize the bacterial diversity present in these enclosed spaces. Of these sequences, only 19,491 were validated in terms of quality, length and absence of chimera sequences. The others were removed from the bioinformatics analysis.

In the three bacterial 16S rDNA libraries, all sequences were distributed within 2,577 phylotypes defined at a threshold of 97% similarity.

The similarities based on comparing the sequences were between 100% and 59%. 95.2% of the sequences displayed at least 97% similarity with known sequences found in the RDP public database. By contrast, only 1.3% of the sequences displayed similarity of less than 90% with previously published sequences.

The indoor air sequences belonging to the bacterial domain were analyzed at the phylum level. The phylogenetic identification of each phylotype, as well as its abundance at the different sites, is shown in Table 17, which presents sequences with more than 90% similarity to the closest sequences in the RDP database. Only phylotypes with an abundance higher than 0.5% were included. A table showing the identification of all bacterial phylotypes is included as supplementary material.

Bacterial diversity was represented by 19 different phyla but was largely dominated by *Proteobacteria* and *Actinobacteria* (Figure 39A). The *Proteobacteria* (*Alpha*, *Beta*, *Gamma*, *Delta* and *Epsilon*) phylum was the best represented in the three air samples, with 51.1%, 46.9% and 38.4% of sequences in samples D1, D157 and D164, respectively.

Indeed, of the 2,577 bacterial phylotypes obtained overall for the three samples, 45.5% were assigned to *Proteobacteria* and 26.5% to *Actinobacteria*. The remaining 28.0% were mainly distributed between *Firmicutes* and *Bacteroidetes*. *Alphaproteobacteria* and *Gammaproteobacteria* were better represented in all samples.

Some phyla were uncommon and found in only one indoor air sample: *Chlamydiae*, *Tenericutes*, *OP10* and *Aquificae*. Two non-cultivated phyla were found: *TM7* (0.15% of sequences in sample D1, 0.04% of sequences in D157 and 0.09% of sequences in D164) and *OP10* (0.03% of sequences in sample D164).

The dominant bacteria were represented by *Actinobacteria* genera and *Paracoccus* sp. (in samples D1 and D157) and *Actinobacteria* genera and *Sphingomonas* sp. (in sample D164). *Paracoccus* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Streptococcus* sp. and *Sphingomas* sp. were present in all the studied samples at proportions ranging from 4% to 9%.

The bacterial diversity of indoor bioaerosols was assessed using several parameters including rarefaction curves (Figure 39B), diversity indices and coverage (Table 16).

Based on rarefaction curves constructed from these data and by the analysis of S_{chao1} values, the samples were estimated to contain a total of 1,257, 1,532 and 1,775 separate Operational Taxonomic Units (OTU) for samples D1, D157 and D164, respectively. Bacterial coverage was similar in the three

samples. The Simpson indexes revealed considerable bacterial diversity in all samples, although it was slightly higher in D157 and D164. Moreover, the values given by S_{chao1} also predicted a much higher bacterial diversity in these two samples. The lowest values of the two diversity indexes were obtained for sample D1.

2.5 Temporal stability of bacterial bioaerosols communities at the phylotype level

Among the 2,577 phylotypes found in the three samples, only 303 were common, representing 11.8% of OTU and 58.4% of the total number of sequences obtained from the three samples (Figure 40). Each sample was similarly represented in this overall percentage (18.4% of diversity in D1, 21.3% in D157 and 18.7% in D164). However, these values corresponded to a majority of the total number of sequences found in each sample (59.0% of sequences in D1, 60.3% in D157 and 55.9% in D164).

The 303 representatives of an OTU common to the three sites and belonging to the bacterial domain were analyzed at the phylotype and sub-group levels. The distribution and abundance of phyla in the 303 OTU were the same as those observed previously among the 2,577 phylotypes, with a predominance of *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroidetes*.

The dominant bacteria were represented by *Actinobacteria* genera, *Paracoccus* sp., *Acinetobacter* sp., *Pseudomonas* sp., *Enhydrobacter* sp., *Sphingomonas* sp., *Staphylococcus* sp. and *Streptococcus* sp.

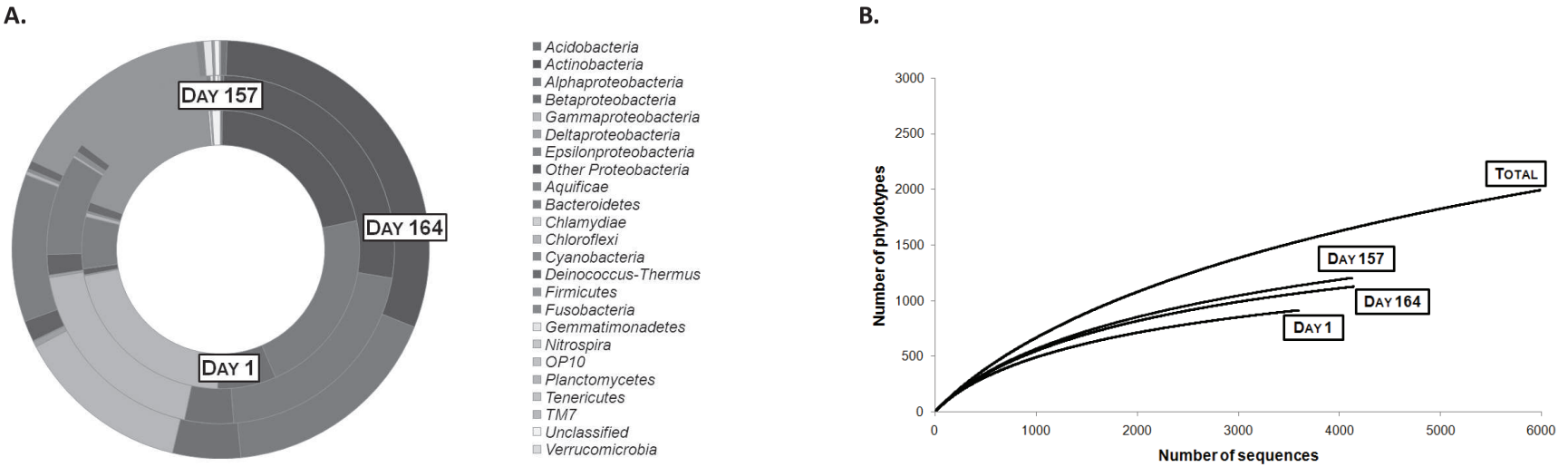


Figure 39 : A. Distribution of microbial phyla in each of the environmental samples. Working from the centre outwards, the circles represent the samples for day 1, day 157 and day 164. B. Rarefaction curves determined from 16S rDNA libraries for indoor bioaerosols.

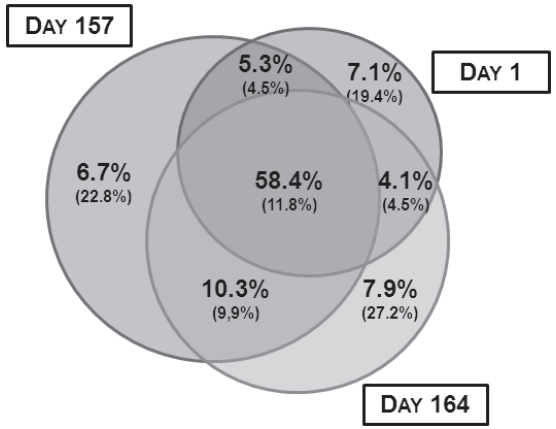


Figure 40 : Sequences were grouped into OTUs at a 97% level of sequence similarity. OTUs overlapping between day 1, day 157 and day 164 samples are shown in the inset. Sequence abundances are presented and OTU abundances are given in brackets.

3. Discussion

During this study, we explored the temporal dynamics of airborne microbes from the Louvre Museum during a 6-month sampling campaign broken down to the day, week and month scales.

Observations performed throughout the campaign were able to link bacterial loads to the total occupancy of the room investigated during the sampling time. Indeed, when room occupancy was low (<100 visitors during the sampling period), the values obtained were below the limit of detection or significantly lower (D2, D3, D4 and D5). Conversely, when room occupancy was higher (during French school holidays, with on average between 150 and more than 300 visitors), the bacterial load could reach values ranging from $3.6x10^3$ to $4.7x10^3$ GE bacteria/m^3 air.

The values obtained were lower than the data obtained previously in the Mona Lisa room of the Louvre Museum and in the Decorative Arts Museum: at the former site, samples yielded between $3.7x10^4$ and $4.1x10^4$ GE bacteria/m^3 air (between $1.5x10^5$ and $1.7x10^5$ 16S copies/m^3 considering a value of 4.1 16S gene copies per genome or cells according to Klappenbach et al. (2001)) and between $5.0x10^4$ and $5.9x10^4$ GE fungi/m^3 air; while in the Decorative Arts Museum, the values were between $2.1x10^4$ and $2.5x10^4$ GE bacteria/m^3 air (between $8.6x10^4$ and $1.0x10^5$ 16S copies/m^3) and between $1.4x10^4$ and $1.7x10^4$ GE fungi/m^3 (C. Gaüzère, unpublished). These differences could be explained by a more pronounced confinement (room occupancy higher and/or its volume smaller). This was in line with data on other enclosed spaces that might be qualified as confined (industrial environments) where recent findings have revealed significantly higher Q-PCR microbial loads in air samples. In such environments, high concentrations of bacteria were revealed by Q-PCR; for example, values of between $7.7x10^7$ and $1.3x10^9$ bacterial cells/m^3 air were found in poultry houses (Oppliger et al., 2008; Just et al., 2011). In the case of flight cabins (where ventilation rates are lower than those applied in museums), the values recorded ranged from 10^6 to 10^7 16S gene copies/m^3 (Osman et al., 2008). Nevertheless, values with a similar order of magnitude for both bacterial and fungal counts were observed on seven sampling dates in urban outdoor air (Lee et al., 2010).

Statistical studies performed on the Q-PCR results also showed no significant variations over time. However, the small number of samples along with problems encountered in analysing certain samples did not enable us to draw a conclusion regarding the dynamics of bacterial and fungal concentrations over time. More exhaustive campaigns will be necessary to provide a realistic picture of the exposure of occupants; and new collection strategies should be considered in order to manage indoor air quality.

Bacterial CE-SSCP findings revealed very similar patterns for the main peaks in all indoor samples. Even with changes to the level of occupancy, no modifications were reflected by the CE-SSCP patterns.

The bacterial diversity encountered during our study appeared to be similar to the bacterial diversity described in studies of other indoor environments which demonstrated a relatively similar breakdown for phyla. Even though the same principal phyla were represented, the proportions differed in all indoor environments and in all samples from the Louvre Museum. Similarities were found regarding the phyla preferentially encountered in various indoor environments (*Actinobacteria*, *Proteobacteria, Firmicutes, Bacteroidetes,* etc.) and the presence of non-cultivated phylum such as *TM7*.

Physical parameters such as temperature, relative humidity and particle counts displayed stability throughout the collection campaign even though variations were seen in the outdoor atmosphere.

Indoor air can be considered as a microenvironment with stable microclimatic parameters such as particle counts, temperature or relative humidity, as well as stable bacterial diversity. According to our observations, the stability of airborne bacteria in the Louvre Museum might have derived from the constant source provided by its occupants. Although, as a general rule, an outdoor air supply is present in about 20% of enclosed spaces, its effect seems to be negligible. Similar conclusions were reached in the study by Nehme et al. (2008) on bioaerosols collected from the air in piggeries buildings. Indeed, their findings suggested that the absence of modifications to DGGE patterns could be linked to a common origin of the bioaerosols. Furthermore, analyses of phylotype diversity showed that the bioaerosol contamination might have derived from pig faeces.

Because the occupants were one of the links found in indoor environments, it is appropriate to consider humans as a potential source of bioaerosols in enclosed spaces. Moreover, studies describing the diversity of the human skin microbiome have highlighted inter- and intra-personal variations at phylum level, with more pronounced inter-personal variations and a stability of these variations over times (Gao et al., 2007; Grice et al., 2009).

These differences might explain the limited number of variations observed between the abundances of phyla.

The structure of the bacterial community displayed a common signal of 55%, while that of the eukaryote community appeared to be more variable, with a common signal of 30% which, on average, represents half that of bacteria. Based on our data, the diversity of eukaryotes varied in an indoor environment. By examining fungal diversity in an urban area, Lee et al. (2010), demonstrated similar shifts over time in an outdoor environment. We advance the hypothesis that external sources might markedly influence indoor eukaryote loads and communities. Moreover, the Simpson diversity index for bacteria produced values between 5.0 and 6.2, which corresponded to the values observed in compost samples, rumen or gut samples (J.-J. Godon, unpublished). Eukaryote values displayed significant variations and ranged from 2 to 5.9.

To date, ecological studies of airborne biological samples have only been carried out using cultivation-based techniques. Conversely, culture-independent studies have demonstrated the prevalence of Gram-negative bacteria that are rarely found in the literature on cultivable bacteria but which might be found in enclosed spaces; these include *Pseudomonas* sp., *Paracoccus* sp., *Sphingomonas* sp or *Acinetobacter* sp. These differences are not new and might be explained by microbes that are preferentially cultivated, such as particularly resistant species, e.g. *Staphylococcus* sp. (Gilbert and Duchaine, 2009). *Acinetobacter* sp. are present in both environmental and commensal skin microflora and were found in all the air samples during our study. Gao et al. (2007) found that they were associated with common skin flora in six individuals.

These results highlight the need to use more exhaustive methods in order to access the preponderant micro-organisms of microbial diversity in enclosed spaces.

This study has provided a characterization of the diversity and dynamics of airborne micro-organisms in the Louvre Museum. To sum up, the indoor airborne community of bacteria remained very stable over time. Its dynamic was therefore mainly driven by the stable parameters of an indoor environment. The analyses carried out emphasised that bacterial diversity could derive from human

sources in enclosed spaces. By contrast, the indoor airborne community of eukaryotes was less stable, its dynamic being partially influenced by variable outdoor parameters.

4. Experimental Procedures

4.1 Measurement site

Indoor bioaerosol measurements were made during a 6-month sampling campaign between 27 September 2010 and 23 March 2011, in the Louvre Museum. All measurements were made in room 36 on the second floor of the museum's "Richelieu" wing (Figure 41).

The number of visitors to the room was counted during measurements in order to determine occupancy, which proved to be moderate (an average of 250 visitors passing through during the collection of micro-organisms). All measurements were made during the autumn and winter.

Monitoring was carried out at different intervals: every day (D1, D2, D3, D4 and D5), every week (D143, D150, D157, D164 and D178) and every month (D3, D87, D115, D143 and D178) (Table 18).

4.2 Physical parameters

Parameters of the microclimate in the room (temperature and relative humidity) were measured continuously with respect to all samplings (Hanwell ML 4106). Particle counts were performed on the samples collected each week using an Optical Particle Counter (Grimm OPC, Model 1.108). The quantities and size distribution of organic dusts were studied throughout the sampling period. The Grimm OPC thus recorded the number, concentration and size distribution of particles every 6 seconds with 16 measurement channels ranging from 0.3 to 20 µm. The results were expressed in particles/m^3.

4.3 Samples collection

Samples were collected using an experimental bioaerosol collector (a cyclone-like device), developed by our laboratory (CSTB) and previously used for the *in situ* sampling of airborne *Legionella* spp. (Mathieu et al., 2006). The aerosols penetrated the sampling device tangentially to the cyclone wall where they impacted. In order to concentrate the aerosol particles collected, the wall was washed constantly with a molecular-grade water flow circulating in a closed loop. The cut-off diameter (d_{50}) of the experimental cyclone was 0.5 µm. Sampling was done under controlled airflow conditions of 1000 L/min for 40 min to sample 40 m^3 of air in 100 mL of molecular-grade water.

The collection system was placed in front of the circulation flow of the room, in a restricted area which separated the room into equal parts. The system was accepted by the public despite the noise generated. The samples were shipped directly to the laboratory where they were pre-treated and stored at -80°C until DNA extraction.

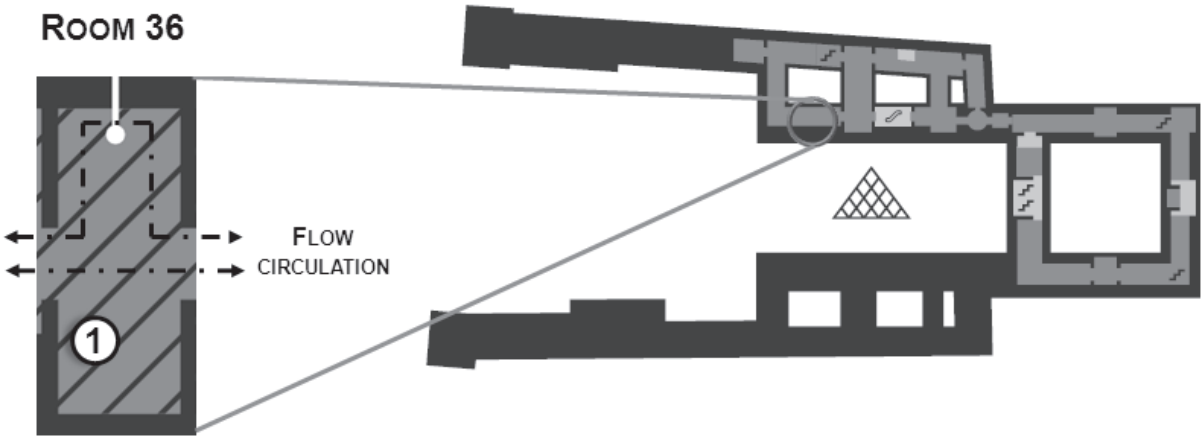


Figure 41 : Location of sampling sites in the Louvre Museum and positioning of the cyclone-like device (reference 1).

Table 18 : Synopsis of information from the samples and the analyses performed.
*** Samples for which Q-PCR values were below limits of detection.**

			Q-PCR analysis	CE-SSCP analysis	Sequencing
Sample	**Date**	**Code**	**(Bacteria and Fungi)**	**(Bacteria and Eukarya)**	**(Bacteria)**
Day 1	27/09/2010	D1	X	X	X
Day 2	28/09/2010	D2	X*	X	-
Day 3	29/09/2010	D3	X*	X	-
Day 4	30/09/2010	D4	X*	X	-
Day 5	01/10/2010	D5	X*	X	-
Day 87	22/12/2010	D87	X	X	-
Day 115	19/01/2011	D115	X	X	-
Day 143	16/02/2011	D143	X	X	-
Day 150	23/02/2011	D150	X	X	-
Day 157	02/03/2011	D157	X	X	X
Day 164	09/03/2011	D164	X	X	X
Day 178	23/03/2011	D178	X	X	-

4.4 Sample preparation

The samples were concentrated by filtering the solution through a 47 mm, 0.2 µm polyestersulfone sterile filter (Supor 200, Pall Corporation, USA). The filter was then cut using a sterile scalpel and each half-filter was placed in a 1.5 mL sterile tube and frozen at -80°C.

4.5 Extraction and purification of total genomic DNA

DNA extraction was performed directly on the filter using a slightly modified version of the protocol described by Moletta et al. (2007). The filter was ground to a powder using a sterile plastic stick while maintained in dry ice. Total DNA was then purified using a QiaAmp DNA microkit (Qiagen, Hilden, Germany) and collected in 50 µL DNA-free water.

After extraction, the DNA was stored at -80°C. An extraction control was produced using molecular-grade water and then passed through all the extraction and amplification stages.

4.6 Real-time PCR (Polymerase Chain Reaction) amplification and quantification

Analysis of the data was carried out on a RotorGene 3000 (Corbett Research, Australia). The calibration curve was generated using RotorGene software, version 6.1.

During this study, two different quantitative real-time PCR systems were used in order to assess concentrations of bacteria and fungi in indoor air. They were all based on 16S and 18S rRNA gene sequences. All primers and the TaqMan® probe were synthesized by Sigma Proligo (Paris, France). The cycle threshold (CT) was calculated as the cycle number at which the reaction became exponential. The cycle threshold of each sample was then compared to a standard curve and the result was expressed as a numerical value of the number of target genomes in the sample. The standard curves were generated by amplifying serial 5-fold dilutions of the total genomic DNA of *Escherichia coli* and *Aspergillus fumigatus*. Concentrations of 2.32×10^6 Genome Equivalent (GE) bacteria/µL and 5.65×10^5 GE fungi/µL of the total genomic DNA solution were determined using the Genequant Pro system (Amersham Biosciences). Each standard was tested in triplicate.

The current specificity of all real-time amplification systems was tested *in silico* using Probematch software for the ARB database and NCBI Blast (Altschul et al., 1990) against all 16S and 18S rRNA gene sequences available in RDP and/or Genbank.

Table 19 : Details of the different primers and probes used for molecular analyses.

Names	Types	Target	Sequences	Positions	References
Real-time Q-PCR					
B01	Primer	Bacteria	5'-TCCTACGGGAGGCAGCAGT-3'	F331-349	Horz et al. (2005)
B02	Primer	Bacteria	5'-GGACTACCAGGGTATCTAATCCTGTT-3'	R772-797	Horz et al. (2005)
B14	Probe	Bacteria	5'-CGTATTACCGCGGCTGCTGGCAC-3'	506-528	Horz et al. (2005)
E07	Primer	Fungi	5'-TTAGCATGGAATAATRRAATAGGA-3'	F817	Borneman and Hartin (2000)
E08	Primer	Fungi	5'-TCTGGACCTGGTGAGTTTCC-3'	R1196	Borneman and Hartin (2000)
PCR-SSCP					
B22	Primer	Bacteria	5'-ACGGTCCAGACTCCTACGGG-3'	F329-348	Zumstein et al. (2000)
B23	Probe	Bacteria	5'-TTACCGCGCTGCTGGCAC-3'	R515-533	Zumstein et al. (2000)
E04	Primer	Eukaryotes	5'-CTTAATTTGACTCAACACGG-3'	F955	Godon et al. (1997)
E12	Probe	Eukaryotes	5'-GGGCATCACAGACCTGTT-3'	R1195	Godon (2004)

4.6.1 Assessment of total bacterial load

A 441 bp (base pair) fragment of bacterial 16S rDNA was amplified with bacteria-directed primers and a probe set (see details in Table 19) (Horz et al., 2005). PCR was performed using the Quantitect Probe PCR kit (Qiagen), with 12.5 µL Master Mix, 0.25 µL forward primer, 0.25 µL reverse primer and 0.25 µL probe, with water being added to a final volume of 20 µL. 5 µL of the sample were added to the PCR mix. Amplification was carried out under the following conditions: 95°C for 15 min, then 50 cycles at 94°C for 15 s, 60°C for 60 s and 72°C for 20 s. No-template-controls with molecular-grade water, rather than DNA, were included in each run.

Serial dilutions of *Escherichia coli* (DSMZ 5695, ATCC 12435) DNA were used as the standard to determine bacterial concentrations with real-time PCR. All samples were included in triplicate in each run. The reaction efficiency of the standard curve for bacterial load evaluations ranged from 85% to 100%, the correlation coefficient R^2 being >0.99.

4.6.2 Assessment of total fungal load

A 379 bp fragment of fungal 18S rDNA was amplified with fungus-directed primers (see details in Table 19). PCR was performed using the Quantitect Sybr Green PCR kit (Qiagen), with: 12.5 µL Master Mix, 2.25 µL forward primer and 2.25 µL reverse primer, with water being added to a final volume of 20 µL. 5 µL of the sample were added to the PCR mix. Amplification was carried out under the following conditions: 95°C for 15 min, then 45 cycles at 94°C for 15 s, 52°C for 30 s and 72°C for 45 s. No-template-controls with molecular-grade water, rather than DNA, were included in each run.

The Q-PCR system used during this study can be used to amplify the 18S rRNA gene sequence of the four major fungal phyla: *Ascomycetes*, *Basidiomycetes*, *Zygomycetes* and *Chytridomycetes* (Borneman and Hartin, 2000).

Serial dilutions of *Aspergillus fumigatus* DNA (Institut d'Hygiène et d'Epidémiologie in Brussels – Mycology Section) were used as the standard to determine fungal concentrations with real-time PCR. All samples were included in triplicate in each run. The reaction efficiency of the standard curve for fungal load evaluations ranged from 91% to 94% and the correlation coefficient was R^2> 0.99.

4.6.3 Limits of quantification

The limit of quantification was defined using the lowest concentration of the standard within the linear range at which 95% of PCR results were positive. The limit of quantification of the number of GE bacteria or GE fungi per cubic metre of collected air was then calculated for each sample using these values, taking account of the equivalent volume of air used for DNA extraction and the dilution factor applied prior to Q-PCR. In the case of bacteria, the limit of detection was 1.9×10^2 GE/m^3 air. For the fungal system, the limit of detection (LD) was 9.0×10^1 GE/m^3 air and was given by the smallest dilution of the amplified range. The limit of detection values found for fungi lower than for bacteria, mainly because of the presence in Q-PCR reagents of contaminating rRNA gene sequences arising from the manufacturing process (Rueckert and Morgan, 2007).

Lower GE numbers (10^2) was observed in four of the five daily samples (D2 to D5) which coincided with difficulties in amplifying sufficient 16S or 18S rRNA gene fragments for subsequent quantification by Q-PCR. These samples were withdrawn from the analysis (Table 18).

The dispersion of values using this collecting system was based on an analysis of the Q-PCR standards performed in triplicate, and were found to be those typical in the quantitative experiment; i.e. 8%. The dispersion observed for Q-PCR triplicate analyses was 3%.

4.7 CE-SSCP (Capillary Electrophoresis Single Strand Conformation Polymorphism) analysis and structure of the microbial community

4.7.1 PCR amplification

Five microliters of total DNA were used for each PCR-SSCP amplification. The B22 and B23* were used to amplify the V3 16S rDNA bacterial region (Zumstein et al., 2000). The eukaryotic primers used for 18S V7 ribosomal DNA were E04 and E12*

Five microlitres of total DNA were used for each PCR-SSCP amplification. B22 and B23* were used to amplify the V3 16S rDNA bacterial region (Zumstein et al., 2000). The eukaryotic primers used for 18S V7 ribosomal DNA were E04 and E12* (see details in Table 19) (Godon et al., 1997; Godon et al., 2004). The PCR-SSCP amplification mix contained 1.25 units of *Pfu Turbo* (Stratagene, La Jolla, California), 5 µL 10X buffer, 200 µM dNTPs, and 130 ng of each primer, with water added to make up a final volume of 50 µL. The thermal profile used to amplify ribosomal RNA genes was as follows: incubation at 94°C for 2 min, then 30 cycles of denaturation at 94°C for 30 s, 61°C for 30 s and 72°C for 30 s and a final elongation of 10 min at 72°C. The same thermal amplification profile was used for Eukaryota, except that the annealing temperature was 51°C.

4.7.2 CE-SSCP analysis

Global microbial communities were studied by CE-SSCP analysis using an ABI310 system (Applied Biosystems) on all samples with 1 µL of extracted DNA, in accordance with CE-SSCP amplification methods previously described (Delbes et al., 2000).

4.7.3 Analysis of CE-SSCP profiles and calculation of the diversity index

Diversity indexes can be calculated by taking account of either the number of peaks on the fingerprint profile or the number of peaks and their relative abundances (area or height under each peak of the fingerprint profile). The following diversity indexes are available: peak number (often named Richness), and Simpson's minus logarithm, under which normalization is calculated as $D = -\log \Sigma a_i^2$ where a_i is the relative abundance of each peak. This ranges from 0 (a single peak) to infinity (an infinite number of peaks of equal abundance). This index was calculated for all the samples analyzed with CE-SSCP fingerprinting using StatFingerprints (Loisel et al., 2006; Vanpeteghem et al., 2008; Michelland et al., 2009).

The minimum value of each scan was extracted from the overall profiles in order to determine a percentage stability of each profile.

4.8 16S rRNA gene sequencing

The samples were amplified using the following primers: bacterial forward primers (5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAGTGCGTGTGCCAGCAGCCGCGGTAATA-3' for D1; 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACGCACTCGTGCCAGCAGCCGCGGTAATA-3' for W3 and 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCACTGTAGGTGCCAGCAGCCGCGGTAATA-3' for W4) and a reverse primer (5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAGGGGCATCACAGACCTGTT-3'). DNA aliquots of each sample were used for a 50 µLl PCR reaction. SSU ribosomal DNA fragments with an average size of 270 pb were amplified to build up three bacteria libraries. The Gene Amp High Fidelity PCR system (Applied Biosystems) was used for PCR under the following conditions: 95 °C for 10 min followed by 30 cycles of 95 °C for 30 s; 50 °C for 30 s and 72 °C for 1 min; and a final elongation step at 72 °C for 10 min. All amplicon products from different samples were purified using Agencourt AmPure XP beads (Agencourt Bioscience Corporation, MA, USA). The purification step was checked on Agilent DNA 1000 chips (Agilent Technologies) and the amplicons were sequenced using 454 GS-FLX Titanium (Roche).

4.9 Sequences analyses

Each sequence was cleaned of its primers, and all shorter sequences (>150 bp) were removed using the initial process of the RDP pyrosequencing pipeline. The sequences were then checked for chimeras, aligned, and a distance matrix was generated using MOTHUR (v.1.20.0) (Schloss et al., 2009). Phylotypes were defined at the level of 97% similarity using the DOTUR program (Schloss and Handelsman, 2005). One representative of each phylotype was compared with the RDP public database (Cole et al., 2007; Cole et al., 2009) using the SEQMATCH program (v.10.27). Rarefaction curves and diversity indexes were generated by DOTUR at 97% of similarity. The identification of common phylotypes in the different air samples was ensured using DOTUR and MOTHUR programs.

One sequence for each phylotype was deposited in the GenBank database. The accession numbers of bacterial nucleotide sequences were FQ077208–FQ079780.

4.10 Statistical analyses

Matched means for size distribution measurements were compared using a non-parametric Wilcoxon test. The type I error rate was 0.05.

The normality of the distribution of Q-PCR results was studied using the Shapiro–Wilk test method.

Due to the small number of samples available (n=24), non-parametric statistical tests were used. In order to compare the means for Q-PCR findings, a Friedman test was run. The type I error rate was 0.05.

Acknowledgements

This research was supported by the DGA, the CSTB, the INRA-LBE and GENOSCREEN. The authors wish to thank all those who contributed to this study and particularly Joëlle Le Roux from the Louvre Museum. They also gratefully acknowledge the contributions of Sabine Gaüzère regarding automated sequences analyses and Valérie Bru-Adan and Anaïs Bonnafous who provided valuable assistance with SSCP analyses.

References:

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic local alignment search tool. J Mol Biol 215: 403-410.

Angenent, L.T., Kelley, S.T., Amand, A.S., Pace, N.R., and Hernandez, M.T. (2005) Molecular identification of potential pathogens in water and air of a hospital therapy pool. Proc Natl Acad Sci U S A 102: 4860-4865.

Augustowska, M., and Dutkiewicz, J. (2006) Variability of airborne microflora in a hospital ward within a period of one year. Ann Agr Env Med 13: 99-106.

Aydogdu, H., Asan, A., Otkun, M.T., and Ture, M. (2005) Monitoring of Fungi and Bacteria in the Indoor Air of Primary Schools in Edirne City, Turkey. Indoor Built Environ 14: 411-425.

Baron, P., and Willeke, K. (2005) Aerosol Measurement; Principles, Techniques, and Applications: John Wiley & Sons.

Borneman, J., and Hartin, R.J. (2000) PCR Primers That Amplify Fungal rRNA Genes from Environmental Samples. Appl Environ Microbiol 66: 4356-4360.

Bru-Adan, V., Wery, N., Moletta-Denat, M., Boiron, P., Delgenes, J.P., and Godon, J.J. (2009) Diversity of bacteria and fungi in aerosols during screening in a green waste composting plant. Curr Microbiol 59: 326-335.

Chen, Y.P., Cui, Y., and Dong, J.G. (2010) Variation of airborne bacteria and fungi at Emperor Qin's Terra-Cotta Museum, Xi'an, China, during the "Oct. 1" gold week period of 2006. Environ Sci Pollut R 17: 478-485.

Cho, J.H., Hee Min, K., and Paik, N.W. (2006) Temporal variation of airborne fungi concentrations and related factors in subway stations in Seoul, Korea. Int J Hyg Envir Heal 209: 249-255.

Cole, J.R., Chai, B., Farris, R.J., Wang, Q., Kulam-Syed-Mohideen, A.S., McGarrell, D.M. et al. (2007) The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data. Nucleic Acids Res 35: 169-172.

Cole, J.R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R.J. et al. (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 37: 141-145.

Delbes, C., Moletta, R., and Godon, J.J. (2000) Monitoring of activity dynamics of an anaerobic digester bacterial community using 16S rRNA polymerase chain reaction - single-strand conformation polymorphism analysis. Environ Microbiol 2: 506-515.

Després, V.R., Nowoisky, J.F., Klose, M., Conrad, R., Andreae, M.O., and Pöschl, U. (2007) Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes. Biogeosciences 4: 1127-1141.

Gao, Z., Tseng, C.-h., Pei, Z., and Blaser, M.J. (2007) Molecular analysis of human forearm superficial skin bacterial biota. Proc Natl Acad Sci U S A 104: 2927-2932.

Gilbert, Y., and Duchaine, C. (2009) Bioaerosols in industrial environments: a review. Can J Civil Eng 36: 1873.

Godon, J.-J., Zumstein, E., Dabert, P., Habouzit, F., and Moletta, R. (1997) Microbial 16S rDNA diversity in an anaerobic digester. Water Sci Technol 36: 49-57.

Godon, J.J., Bayle, S., Leclerc, M., and Delgenes, J.P. (2004) Comparison of 16S ribosomal DNA structure of ten anaerobic digestors. In Final Program Abstracts, 10th ISME, Cancun, Mexico: pp. 176.

Gorny, R.L., Reponen, T., Willeke, K., Schmechel, D., Robine, E., Boissier, M., and Grinshpun, S.A. (2002) Fungal fragments as indoor air biocontaminants. Appl Environ Microbiol 68: 3522-3531.

Grice, E.A., Kong, H.H., Conlan, S., Deming, C.B., Davis, J., Young, A.C. et al. (2009) Topographical and Temporal Diversity of the Human Skin Microbiome. Science 324: 1190-1192.

Horz, H.P., Vianna, M.E., Gomes, B.P., and Conrads, G. (2005) Evaluation of universal probes and primer sets for assessing total bacterial load in clinical samples: general implications and practical use in endodontic antimicrobial therapy. J Clin Microbiol 43: 5332-5337.

Just, N., Kirychuk, S., Gilbert, Y., Létourneau, V., Veillette, M., Singh, B., and Duchaine, C. (2011) Bacterial diversity characterization of bioaerosols from cage-housed and floor-housed poultry operations. Environ Res 111: 492-498.

Kejun, L. (2011) Molecular comparison of the sampling efficiency of four types of airborne bacterial samplers. Sci Total Environ 409: 5493-5498.

Klappenbach, J.A., Saxman, P.R., Cole, J.R., and Schmidt, T.M. (2001) rrndb: the Ribosomal RNA Operon Copy Number Database. Nucleic Acids Res 29: 181-184.

La Duc, M.T., Stuecker, T., and Venkateswaran, K. (2007) Molecular bacterial diversity and bioburden of commercial airliner cabin air. Can J Microbiol 53: 1259-1271.

LeBouf, R., Yesse, L., and Rossner, A. (2008) Seasonal and diurnal variability in airborne mold from an indoor residential environment in northern New York. J Air Waste Manag Assoc 58: 684-692.

Lee, S.-H., Lee, H.-J., Kim, S.-J., Lee, H.M., Kang, H., and Kim, Y.P. (2010) Identification of airborne bacterial and fungal community structures in an urban area by T-RFLP analysis and quantitative real-time PCR. Sci Total Environ 408: 1349-1357.

Loisel, P., J, H., Zemb, O., Latrille, E., Lobry, C., Delgenes, J., and Godon, J. (2006) Denaturing gradient electrophoresis (DGE) and single-strand conformation polymorphism (SSCP) molecular fingerprintings revisited by simulation and used as a tool to measure microbial diversity. Environ Microbiol. 8: 720-731.

Maron, P.A., Mougel, C., H. Lejon, D.P., Carvalho, E., Bizet, K., Marck, G. et al. (2006) Temporal variability of airborne bacterial community structure in an urban area. Atmos Environ 40: 8074-8080.

Mathieu, L., Robine, E., Deloge-Abarkan, M., Ritoux, S., Pauly, D., Hartemann, P., and Zmirou-Navier, D. (2006) Legionella Bacteria in Aerosols: Sampling and Analytical Approaches Used during the Legionnaires Disease Outbreak in Pas-de-Calais. J Infect Dis 193: 1333-1335.

Michelland, R.J., Dejean, S., Combes, S., Fortun-Lamothe, L., and Cauquil, L. (2009) StatFingerprints: a friendly graphical interface program for processing and analysis of microbial fingerprint profiles. Mol Ecol Resour 9: 1359-1363.

Moletta-Denat, M., Bru-Adan, V., Delgenes, J.-P., Hamelin, J., Wéry, N., and Godon, J.-J. (2010) Selective microbial aerosolization in biogas demonstrated by quantitative PCR. Bioresource Technol 101: 7252-7257.

Moletta, M., Delgenes, J.-P., and Godon, J.-J. (2007) Differences in the aerosolization behavior of microorganisms as revealed through their transport by biogas. Sci Total Environ 379: 75-88.

Nehme, B., Létourneau, V., Forster, R.J., Veillette, M., and Duchaine, C. (2008) Culture-independent approach of the bacterial bioaerosol diversity in the standard swine confinement buildings, and assessment of the seasonal effect. Environ Microbiol 10: 665.

Oppliger, A., Charriere, N., Droz, P.-O., and Rinsoz, T. (2008) Exposure to Bioaerosols in Poultry Houses at Different Stages of Fattening; Use of Real-time PCR for Airborne Bacterial Quantification. Ann Occup Hyg 52: 405-412.

Osman, S., La Duc, M.T., Dekas, A., Newcombe, D., and Venkateswaran, K. (2008) Microbial burden and diversity of commercial airline cabin air during short and long durations of travel. ISME J 2: 482-497.

Radosevich, J.L., Wilson, W.J., Shinn, J.H., DeSantis, T.Z., and Andersen, G.L. (2002) Development of a high-volume aerosol collection system for the identification of air-borne micro-organisms. Lett Appl Microbiol34: 162-167.

Rinsoz, T., Duquenne, P., Greff-Mirguet, G., and Oppliger, A. (2008) Application of real-time PCR for total airborne bacterial assessment: Comparison with epifluorescence microscopy and culture-dependent methods. Atmos Environ 42: 6767-6774.

Rueckert, A., and Morgan, H.W. (2007) Removal of contaminating DNA from polymerase chain reaction using ethidium monoazide. J Microbiol Meth 68: 596-600.

Schloss, P.D., and Handelsman, J. (2005) Introducing DOTUR, a Computer Program for Defining Operational Taxonomic Units and Estimating Species Richness. Appl Environ Microbiol 71: 1501-1506.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B. et al. (2009) Introducing mothur: Open Source, Platform-independent, Community-supported Software for Describing and Comparing Microbial Communities. Appl Environ Microbiol 75: 7537-7541.

Tringe, S.G., Zhang, T., Liu, X., Yu, Y., Lee, W.H., Yap, J. et al. (2008) The airborne metagenome in an indoor urban environment. PLoS ONE 3: 1-10.

Vanpeteghem, D., Zemb, O., and Haegeman, B. (2008) Dynamics of neutral biodiversity. Math Biosci 212: 88-98.

Wang, Y.-F., Wang, C.-H., and Hsu, K.-L. (2010) Size and seasonal distributions of airborne bioaerosols in commuting trains. Atmos Environ 44: 4331-4338.

Zumstein, E., Moletta, R., and Godon, J.-J. (2000) Examination of two years of community dynamics in an anaerobic bioreactor using fluorescence polymerase chain reaction (PCR) single-strand conformation polymorphism analysis. Environ Microbiol 2: 69-78.

II.2 ARTICLE 3 : SIGNATURE BACTERIENNE ET ORIGINE DES BACTERIES DE L'AIR DES ENVIRONNEMENTS INTERIEURS.

Les résultats obtenus et décrits dans la partie précédente, permettent d'envisager une analyse de la microbiologie de l'air pertinente et réaliste par le biais d'un système intégré. Celui-ci a donc été employé pour différents sites d'études : un lieu dit sensible de part une forte concentration de population (un musée) ou de part la vulnérabilité des populations qu'on y rencontre (un établissement de soins), enfin un environnement où les occupants sont susceptibles de subir une durée d'exposition prolongée (un bureau).

Cette stratégie permet ainsi de collecter les aérosols sur une zone plus étendue tout en limitant au maximum la gêne des occupants et en collectant une matrice ADN suffisante pour les analyses envisagées.

Actuellement, le manque de données moléculaires environnementales sur les microorganismes aéroportés dans les espaces clos ainsi que sur l'environnement d'origine des séquences, ne permet pas une description de la diversité bactérienne de l'air intérieur dans sa globalité.

Dans ce contexte, l'objectif de cette étude était de caractériser la diversité bactérienne de l'air intérieur, en particulier, les « *core species* », les pathogènes opportunistes et l'origine des bactéries à l'intérieur, dans trois différents espaces clos, avec trois niveaux d'occupation (faible pour le bureau, élevé pour le musée et moyenne pour l'hôpital).

Les résultats obtenus dans le cadre de cette étude, sont présentés dans un article soumis à *FEMS Microbiology Ecology*.

'CORE SPECIES' IN THREE SOURCES OF INDOOR AIR BELONGING TO THE HUMAN MICRO-ENVIRONMENT TO THE EXCLUSION OF OUTDOOR AIR.

'Core species' in indoor air

Carole Gaüzère[a*], Jean-Jacques Godon[b],Hélène Blanquart[c], Stéphanie Ferreira[c], Stéphane Moularat[a], Enric Robine[a], Marina Moletta-Denat[a]

[a]Université Paris-Est, Centre Scientifique et Technique du Bâtiment (CSTB), Laboratoire de Recherche et d'Innovation pour l'Hygiène des Bâtiments, 84, avenue Jean Jaurès - Champs-sur-Marne, 77447 Marne-la-Vallée Cedex 2, France.

[b]INRA, UR50, Laboratoire de Biotechnologie de l'Environnement (LBE), Avenue des Etangs, 11100 Narbonne, France.

[c]Genoscreen, Genomic Platform and R&D, Campus de l'Institut Pasteur, 1 rue du Professeur Calmette, 59000 Lille, France.

[*]Corresponding author: carole.gauzere@gmail.com
Telephone number: +33(0).1.64.68.85.17
Fax number: +33(0).1.64.68.88.23

Bioaerosols, 'core species', indoor air quality, pyrosequencing

Abstract

Although we spend the majority of our lives indoors, the airborne microbial content of enclosed spaces is still poorly described. In this context, the objective of this study was to characterize the bacterial diversity of indoor air in three different enclosed spaces with three levels of occupancy and, in particular, to highlight the 'core species', opportunistic pathogens and sources of indoor bacteria. Our findings provide an overall description of bacterial diversity in these indoor environments. Data gathered from the three enclosed spaces revealed the presence of a common indoor signature (60% of total sequences in common). This work will provide a clearer understanding of the dominant groups of bacteria encountered in enclosed spaces: *Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes*. Thus, certain evidence revealed a connection between 'core species' and the human micro-environment (20% of phylotypes and 12% of sequences of human origin). Overall PCA analysis showed that the indoor environment may be influenced mainly by the microbial diversity from nose and skin. Among the 'core species' found during this study, a large number (72% of all pathogen-related sequences were concentrated in 'core species') of genera and species are known to be responsible for opportunistic or nosocomial diseases or to include human commensal bacteria such as *Mycobacterium* sp., *Acinetobacter baumanii*, *Aerococcus viridians*, *Thermoactinomyces vulgaris* or *Clostridium perfringens*.

1. Introduction

Which element is the most widely shared by human beings? Neither food nor water but air. This unavoidable sharing, a potential vector of many diseases, has increased exponentially in recent years due to changes to our lifestyle. Indoor air accounts for only 0.3 millionth of tropospheric air. The Earth's population continues to grow, people are travel more, but 50% of them live in urban areas and spends up to 90% of their lifetime indoors (children in day care, nursing homes, dwellings, offices, transport systems, public buildings, etc.). This is why assessing the risks associated with micro-organisms in enclosed spaces has become so necessary (Höppe and Martinac, 1998). Despite concern with public health, the microbial content of indoor air as well as the role of air in spreading pathogens remain poorly described in comparison to other environments such as outdoor air, soil, food or water. The result is that indoor air is a scientific field that is largely unexplored. And yet the transmission of infectious agents cannot always be avoided because controlling the dissemination of airborne pathogens is far more difficult than preventing the pathogenic contamination of surfaces, water or food.

Most studies of enclosed spaces have linked the indoor environment with the outdoors, entailng a description of both settings. But some studies have presented indoor air quality as being closely linked to major sources indoors, including human activity or mere occupantion (Zuraimi and Tham, 1994; Kotzias *et al*., 2009; Wichmann *et al*., 2010). The particle concentrations found suggest that inadequate ventilation systems may lead to an increased of concentration pollutants in indoor environments (Daisey *et al*., 2003; Fromme *et al*., 2007; Almeida *et al*., 2011). Moreover, physical activities in an indoor space cause the re-suspension of sedimented particles from indoor floor dust (Fromme *et al*., 2007; Fromme *et al*., 2008; Almeida *et al*., 2011). The study by Hospodsky *et al*. (2011) reached a similar conclusion, suggesting that bacterial populations recovered from indoor air showed greater similarity to the populations found in floor dust than to those in outdoor air. Zuraimi and Tham (1994) showed that higher levels of human-related bacteria were associated with high

occupancy rates, irregular floors and the frequency of surface cleaning. Liu *et al*. (2000) investigated bacterial concentrations in the indoor air of two elementary schools and hypothesized that the children and teachers might be the principal source of bacterial contamination. They also showed that indoor air contained many opportunistic culturable bacteria that could be infectious to exposed occupants. Furthermore, a comparison between airborne micro-organisms found in shopping centres and those in other environments located nearby indicated that indoor-air micro-organisms seemed to arise from indoor domestic niches and were not related to any inward transfer from outdoor environments. They also suggested an interaction between airborne microflora and human occupants in indoor environments (Tringe *et al*., 2008).

Most studies employ culture methods that do not fully describe microbial diversity. Indeed, only 1% of environmental micro-organisms can currently be cultivated (Amann *et al*., 1995) and microbial aerosols appear to be particularly recalcitrant because of the constraints hampering the methods used for their collection (Wang *et al*., 2001; Radosevich *et al*., 2002). The collection of airborne micro-organisms, because they are present at low concentrations, is a challenging task, particularly when subsequently using molecular tools. Collection systems with high flow rates may be necessary to permit molecular analysis under optimum conditions (Gaüzère *et al*., 2011). Sampling larger volumes of air using integrated systems may also be an efficient alternative (Tringe *et al*., 2008). Although the diversity of cultivable bacteria in confined spaces is relatively well-documented, the use of molecular methods remains rare. Only three studies have investigated the microbial diversity of air in indoor environments since the first such inventory was made by Radosevich *et al*. in 2002: in a therapeutic swimming pool (Angenent *et al*., 2005), in two shopping centres in Singapore (Tringe *et al*., 2008) and in commercial airline cabins (Osman *et al*., 2009). The data obtained with molecular methods on the microbial diversity of indoor air have demonstrated the presence of all microbial domains (*Bacteria*, *Eukarya* and *Archaea*). Examination of these data reveals a bacterial diversity which differs from that observed using culture methods: bacterial diversity is mainly dominated by *Alpha-*, *Beta-* and *Gamma-Proteobacteria*. There are similarities regarding the groups preferentially found in various environments (*Actinobacteria*, *Proteobacteria,* etc.) and the presence of non-cultivated phylum such as *TM7*. There appears to be a specific diversity of indoor air. In terms of the health risks associated with the presence of pathogenic species in air, these studies highlighted the over-representation of opportunistic pathogen species such as *Propionibacterium acnes*, *Staphylococcus* spp., *Streptococcus* spp. or *Stenotrophomonas maltophila*. The pathogenic species found in air are often associated with nosocomial infections (*Acinetobacter baumanii*, *Clostridium jejeikieum,* etc.) and respiratory infections (*Mycobacterium* spp.). But no virulent pathogens such as *Bacillus anthracis* or *Legionella pneumophila*, for example, were encountered during these studies.

There is currently a lack of molecular data both on the environmental airborne species found in enclosed spacesand on the environmental origin of the sequences present.

In this overall context, the aim of this study was to characterize the bacterial diversity of indoor air and, in particular, to highlight 'core species', opportunistic pathogens as well as the origin of indoor bacteria found in three different enclosed spaces with three different levels of occupancy (low: office, high: museum and average: hospital).

2. Materials and Methods

2.1. Measurement sites

The measurement of the indoor bioaerosols was done during four-week sampling periods in 2010: 14 January – 11 February in Lagny Hospital (H), 16 July – 13 August in an office in Champs-sur-Marne (O) and 30 September – 28 October in the Louvre Museum in Paris (M).

These three sites differ in terms of their levels of occupancy (on average: 10,000 people in the museum (M), 1,000 people in the Emergency Paediatric Department of the hospital (H) and 20 people in the office (O)).

Only the entrance and waiting room of the Paediatric Emergency Department in the hospital were investigated. This site is considered to be highly sensitive to the presence of pathogens. In the Louvre Museum, measurements were performed on the second floor of the Richelieu wing, which represents a huge sampling volume. The office was an open-plan area occupied during working hours (5 days a week between 08:00 and 19:30).

2.2. Collection of samples

A sampling device was developed that could be connected to the existing ventilation system of the buildings (Figure 42). This filtration device operated at a rate of 6.3 m^3/h. The duration of each sampling period was 4 weeks and an average volume of 4200 m^3 air was filtered. A cellulose HEPA 13 filter (CAMFIL FARR) was used, its diameter 200 mm, and its collection efficiency 99.95% for particles of 0.3 microns. The filters were then scraped into molecular-grade water.

A control filter was stored at 4°C during the 4-week collection period and was then subjected to all the analytical stages.

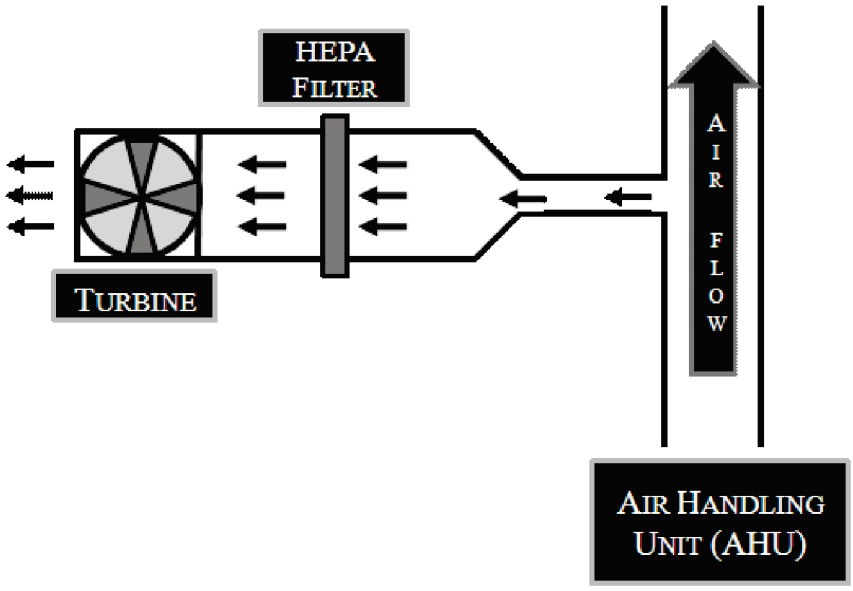


Figure 42 : A schematic view of the sampling filtration system used at the three sites.

2.3. Sample preparation

The samples were concentrated by filtering the solution through a sterile 47 mm x 0.2 µm polyestersulfone filter (Supor 200, Pall Corporation, USA). The filter was then cut in half using a sterile scalpel and each was placed in a 1.5 mL sterile tube and frozen at a temperature of -80°C.

2.4. Extraction and purification of total genomic DNA

DNA extraction was performed directly on the filter using a slightly modified version of the protocol described by Moletta *et al*. (2007). The filter was ground to a powder using a sterile plastic stick while maintained in dry ice. Total DNA was then purified using a QiaAmp DNA microkit (Qiagen, Hilden, Germany) and collected in 50 µL DNA-free water.

After extraction, the DNA was stored at -80°C. An extraction control was produced using molecular-grade water and then passed through all the extraction and amplification stages.

2.5. 16S rRNA gene sequencing

The samples were amplified using the following primers: a bacterial forward primer (5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGCTCGCGTGTCGTGCCAGCAGCCGCGGTAATA-3') and a reverse primer (5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAGGCGTGGACTACCAGGGTATCT-3'). DNA aliquots of each sample were used for a 50 µl PCR reaction. SSU ribosomal DNA fragments with an average size of 270 pb were amplified to build up three bacteria libraries. The Gene Amp High Fidelity PCR system (Applied Biosystems) was used for PCR. All amplicon products from different samples were purified using Agencourt AmPure XP beads (Agencourt Bioscience Corporation, MA, USA). The purification step was checked on a Agilent DNA 1000 chip (Agilent Technologies) and the amplicons were sequenced using 454 GS-FLX Titanium (Roche).

2.6. Sequence analyses

In the initial step of the overall process, each sequence was cleaned of its primers and all shorter sequences (>= 150 bp) were removed using RDP's pyrosequencing pipeline. Then the sequences were checked for chimeras and aligned and a distance matrix was generated using MOTHUR (v.1.20.0) (Schloss *et al*., 2009). Phylotypes were defined at the level of 97% similarity using the DOTUR program (Schloss and Handelsman, 2005). One representative of each phylotype was compared with the RDP public database (Cole *et al*., 2007; Cole *et al*., 2009) using the SEQMATCH program (v.10.27). Rarefaction curves and diversity indexes were generated by DOTUR at 97% similarity. The identification of common phylotypes in the different air samples was carried using the DOTUR and SONS programs (Schloss and Handelsman, 2006).

Of 42,753 raw 16S rRNA sequences, only 26,297 were validated in terms of quality, length and absence of chimera sequences. The others were removed from the bioinformatics analysis.

For each phylotype, one sequence was deposited in the GenBank database. The accession numbers of bacterial nucleotide sequences were FQ074240–FQ077207.

2.7. Statistical analysis of data: Principal Component Analysis (PCA)

A matrix of 15 lines or observations (samplings) and 31 columns or variables (phyla) was constructed using sequences previously published in different databases, originating from humans, indoor air and outdoor air, (Hold *et al*., 2002; Radosevich *et al*., 2002; Maron *et al*., 2005; Paez-Rubio *et al*., 2005; Brodie *et al*., 2007; Grice *et al*., 2009; Chen *et al*., 2010; Frank *et al*., 2010; Louis *et al*., 2010; Durbán *et al*., 2011). The dimensions of this data matrix were such that it was impossible to detect directly any similarities in statistical behaviour between the samples (individuals) or the phyla encountered (variables). Principal Component Analysis (PCA) (Lebart *et al*., 1997) was chosen to analyze these results. The data analysis software used for these analyzes was the SPAD version 3.5 (Décisia).

3. Results

3.1. Bacterial diversity in indoor air

In the three bacterial 16S rDNA libraries, all sequences were distributed in 2,971 phylotypes defined with a threshold 97% of similarity.

The similarities based on a comparison of the sequences were between 100% and 50%. 93.8% of the sequences displayed a similarity matching at least 97% with known sequences found in the RDP public database. By contrast, only 2.5% of the sequences displayed similarity of less than 90% with previously published sequences.

The sequences of indoor air belonging to the bacterial domain were analyzed at the phylum level. The phylogenetic identification of each phylotype, as well as its abundance at the different sites, is shown in Table 20, which presents sequences with more than 90% similarity to the closest sequences in the RDP database. Only phylotypes with an abundance higher than 1% were included. A table showing the identification of all bacterial phylotypes is included as supplementary material.

Table 20 : Phylogenetic positioning and abundance of bacterial phylotypes in the three indoor bioaerosols. Only phylotypes with an abundance of more than 1% in one bioaerosol or more are presented. Phylotypes found at three, two or one sites are presented respectively in white-on-black, dark grey and light grey shading.

	Percentage of sequences (%)							
Phylotypes	**Museum**	**Office**	**Hospital**	**Total**	**Closest relatives**	**Accession number**	**Similarity (%)**	**Origin**
Actinobacteria (640 phylotypes, 8354 sequences)								
IoNA 3	7.9	0	0.2	2.7	*uncultured bacterium*	GQ111666	100	Skin
IoNA 25	0.8	1.2	0.2	0.7	*Kocuria carniphila (T)*	AJ622907	100	ND
IoNA 37	3.2	36.3	5.2	14.9	*Micrococcus luteus*	AB023371	100	ND
IoNA 61	0.3	3.2	0.2	1.2	*uncultured bacterium*	FM873583	100	Dust
IoNA 93	2.4	1.7	1.4	1.8	*uncultured bacterium*	FM873570	100	Dust
IoNA 97	1.3	0.7	0.4	0.8	*unidentified bacterium*	AY345490	99	Sediment
IoNA 105	0.1	1.5	0.1	0.6	*Crocebacterium ilecola*	DQ826511	100	OE
IoNA 149	0.1	1	0.2	0.4	*Micrococcus luteus*	GQ260074	100	Human
IoNA 153	2.6	2	1.3	2.0	*uncultured bacterium*	GQ034508	100	Skin
IoNA 191	1.3	0.6	0.5	0.8	*Corynebacterium sp. 47081*	AF227825	100	OE
IoNA 218	0.2	3.8	0.3	1.4	*Curtobacterium herbarum (T)*	AJ310413	100	Soil
IoNA 246	0.2	1.5	0.2	0.6	*Micrococcus antarcticus (T)*	AJ005932	100	OE
IoNA 271	0.6	0.1	0	0.2	*Streptomyces sp. s8-203*	EF012134	100	Water
IoNA 366	0.2	2.1	0.2	0.8	*Micrococcus sp. TUT1210*	AB188213	100	Compost
IoNA 447	0.1	1.2	0.1	0.5	*Rhodococcus fascians*	Y11196	100	ND
IoNA 476	0.4	1	0.1	0.5	*uncultured actinobacterium*	EF016815	100	Soil
IoNA 587	0.4	2.1	0.3	0.9	*Rathayibacter caricis (T)*	AF159364	100	OE
IoNA 653	0.2	1.9	1.6	1.2	*uncultured bacterium*	AM697552	100	Dust
IoNA 1056	0.1	1	0	0.4	*Micrococcus sp. OS6*	EF491956	100	OE
Gammaproteobacteria (279 phylotypes, 4677 sequences)								
IoNA 16	4.9	1.9	2.8	3.2	*uncultured Pseudomonas sp.*	DQ234206	98	Water

IoNA 27	1.5	3.1	6.6	3.7	*unidentified bacterium*	Z93992	100	Waste
IoNA 39	3.3	3.2	3.9	3.5	*uncultured gamma proteobacterium*	AJ619051	100	OE
IoNA 94	1.5	2.1	10.7	4.8	*Acinetobacter lwoffii*	AF188302	100	Water
IoNA 122	0.4	1.8	0.8	1.0	*Pseudomonas sp. K15*	AB088755	100	ND
IoNA 129	0.8	1	2.3	1.4	*uncultured bacterium*	AY907718	100	Water
IoNA 214	0.4	0.2	1.2	0.6	*Pantoea sp. NCCP-116*	AB574400	100	OE
IoNA 230	0.6	0.2	1.8	0.9	*Pseudomonas sp.*	AJ387903	100	ND
IoNA 296	1	3.3	0.8	1.7	*uncultured bacterium*	FJ152954	100	Soil
IoNA 570	< 0.1	0	1.3	0.4	*uncultured bacterium*	EU842126	100	OE
IoNA 790	0.4	0.5	1.7	0.9	*Pantoea sp. NIIST-167*	FJ445213	100	Soil
Alphaproteobacteria (469 phylotypes, 4197 sequences)								
IoNA 30	4.2	1.1	1.6	2.3	*Paracoccus yeei*	AY014169	100	OE
IoNA 59	0.6	1.6	0.5	0.9	*uncultured alpha proteobacterium*	GQ850582	100	Water
IoNA 117	2.5	6.3	2.3	3.7	*Sphingomonas aurantiaca*	AJ429237	100	Air
IoNA 179	1.9	1.1	1.4	1.5	*Paracoccus carotinifaciens (T)*	AB006899	100	ND
IoNA 268	1.2	1.6	0.6	1.1	*Paracoccus sp. PA216*	AM900779	100	Dust
IoNA 436	0.3	1.1	0.3	0.6	*Methylobacterium sp. OS-16.b*	AM237344	99	Dust
IoNA 508	1.7	2.5	0.8	1.7	*Paracoccus sp. MBIC4036*	AB025192	100	Water
Betaproteobacteria (145 phylotypes, 1735 sequences)								
IoNA 41	1.9	1.4	1.3	1.5	*uncultured eubacterium WD285*	AJ292644	100	Soil
IoNA 175	1.4	0	0	0.5	*uncultured Bacillus sp.*	EU567044	99	Soil
IoNA 286	0.3	1.1	0.2	0.5	*uncultured proteobacterium OCS7*	AF001645	100	OE
IoNA 297	0.3	1.8	0.8	1.0	*uncultured proteobacterium OCS7*	AF001645	100	OE
IoNA 352	0.4	0.4	1.1	0.6	*uncultured bacterium*	EU431681	100	OE
IoNA 509	0.1	1.2	0.9	0.7	*uncultured hydrocarbon seep bacterium BPC087*	AF154097	100	Sediment

Firmicutes (534 phylotypes, 4002 sequences)							
IoNA 5	1.1	0.2	0.1	0.5			
IoNA 57	1	1.1	1.2	1.1	*Staphylococcus haemolyticus*	FN393802	100	Clean Room
IoNA 120	1.8	1.7	3.6	2.4	*Thermoactinomyces vulgaris*	EU430570	100	OE
IoNA 139	0.7	1	2.1	1.3	*Staphylococcus sp. NCCP 255*	AB619597	100	OE
IoNA 147	1.4	0.3	0.2	0.6	*uncultured bacterium*	AF371691	100	OE
IoNA 322	0.9	0.5	2.1	1.2	*Streptococcus oralis*	GU561395	100	Skin
IoNA 379	0.3	1.7	0.1	0.7	*Thermoactinomyces intermedius (T)*	AF138734	100	OE
IoNA 542	1.5	2.7	2.2	2.1	*uncultured bacterium*	FM875342	98	Dust
Bacteroidetes (417 phylotypes, 2048 sequences)							
IoNA 151	1	< 0.1	1	0.7	*Cytophaga sp. An36*	AJ551174	100	Sediment
IoNA 1595	0	2.4	0	0.8			
Deinococcus-Thermus (29 phylotypes, 296 sequences)							
IoNA 19	2.1	0	0.1	0.7	*Thermus sp.*	L09661	100	ND
IoNA 89	1.4	0.3	< 0.1	0.6	*Thermus brockianus*	Y18409	99	OE

Bacterial diversity, made up of by 22 different phyla, was largely dominated by *Proteobacteria* and *Actinobacteria* (Figure 43A). The *Proteobacteria* (*Alpha*, *Beta*, *Gamma*, *Delta* and *Epsilon*) phylum was the best represented phylum in two air samples, with respectively, 42.1% and 47.5% of sequences in the museum and hospital samples. The office library was dominated by *Actinobacteria*, which accounted for 41.8% of sequences, while *Proteobacteria* represented 36.2% of the sequences.

Indeed, of the 2,971 bacterial phylotypes obtained overall at the three sites, 40.3% were assigned to *Proteobacteria* and 31.8% to *Actinobacteria*. The remaining 27.9% were mainly distributed between *Firmicutes* and *Bacteroidetes* (Figure 43B). Although the same trend was observed at the three sites with respect to *Proteobacteria* (36.2% to 47.5%), differences were observed in class abundance from one site to another. *Alphaproteobacteria* were dominant in the museum and office locations, while *Gammaproteobacteria* were better represented in the hospital samples.

Some phyla were uncommon and were found only in one indoor air sample: *Spirochaetes*, *Nitrospira*, *OP11* and *Aquificae*. Three non-cultivated phyla were found: *TM7* (0.15% of sequences in the museum, 0.08% of sequences in the office and 0.11% of sequences in the hospital), *OP10* (0.12% in the museum, 0.05% in the office and 0.02% in the hospital) and *OP11* (0.02% of sequences in the museum).

The dominant bacteria were represented by *Actinobacteria* genera and *Micrococcus* sp. (particularly in the office where *Micrococcus* predominated). *Paracoccus* sp., *Pseudomonas* sp., *Acinetobacter* sp. and *Sphingomas* sp. were present at all the sites studied in proportions ranging from 2% to 6%.

The bacterial diversity of indoor bioaerosols was assessed using several parameters including rarefaction curves (Figure 43C), diversity indices and coverage (Table 21).

Based on the rarefaction curves built up from these data and by the analysis of S_{chao1} values, the samples were estimated to contain a total of respectively, 1,875, 1,449 and 1,851 separate OTU in the museum, office and hospital. Bacterial coverage was similar at the three sites. Simpson index at all three sites revealed considerable bacterial diversity although it was slightly higher in the museum and hospital. In fact, the values given by S_{chao1} predicted a much higher bacterial diversity for these two sites. The lowest values of the two diversity indexes were obtained in the office environment.

Table 21 : Analysis of bacterial diversity in the air of indoor environments.

				Diversity indexes	
Sites	**Number of sequences**	**Number of phylotypes**	**Coverage C (%)**	**S_{chao1}**	**Simpson**
Museum	10406	1589	63	1875	6.1
Hospital	8122	1164	63	1851	6.4
Office	7769	1518	63	1449	5.6

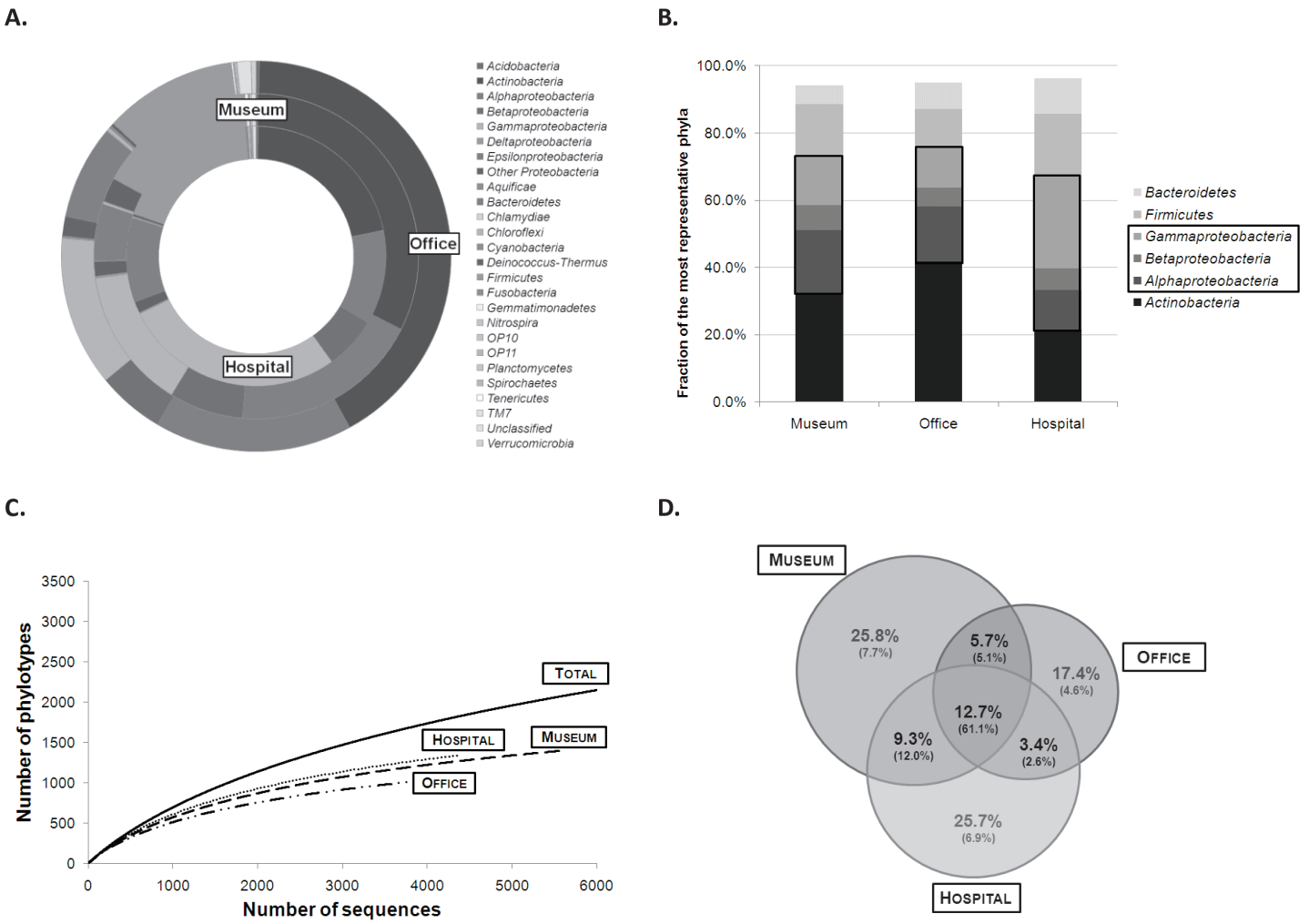


Figure 43 : A. Distribution of microbial phyla in each of the environmental samples. Circles represent, from the centre outwards, the hospital, the museum and the office area. B. The most representative phyla found in each sample. C. Rarefaction curves determined from 16S rDNA libraries for indoor bioaerosols. D. Sequences were grouped into OTUs at a 97% level of sequence similarity. OTUs overlapping between the museum, office and hospital are shown in the inset. Sequence abundances are given in brackets.

3.2. Airborne pathogen species in indoor environments

A total of 43 different genera, including some closely related to known pathogens, were detected among the indoor air sequences. The 43 pathogen-related genera along with the 32 species found during this analysis are shown in Table 22. Pathogen-related genera accounted for 6%, 8% and 7% ,respectively, of all the sequences analyzed from the museum, office and hospital. Different pathogen-related genera tended to dominate at each site. Indeed, four genera that may be related to pathogens (*Bacillus* sp., *Micrococcus* sp., *Corynebacterium* sp. and *Pseudomonas* sp.) were recovered at frequencies higher than 10% from the museum pathogen-related sequence library. The office was largely represented by *Micrococcus* sp. (63.8% of pathogen-related sequences), while *Acinetobacter* sp., *Micrococcus* sp. and *Pseudomonas* sp. were mostly encountered in the hospital samples. With regard to the presence of pathogen-related bacterial species, it should be noted that the genera and species encountered were those which are often responsible for opportunistic or nosocomial diseases or form part of human commensal bacteria. Species such as *Haemophilus influenzae* or *Acinetobacter baumanii* or *lwoffi* are opportunistic pathogens which cause respiratory complaints in the immuno-compromised and nosocomial infections in sensitive occupants (the elderly, children, sick people, etc.). *Aerococcus viridians* may also be an opportunistic pathogen in sensitive individuals. *Thermoactinomyces vulgaris*, which was found at all sites, is a causal agent of hypersensitivity pneumonitis. *Clostridium perfringens* is a commensal micro-organism found in normal human intestinal flora but it is also a pathogen that can cause a range of gastro-intestinal disorders (some of which may be extremely severe).

Some sequences were affiliated to virulent and opportunistic pathogenic genera, such as *Borrelia* sp., *Burkholderia* sp., *Legionella* sp., *Neisseria* sp. and *Mycobacterium* sp., but the level of sequence identity was not sufficient to predict the species concerned.

Table 22 : Sequence numbers per thousand of known pathogenic genera at each site. The site where pathogenic species were identified is shown in brackets. Bold numbers indicate the most numerous per thousand for each site.

Genus	Museum	Office	Hospital	Total	Pathogenic species	
Achromobacter sp.	1.2	3.7	3.1	2.7		
					xylosoxidans (M, O, H)	Opportunistic
Acinetobacter sp.	76.3	47.6	**253.3**	125.7		
					baumanii (M, H)	Opportunistic
					calcoaceticus (M, O, H)	Commensal
					johnsonii (M, O, H)	Opportunistic
					lwoffii (M, O, H)	Opportunistic
					schindleri (M, O, H)	Opportunistic
Actinomadura sp.	13.1	0.0	4.2	5.8		Opportunistic
Actinomyces sp.	13.1	0.0	4.7	5.9		Opportunistic
Aerococcus sp.	5.4	1.4	15.7	7.5		
					viridans (M, O, H)	Opportunistic
Aeromonas sp.	4.8	6.9	16.7	9.4		
					caviae (H)	Pathogen
					schubertii (M)	(Pathogen)
Alcaligenes sp.	0.6	0.9	4.7	2.1		
					faecalis (M, O, H)	Opportunistic
Bacillus sp.	**162.1**	40.2	24.0	75.5		(Pathogen)
Bacteroides sp.	0.0	2.3	1.0	1.1		
					fragilis (H)	Opportunistic
Bifidobacterium sp.	3.0	0.0	5.2	2.7		Commensal
Borrelia sp.	0.0	1.4	0.0	0.5		Pathogen
Burkholderia sp.	17.9	7.8	7.8	11.2		Pathogen
Clostridium sp.	24.4	5.0	27.2	18.9		
					butyricum (M, O, H)	Opportunistic

					glycolicum (O)	Opportunistic
					perfringens (M, H)	Pathogen
Corynebacterium sp.	113.2	29.3	53.8	65.4		
					xerosis (H)	Opportunistic/Pathogen
Enterococcus sp.	1.2	3.2	3.1	2.5		
					durans (H)	Commensal
Escherichia sp.	4.8	1.4	0.0	2.0		
					coli (M, O)	Commensal/Pathogen
Eubacterium sp.	6.6	3.2	8.4	6.0		Commensal
Flavobacterium sp.	39.3	0.9	25.6	21.9		Commensal/Opportunistic
Francisella sp.	1.2	0.0	0.0	0.4		(Pathogen)
Fusobacterium sp.	0.6	0.0	3.7	1.4		(Pathogen)
Gemella sp.	0.6	0.0	0.0	0.2		Commensal
Haemophilus sp.	1.8	0.0	5.7	2.5		
					haemolyticus (M, H)	Pathogen
Klebsiella sp.	6.6	9.1	7.8	7.8		Opportunistic
Lactobacillus sp.	16.1	11.9	24.5	17.5		Commensal
Legionella sp.	3.6	0.0	1.6	1.7		Pathogen
Micrococcus sp.	137.7	**639.7**	112.8	296.7		Commensal/Opportunistic
Mycobacterium sp.	3.6	3.2	6.8	4.5		Pathogen/Opportunistic
Mycoplasma sp.	0.6	0.0	2.1	0.9		Commensal/Opportunistic
Neisseria sp.	8.3	0.5	3.7	4.2		Commensal/(Pathogen)
Nocardia sp.	1.2	0.0	0.0	0.4		
					asteroides (M)	Opportunistic
Pasteurella	0.0	0.0	1.0	0.3		
					aerogenes (H)	Pathogen
Peptostreptococcus sp.	1.8	1.8	14.6	6.1		Opportunistic
Porphyromonas sp.	0.0	0.0	9.4	3.1		Commensal/(Pathogen)

Prevotella sp.	1.2	0.0	9.9	3.7		(Pathogen)
Propionibacterium sp.	2.4	0.0	0.0	0.8		
					acnes (M)	Commensal
					granulosum (M)	Commensal
Proteus sp.	0.0	0.5	0.5	0.3		
					vulgaris (O, H)	Pathogen
Pseudomonas sp.	109.7	41.2	103.9	84.9		
					aeruginosa (M, H)	Opportunistic
					alcaligenes (M)	Opportunistic
					pseudoalcaligenes (M, O, H)	Opportunistic
					putida (M, O, H)	Opportunistic
					stutzeri (M, H)	Opportunistic
Rhodococcus sp.	13.1	23.8	4.7	13.9		(Pathogen)
Rickettsia sp.	0.6	0.0	0.0	0.2		
					prowazekii (M)	Pathogen
Serratia sp.	2.4	1.8	4.7	3.0		
					marcescens (M, O, H)	Opportunistic
Staphylococcus sp.	62.6	35.2	67.9	55.2		
					haemolyticus (M, O, H)	Pathogen
Streptococcus sp.	42.3	8.2	67.4	39.3		(Pathogen)
Thermoactinomyces sp.	95.4	68.1	88.8	84.1		
					vulgaris (M, O, H)	Opportunistic

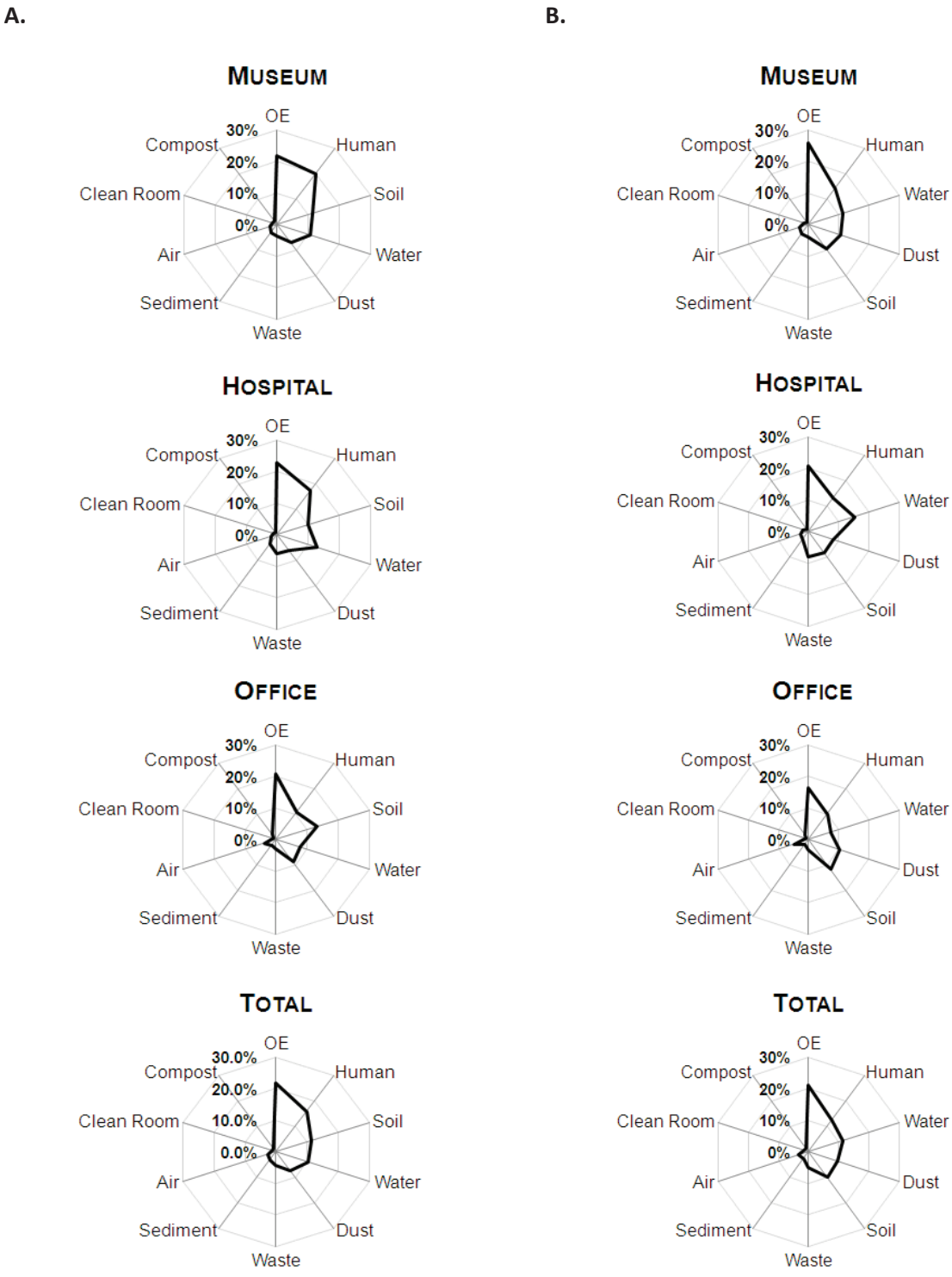


Figure 44 : Environmental origin of the closest bacterial relatives in public databases according to 16S rDNA similarities. A. Percentage abundances for all sequences. B. Percentage abundances for common sequences. The closest bacterial relatives recovered from several environments are shown under 'other environments (OE)'. Sequences for which the environment of the closest relatives was not found in the database are not presented. The term 'Waste' was used for aerobic and anaerobic treatment processes, biogas and digestors.

3.3. Comparison of sequence origins in the database

It is generally assumed that indoor airborne micro-organisms may arise from nearby primary environments such as soil or water as well as from occupants, dust or outdoor air. In order to determine whether the sequences obtained were affiliated to bacterial phylotypes previously identified in hypothetical sources of indoor contamination, the origin of the closest sequences present in public databases was collected for each phylotype, based on the data available on the Genbank sequence page. The origins determined for each phylotype are shown in Table 20 and Figure 44A.

Of the total number of indoor air sequences considered, 15.9% were of human origin (skin, faeces, oral, nasal, etc.). In addition, sequences displaying similarity of 95% or more with sequences of air or dust origin accounted for 2.7% and 7.3% of sequences, respectively. The other sequences were close to sequences found in sea and fresh water, waste, compost, plants, clinical isolates, animal faeces and biofilms.

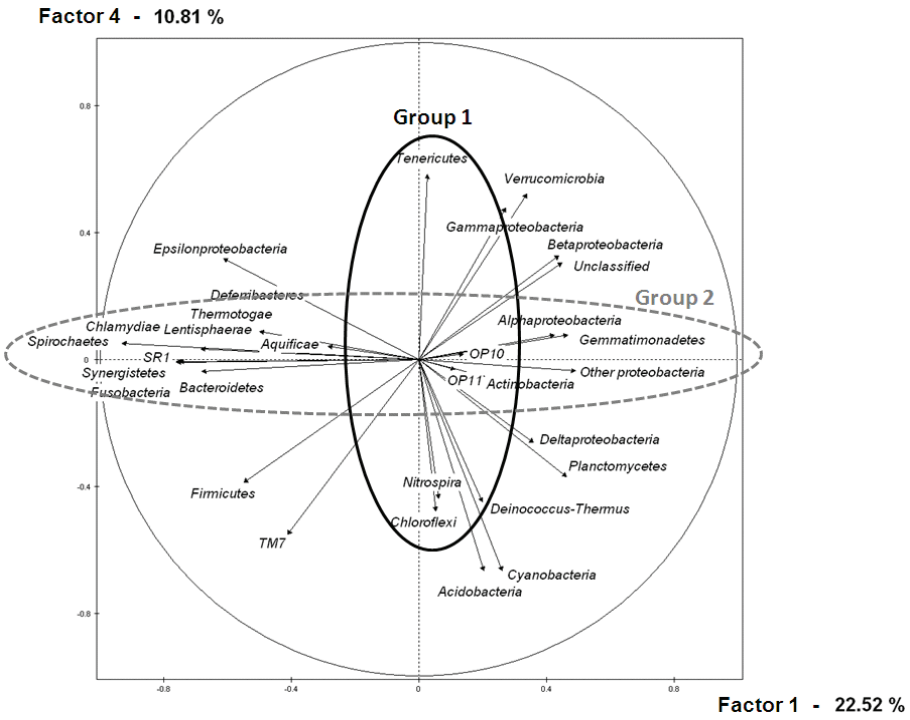
With the exception of the composite group of sequences of diverse origins (OE), the three sources found were mainly soil, water and humans.

3.4. Global analysis of different bacterial libraries

Analysis of the distribution of phyla at the different sampling sites generated a 31-column matrix (corresponding to the 31 different phyla retrieved from the bacterial libraries investigated). The results of PCA performed on this matrix showed that axis 1 represented 7 out of 31 variables. The two factors selected accounted for more than 33% of global variance, the principal plane thus grouping most of the information. In order to identify the variables responsible for the pattern observed, the 31 phyla were then projected onto a correlation circle (Figure 45A). Phyla from group 1 defined factor 1 while phyla from group 2 defined factor 4. The other phyla made an identical contribution to the construction of axes 1 and 4.

Figure 45B is a diagrammatical representation of a PCA. Each point represents a sampling. Three distinct groups could be observed: A (oral and faeces of human origin), B (nasal and skin of human origin) and C (two shopping centres, the therapeutic swimming pool, commercial airline cabins, hospital, museum and offices). As can be seen from Figure 45B, it was mainly the first factor that separated groups B and C from group A. The fourth factor separated groups A, B and C and the rural site from other outdoor sites. This representation also grouped the two shopping centres and the two urban sites. PCA thus revealed the presence of specific bacterial microflora common to the indoor environments. The matrix used to construct the PCA (based on the percentage abundance of phyla) and the differences or links observed between the groups may be due to the microbial diversity observed in each environment. The PCA analysis linked these microflora mainly to human nasal and skin microflora. It also highlighted the correlation between groups B and C. Group A was characterized by phyla from group 2 where there was a higher abundance of *Spirochaetes*, *Thermotogae*, *Deferribacteres*, *Synergistetes*, *Fusobacteria*, *Bacteroidetes*, *SR1*, *Lentisphaerae* and *Chlamydiae*. Group A was defined by a lower abundance of *Alphaproteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, other *Proteobacteria*, *OP10* and *OP11* whereas group C was characterized by a higher abundance of these phyla.

A.



B.

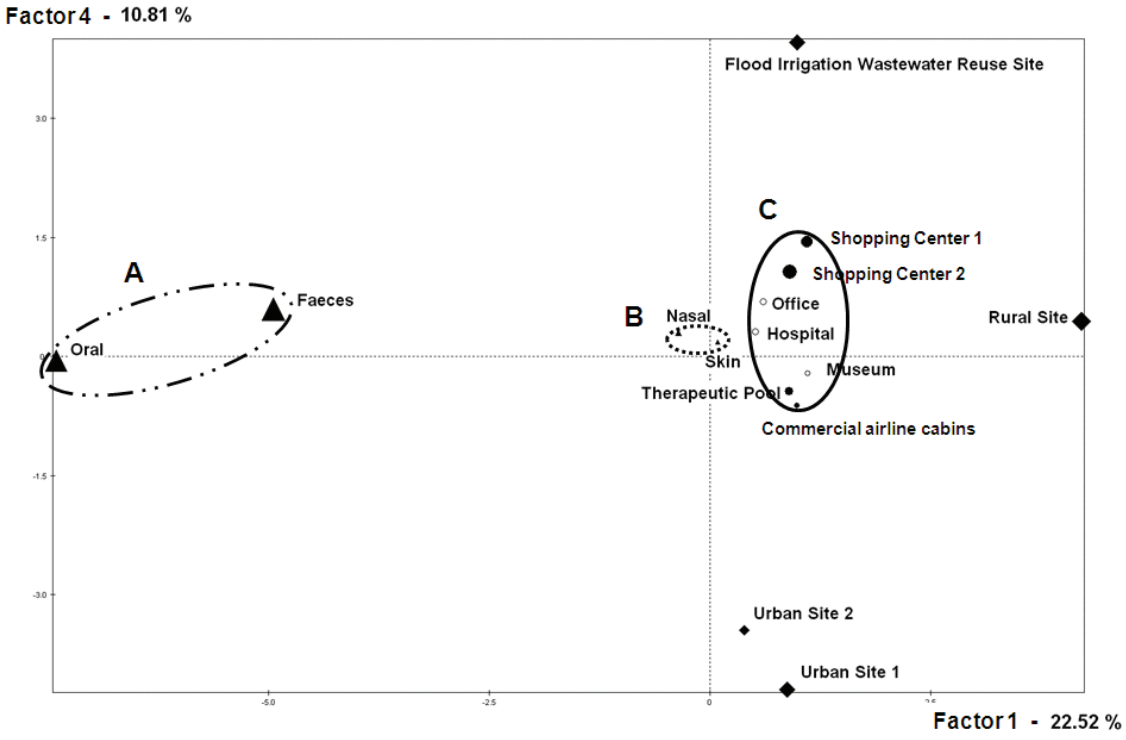


Figure 45 : A. Phyla were projected onto a correlation circle (1st and 4th factor: factors enabling a better representativity of indoor samples). B. Diagrammatic representation of PCA on samples from 15 sampling sites and 31 phyla: ● Indoor sites cited in the literature (6,014 sequences), ○ Indoor sites investigated during this study (26,297 sequences), ▲ human origin (154,583 sequences), ◆ outdoor sites cited in the literature (735 sequences).

3.5. Identification of common 'core species' in airborne bacteria from enclosed spaces

Of the 2,971 phylotypes found at the three sites, only 378 were common to the museum, the office and the hospital and they accounted for 12.7% of the OTU (Operational Taxonomic Units) and 61.1% of the total number of sequences obtained for the three indoor environments (Figure 43D). Each environment was represented more or less equally in this overall percentage (22.0% of diversity in the museum, 21.1% in the office and 18.0% in the hospital). However, these values corresponded to a majority of the total number of sequences found in each environment (55.7% of sequences in the museum, 71.4% in the office and 58.1% in the hospital).

The 378 phylotypes representative of an OTU common to the three sites and belonging to the bacterial domain were analyzed at phylotype and sub-group levels. The distribution and abundance of phyla in the 378 OTU were the same as those observed previously, with a predominance of *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroidetes*.

The dominant bacteria were represented by *Actinobacteria* genera, *Paracoccus* sp., *Acinetobacter* sp., *Micrococcus* sp., *Pseudomonas* sp., *Enhydrobacter* sp., *Staphylococcus* sp. and *Thermoactinomyces* sp.

The *Actinobacteria* dominant in the office mainly corresponded to *Micrococcus* sp., although differences from one site to another were observed. The most abundant airborne microbes in the hospital included several species of *Acinetobacter* sp., some of which were pathogen-related species (Table 22). The office samples were largely dominated by *Micrococcus* species, some of them known as pathogens. *Actinobacteria* genera and *Paracoccus* species represented almost identical percentages of the sequences found in the museum (12.8% and 12.4%, respectively).

In order to determine whether common bacterial flora in indoor air represented a mere copy of bacterial flora of human origin, the 378 bacterial phylotypes were considered relative to the ecosystem producing the closest sequence found in the database. Interestingly, this comparison showed that in each indoor air library, 63 phylotypes presented more than 97% similarity with the database sequences found in humans (skin, oral, etc.). Of the total number of indoor air sequences reviewed, 20% of the phylotypes and 12% of the sequences were of human origin (Figure 44B). Among the 378 phylotypes presenting similarity of 95% or higher, nine were associated with air and 34 with dust, accounting for 3% and 10% of the indoor air sequences, respectively (Figure 44B).

Pathogen-related genera were also investigated in the common diversity established for the three sites: the total proportion of the 66 common pathogenic phylotypes represented 26.7% of common sequences and 72% of all pathogen-related sequences (58% for the museum, 89% for the office and 69% for the hospital).

Genera such as *Micrococcus* sp., *Corynebacterium* sp., *Thermoactinomyces* sp., *Pseudomonas* sp. and *Acinetobacter* sp. accounted for more than 75% of pathogen-related sequences.

4. Discussion

To date, the microbial content of air in enclosed spaces has been little described. Environmental data resulting from an investigation of sources and pathogens are extremely rare despite their health implications. Our study addressed these issues. The results of sequencing of the data collected at three enclosed spaces revealed a common microflora (more than 60% of all sequences). Among these common phyla, four were in a majority: *Actinobacteria*, *Proteobacteria* (*Alpha*, *Beta* and *Gamma*), *Firmicutes* and *Bacteroidetes*. These findings are in agreement with data in the literature. Thus, of the 13 phyla identified during studies in two shopping centres, airline cabins and a therapeutic swimming pool, the same four phyla were common to all air samples (Angenent *et al*., 2005; Tringe *et al*., 2008; Osman *et al*., 2009). During our study, a comparison with the Genbank database of the 'core species' found in our three indoor environments showed that several of our sequences (22%) had been determined during other studies involving the human micro-environment (skin, oral, etc.) and dust. This observation is consistent with the findings of Hospodsky *et al*. (2011), who suggested that bacterial populations in indoor air show greater similarity to the populations found in floor dust than to populations from outdoor air. This indicates that human occupancy is a major source of indoor aerosols and that the re-suspension of floor dust might also be an important source of aerosols in enclosed spaces. This conclusion is in line with several other observations in indoor environments that have demonstrated an increase in the concentration of micro-organisms linked to human occupancy (Pastuszka *et al*., 2000; Jo and Seo, 2005; Hospodsky *et al*., 2011), even where controlled spaces are involved such as the International Space Station (ISS) and operating theatres, where bioaerosols are mainly associated with human emissions (skin, hair and respiratory tract) (Favero *et al*., 1968; Novikova *et al*., 2006). In fact, the human body hosts numerous bacteria and may constitute an important source of dust and microbial aerosols, which can be found on the skin, on mucous membranes in the respiratory tract, in the mouth, in nasal passages and in the gastrointestinal tract (Hold *et al*., 2002; Grice *et al*., 2009; Chen *et al*., 2010; Frank *et al*., 2010; Louis *et al*., 2010; Durbán *et al*., 2011). The convective plume around a human body carries re-suspended particles (skin scales, textile fibers, bacteria, etc.) that may contribute to dust production (Scheinder, 2008; Clark and de Calcina-Goff, 2009; Täubel *et al*., 2009). Indeed, the entire outer skin layer of humans is shed every 1 to 2 days, which can result in the release of several million skin scales per minute (Schneider, 2008). Moreover, certain human activities such as talking, coughing or sneezing can generate enormous quantities of droplets: one sneeze, for example produces about 100,000–1,000,000 droplets, many of which carry bacteria (Xie *et al*., 2007).

Our study thus confirmed these observations by showing that the majority of sequences were of various human origins in both the global database (15.9% of sequences) and 'core species' database (12.0%). Hence, PCA analysis linked this microflora mainly to human nasal and skin microflora.

The analysis of the species involved showed that of the 61.1% sequences found in all three environments, 26.7% were pathogen-related and common to all sites. Moreover, the results obtained by studying pathogen-related sequences among the 'core species' revealed an increase in the number of pathogen-related species, and particularly of opportunistic species.

Acinetobacter sp. and *Staphylococcus* sp. are present in both environmental and commensal skin microflora and were found in all the air samples we studied. Together with *Propionibacterium* spp.and *Corynebacteria* spp., they represent 2.2% of the diversity of the skin flora common to six people (Gao *et al*., 2007). During a broader study on samples from 20 different skins sites,

Actinobacteria genera and *Staphylococcus* sp. were encountered in all samples (Grice *et al*., 2009). During the present study, *Pseudomonas* sp. was also found in all the air samples. Lee *et al*. (2007) pointed out that *Pseudomonas* sequences were the most abundant in their clone libraries obtained from samples collected from the surfaces of toys in a child-care facility. In the office samples of our study, *Micrococcus* was more common than other genera. This was in line with the findings of Bouillard *et al*. (2005), who investigated healthy office buildings and found that the two most frequent cultivable species in air samples were *Micrococcus* spp. and *Staphylococcus* spp.

The predominance of some Gram-negative bacteria in indoor air raises questions about their effect on health and, particularly, the allergenic potential of cell-wall fragments or endotoxins associated with these bacteria.

Airborne micro-organisms are sometimes directly implicated in health emergencies. Indeed, *Mycobacterium* spp. was identified using molecular methods in the air of a therapeutic swimming pool and incriminated in the pulmonary infections caught by some of the staff (Angenent *et al*., 2005). Managing and controlling the quality of indoor air must be suited to the actual use of a building, in particular to its occupancy levels and energy requirements.

Although the office space was the least frequented, the diversity recorded seems to be similar to that observed in the museum and hospital. This diversity is clearly linked to the similar anthropogenic and endogenous sources of micro-organisms. There is no correlation between the level of occupancy and the diversity found at the three sites investigated. Nevertheless, the percentage of pathogen-related organisms in office air was much higher than that found in either the museum or the hospital. Although occupancy was lower in the office area, its volume was also the smallest, leading to an increased load of contaminants and, consequently, to a higher concentration of opportunistic and pathogenic species (Daisey *et al*., 2003). If the bacterial pollution arose from an interaction between the occupants and other indoor sources (dust of human origin), indoor contamination might be explained by the use of an inadequate or insufficient ventilation system.

The determination of common pathogenic species may well make it possible to identify specific indicators crucial for managing the bacterial quality of air in indoor environments or for evaluating the efficiency of ventilation systems. A similar approach was adopted in the study of bioaerosols in composting facilities, where it was possible to determine phylogenetic groups common to different aerosols and thus define microbial indicators for dispersal or risk assessment analysis (Le Goff *et al*., 2009). However, given the large number of commonly occuring phylotypes present in the air of indoor environments, it will be necessary to increase the variety of environments studied in order to draw up a workable list of specific indicators that will effectively improve the management of indoor air quality.

This study is the first to have gathered a large dataset on bioaerosols found in three different enclosed spaces (museum, office and hospital). It has thus improved our knowledge of the dominant bacteria encountered in the air of such environments and highlighted the predominance of *Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes* and the specific phyla signatures of the indoor environments studied.

Comparison of the sequences for the three different sites revealed a common bacterial signature (more than 60% of all sequences) and the presence of ‘core species’ arising from the human microenvironment and not from outdoors. The detection of pathogenic species common to all the

sites means that it may be possible to use them to define indicators and, subsequently, an index to be used as a basis for the development of tools for managing indoor air quality.

Acknowledgements

This research was supported by the DGA, the CSTB, the INRA-LBE and GENOSCREEN. The authors gratefully acknowledge the assistance provided by Ms Sabine Gaüzère through her helpful contribution to automated sequence analyses and the help from Mrs Joëlle Le Roux of the Louvre Museum.

References

Almeida SM, Canha N, Silva A, Freitas MdC, Pegas P, Alves C *et al* (2011). Children exposure to atmospheric particles in indoor of Lisbon primary schools. *Atmos Environ* **45:** 7594–7599.

AmannRI, Ludwig W, Schleifer KH (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* **59:** 143-169.

Angenent LT, Kelley ST, AmandAS, Pace NR, HernandezMT (2005). Molecular identification of potential pathogens in water and air of a hospital therapy pool. *Proc Natl Acad Sci USA* **102:** 4860-4865.

Bouillard L, Michel O, Dramaix M, Devleeschouwer M (2005). Bacterial contamination of indoor air, surfaces, and settled dust, and related dust endotoxin concentrations in healthy office buildings. *Ann Agr Env Med* **12:** 187-192.

Brodie EL, DeSantis TZ, Parker JPM, Zubietta IX, Piceno YM, Andersen GL (2007). Urban aerosols harbor diverse and dynamic bacterial populations. *Proc Natl Acad Sci USA* **104:** 299-304.

Chen T, Yu WH, Izard J, Baranova OV, Lakshmanan A, Dewhirst FE (2010) The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information. *Database*. doi: 10.1093/database/baq013

Clark RP, de Calcina-Goff ML (2009). Some aspects of the airborne transmission of infection. *J R Soc Interface* **6:** S767-S782.

Cole JR, Chai B, Farris RJ, Wang Q, Kulam-Syed-Mohideen AS, McGarrell DM *et al* (2007). The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data. *Nucleic Acids Res* **35:** 169-172.

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ *et al* (2009). The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res* **37:** 141-145.

Daisey JM, Angell WJ, Apte MG (2003). Indoor air quality, ventilation and health symptoms in schools: an analysis of existing information. *Indoor Air* **13:** 53-64.

Durbán A, Abellán J, Jiménez-Hernández N, Ponce M, Ponce J, Sala T *et al* (2011). Assessing Gut Microbial Diversity from Feces and Rectal Mucosa. *Microb Ecol* **61:** 123.

Favero MS, Puleo JR, Marshall JH, Oxborrow GS (1968). Comparison of Microbial Contamination Levels Among Hospital Operating Rooms and Industrial Clean Rooms. *Appl Environ Microb* **16:** 480-486.

Frank DN, Feazel LM, Bessesen MT, Price CS, Janoff EN, Pace NR (2010). The Human Nasal Microbiota and *Staphylococcus aureus* Carriage. *PLoS ONE* **5:** 1-15.

Fromme H, Diemer J, Dietrich S, Cyrys J, Heinrich J, Lang W *et al* (2008). Chemical and morphological properties of particulate matter (PM10, PM2.5) in school classrooms and outdoor air. *Atmos Environ* **42:** 6597-6605.

Fromme H, Twardella D, Dietrich S, Heitmann D, Schierl R, Liebl B *et al* (2007). Particulate matter in the indoor air of classrooms-exploratory results from Munich and surrounding area. *Atmos Environ* **41:** 854-866.

Gao Z, Tseng C-h, Pei Z, Blaser MJ (2007). Molecular analysis of human forearm superficial skin bacterial biota. *Proc Natl Acad Sci* **104:** 2927-2932.

Gaüzère, C, Moletta-Denat, M, Bousta, F, Moularat, S, Orial, G., Ritoux, S *et al*. (2011). Etude de la diversité microbienne dans les espaces clos par une approche moléculaire : résultats préliminaires dans un bureau paysager et un musée. *26ème CFA*, Paris, France.

Gilbert Y, Duchaine C (2009). Bioaerosols in industrial environments: a review. *Can J Civil Eng* **36:** 1873-1886.

Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC *et al* (2009). Topographical and Temporal Diversity of the Human Skin Microbiome. *Science* **324:** 1190-1192.

Hold GL, Pryde SE, Russell VJ, Furrie E, Flint HJ (2002). Assessment of microbial diversity in human colonic samples by 16S rDNA sequence analysis. *FEMS Microbiol Ecol* **39:** 33.

Höppe P, Martinac I (1998). Indoor climate and air quality. *Int J Biometeorol* **42:** 1-7.

Hospodsky D, Qian J, Yamamoto N, Nazaroff WW, Peccia J (2011). Size-fractionated emissions and microbial population characterization to reveal sources of bacteria in indoor air. *In Final Program Abstracts, 12th International Conference on indoor air quality and climate, Austin, Texas*.

Jo WK, Seo YJ (2005). Indoor and outdoor bioaerosol levels at recreation facilities, elementary schools, and homes. *Chemosphere* **61:** 1570-1579.

Kotzias D, Geiss O, Tirendi S, Barrero-Moreno J, Reina V, Gotti A *et al* (2009). Exposure to multiple air contaminants in public buildings, schools and kindergartens-The European Indoor Air Monitoring and Exposure Assessment (AIRMEX) Study. *Fresenius environmental bulletin* **18:** 670-681.

Lebart L, Morineau A, Piron M (1997). *Statistique exploratoire multidimensionnelle - 2eme édition*, Dunod Edition: Paris.

Lee L, Tin S, Kelley ST (2007). Culture-independent analysis of bacterial diversity in a child-care facility. *BMC Microbiol* **7:** 27-40.

Le Goff O, Bru-Adan V, Bacheley H, Godon JJ, Wéry N (2009). The microbial signature of aerosols produced during the thermophilic phase of composting. *J Appl Microbiol* **108:** 325-340.

Liu LJ, Krahmer M, Fox A, Feigley CE, Featherstone A, Saraf A *et al* (2000). Investigation of the concentration of bacteria and their cell envelope components in indoor air in two elementary schools. *J Air Waste Manag Assoc* **50:** 1957-1967.

Louis P, Young P, Holtrop G, Flint HJ (2010). Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. *Environ Microb* **12:** 304.

Maron P-A, Lejon DPH, Carvalho E, Bizet K, Lemanceau P, Ranjard L *et al* (2005). Assessing genetic structure and diversity of airborne bacterial communities by DNA fingerprinting and 16S rDNA clone library. *Atmos Environ* **39:** 3687-3695.

Moletta M, Delgenes J-P, Godon J-J (2007). Differences in the aerosolization behavior of microorganisms as revealed through their transport by biogas. *Sci Total Environ* **379:** 75-88.

Novikova N, De Boever P, Poddubko S, Deshevaya E, Polikarpov N, Rakova N *et al* (2006). Survey of environmental biocontamination on board the International Space Station. *Res Microbiol* **157:** 5-12.

Osman S, La Duc MT, Dekas A, Newcombe D, Venkateswaran K (2008). Microbial burden and diversity of commercial airline cabin air during short and long durations of travel. *ISME J* **2:** 482-497.

Paez-Rubio T, Viau E, Romero-Hernandez S, Peccia J (2005). Source bioaerosol concentration and rRNA gene-based identification of microorganisms aerosolized at a flood irrigation wastewater reuse site. *Appl Environ Microb* **71:** 804-810.

Pastuszka JS, Kyaw Tha Paw U, Lis DO, Wlazlo A, Ulfig K (2000). Bacterial and fungal aerosol in indoor environment in Upper Silesia, Poland. *Atmos Environ* **34:** 3833-3842.

Radosevich JL, Wilson WJ, Shinn JH, DeSantis TZ, Andersen GL (2002). Development of a high-volume aerosol collection system for the identification of air-borne micro-organisms. *Lett Appl Microbiol* **34:** 162-167.

Schloss PD, Handelsman J (2005). Introducing DOTUR, a Computer Program for Defining Operational Taxonomic Units and Estimating Species Richness. *Appl Environ Microb* **71:** 1501-1506.

Schloss PD, Handelsman J (2006). Introducing SONS, a tool for operational taxonomic unit-based comparisons of microbial community memberships and structures. *Appl Environ Microb* **72:** 6773-6779.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB *et al* (2009). Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Appl Environ Microbiol* **75:** 7537-7541.

Schneider T (2008). Dust and fibers as a cause of indoor environment problems. *Scand J Work Env Hea* **34:** 10-17.

Täubel M, Rintala H, Pitkäranta M, Paulin L, Laitinen S, Pekkanen J *et al* (2009) The occupant as a source of house dust bacteria. *J Allergy Clin Immunol* **124:** 834-840.

Tringe SG, Zhang T, Liu X, Yu Y, Lee WH, Yap J *et al* (2008). The airborne metagenome in an indoor urban environment. *PLoS ONE* **3:** 1-10.

Wang Z, Reponen T, Grinshpun SA, Górny RL, Willeke K (2001). Effect of sampling time and air humidity on the bioefficiency of filter samplers for bioaerosol collection. *J Aerosol Sci* **32:** 661-674.

Wichmann J, Lind T, Nilsson MAM, Bellander T (2010) PM2.5, soot and NO2 indoor-outdoor relationships at homes, pre-schools and schools in Stockholm, Sweden. *Atmos Environ* **44:** 4536-4544.

Xie X, Li Y, Chwang AT, Ho PL, Seto WH (2007) How far droplets can move in indoor environments - revisiting the Wells evaporation falling curve. *Indoor Air* **17:** 211–225.

Zuraimi MS, Tham KW (2008). Indoor air quality and its determinants in tropical child care centers. *Atmos Environ* **42:** 2225-2239.

II.3 RESULTATS SUPPLEMENTAIRES : ANALYSE DE LA DIVERSITE EUCARYOTES

II.3.A Matériel et Méthodes

Ce chapitre présente la caractérisation de la diversité eucaryote présente dans l'air de deux environnements intérieurs. En particulier, il détaille la dynamique temporelle et spatiale de cette diversité dans le musée du Louvre et un bureau paysager.

II.3.A.a *Séquençage*

Les échantillons ont été amplifiés en utilisant les amorces suivantes : l'amorce eucaryote sens (5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAGTGCGTCTTAATTTGACTCAACACGG-3 ') pour l'échantillon J1 et (5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGATCAGACACGCTTAATTTGACTCAACACGG-3 ') pour l'échantillon J164 et l'amorce eucaryote anti-sens (5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAGGGGCATCACAGACCTGTT -3'). Pour les échantillons bureau et musée, l'amorce eucaryote sens (5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGTGATACGTCTCTTAATTTGACTCAACACGG -3') et l'amorce eucaryote anti sens (5'- CCTATCCCCTGTGTGCCTTGGCAGTCTCAGGGGCATCACAGACCTGTT -3') ont été utilisées. Des aliquots d'ADN de chaque échantillon ont été utilisés pour une réaction PCR de 50 µL. Des séquences avec une taille moyenne de 220 pb ont été amplifiées afin de construire quatre banques eucaryotes. Le système Gene Amp High Fidelity PCR (Applied Biosystems) a été utilisé pour la PCR dans les conditions suivantes : 95°C pendant 10 min, suivie de 30 cycles de 95°C pendant 30 s, 50°C pendant 30 s et 72°C pendant 1 min et une étape finale d'élongation à 72°C pendant 10 min. Tous les amplicons produits à partir d'échantillons différents ont été purifiés à l'aide de billes Agencourt AmPure XP (Agencourt Bioscience Corporation, MA, Etats-Unis). A l'issue de cette étape de purification, une partie des amplicons est contrôlée sur puce Agilent DNA 1000 (Agilent Technologies), afin de contrôler l'absence d'un pic aux environs de 120 pb, représentatif de la présence de dimères d'amorces. Les amplicons ont été séquencés en utilisant le 454 GS-FLX Titanium (Roche).

II.3.A.b *Analyses phylogénétiques*

Chaque séquence a été nettoyée de toutes les amorces et les séquences plus courtes (> = 150 pb) ont été enlevées à l'aide de la plateforme dédiée de RDP. Les séquences ont été vérifiées pour les chimères, ont été alignées et une matrice de distance a été générée en utilisant MOTHUR (v.1.20.0) (Schloss *et al*., 2009). Les phylotypes ont été définis sur la base de 97% de similarité par le programme DOTUR (Schloss et Handelsman, 2005). Un représentant de chaque phylotype a été comparé à la base de données publique NCBI avec le programme MOTHUR (v.10.27). Les courbes de raréfaction et les indices de diversité ont été obtenus par le biais du programme DOTUR à 97% de similarité.

Un total de 55 339 séquences d'ARN ribosomique 18S a été analysé dans le but d'accéder à la diversité eucaryote présente dans l'air des environnements intérieurs. Parmi elles, 21 733 étaient

affiliées à différents domaines des plantes ou encore aux *Metazoa*. Ces séquences n'ont pas été gardées pour l'analyse suivante.

II.3.B Résultats

II.3.B.a *Diversité temporelle des organismes eucaryotes dans l'air du Musée du Louvre*

Deux banques d'ADNr 18S ont été construites à partir des prélèvements ponctuels réalisés les jours 1 et 164 dans le Musée du Louvre. Un total de 14 329 séquences pour l'échantillon J1 et de 15 499 séquences pour J164, ont été analysées sur la base de 97% de similarité. Ces séquences sont distribuées en 300 et 736 phylotypes pour J1 et J164, respectivement.

- Analyse au niveau du groupe

Les différentes séquences appartenant aux *Eukarya* ont été analysées au niveau groupe. On retrouve dans l'air du musée une faible diversité de groupes (2 à 3 pour les échantillons J1 et J164, respectivement) (Figure 46).

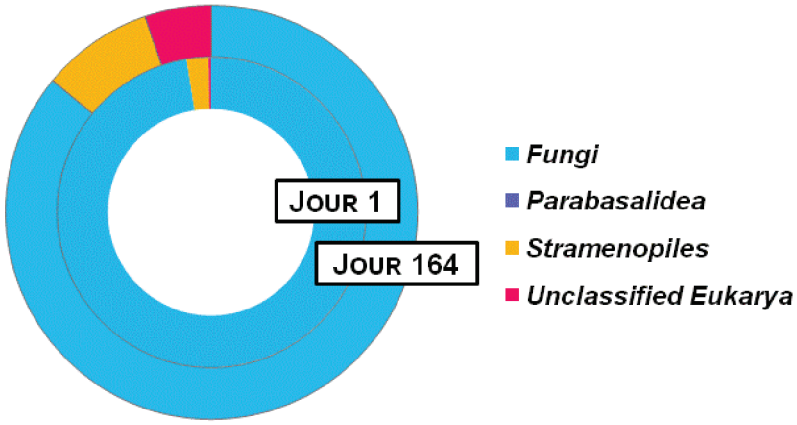


Figure 46 : Répartition des phyla eucaryotes dans chacun des échantillons d'air du musée du Louvre. Les cercles représentent, en partant de l'intérieur, l'échantillon J1 et J164.

Néanmoins, la diversité eucaryote du Musée du Louvre est majoritairement dominée par le groupe des *Fungi* (97,5% des séquences pour J1 et 86,0% des séquences pour J164). Le second groupe retrouvé dans les deux échantillons est celui des *Stramenopiles* (2,3% des séquences pour J1 et 8,8% des séquences pour J164). Le groupe des *Parabasalidea* a été retrouvé dans un seul des échantillons d'air intérieur (0,02% des séquences de l'échantillon J164).

➢ Analyse au niveau phylum et phylotype

La diversité eucaryotes de l'air intérieur du Musée du Louvre est dominée par les champignons affiliés aux *Ascomycota* (pour 92,2% J1 et 64,9% pour J164) et aux *Basidiomycota* (7,3% pour J1 et 32,3% pour J164) quelque soit la date du prélèvement (Figure 47).

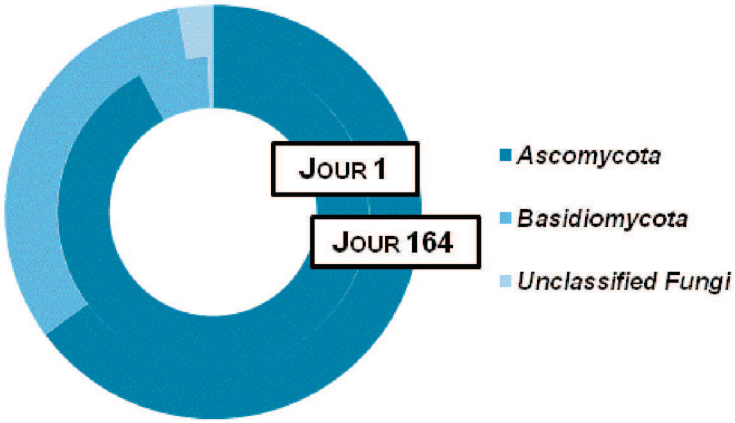


Figure 47 : Répartition des phyla fongiques de chacun des échantillons d'air du musée du Louvre. Les cercles représentent, en partant de l'intérieur, l'échantillon J1 et J164.

La diversité eucaryote des bioaérosols intérieurs a été évaluée à l'aide de plusieurs paramètres tels que les indices de diversité (Simpson et S_{chao1}), le recouvrement (C) (Tableau 23) et les courbes de raréfaction (Figure 48).

Tableau 23 : Analyse de la diversité eucaryote de deux échantillons d'air intérieur (J1 et J164).

				Indices de diversité	
Echantillons	**Nombre de séquences**	**Nombre de phylotypes**	**Recouvrement C (%)**	**S_{chao1}**	**Simpson**
Jour 1	14329	300	65	429	3.1
Jour 164	15499	736	61	1232	5.3

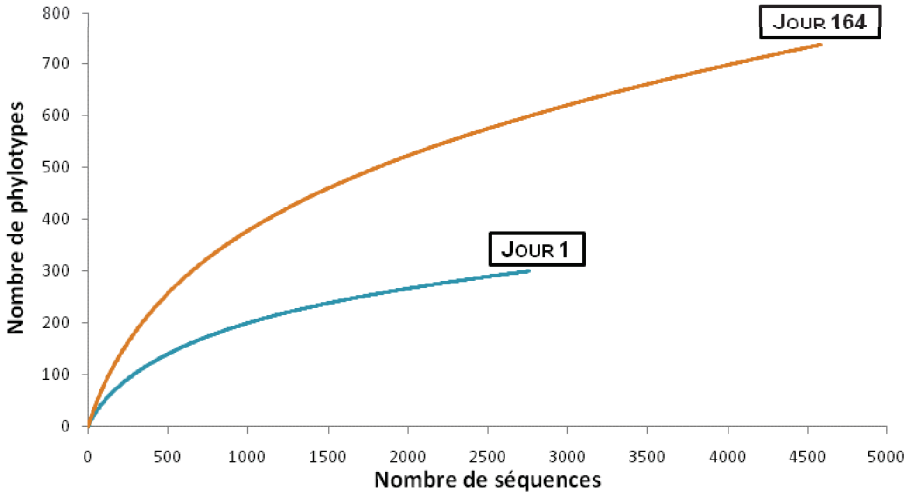


Figure 48 : Courbes de raréfaction déterminées à partir de bibliothèques ADNr 18S pour les échantillons d'air J1 et J164.

En se basant sur les courbes de raréfaction construites à partir des données et de l'analyse des valeurs, les échantillons devraient contenir au total 429 et 1 232 OTU distincts pour J1 et J164, respectivement. Le recouvrement calculé est similaire pour les deux échantillons. Les indices de Simpson ont montré que la diversité eucaryote différait en fonction du jour de prélèvement. L'indice de Simpson est plus élevé pour l'échantillon J164. Par ailleurs, les valeurs données par S_{chao1} prédisent une diversité eucaryote trois fois plus élevée pour J164.

Les données mettent en évidence des différences de diversité au niveau phylum et phylotype entre les échantillons J1 et J164, soulignant une variabilité temporelle des eucaryotes dans l'air.

II.3.B.b *Diversité spatiale des organismes eucaryotes dans l'air de deux espaces clos*

Deux banques d'ADNr 18S ont été construites à partir des prélèvements intégrés réalisés dans le musée et dans le bureau. Un total de 13 064 séquences pour le musée et de 10 447 séquences pour le bureau ont été analysées sur la base de 97% de similarité. Ces séquences sont distribuées en en 722 et 1 029 phylotypes pour le musée et le bureau, respectivement.

- Analyse au niveau du groupe

La diversité eucaryote est représentée par trois groupes différents, mais reste largement dominée par le groupe des *Fungi* (Figure 49). Le groupe des *Fungi* apparaît comme le groupe le mieux représenté dans les deux échantillons d'air, avec 97,8% et 99,6% des séquences dans le musée et le bureau, respectivement.

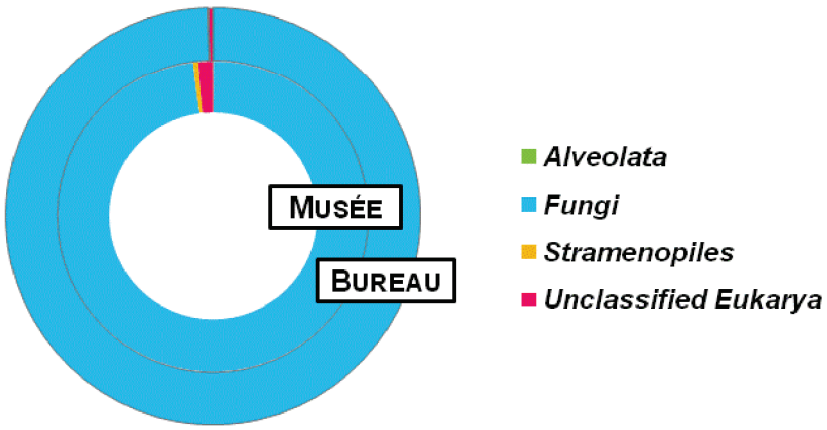


Figure 49 : Répartition des phyla eucaryotes dans chacun des échantillons d'air intérieur. Les cercles représentent, en partant de l'intérieur, le musée et le bureau.

➢ Analyse au niveau phylum et phylotype

La diversité eucaryotes de l'air intérieur du musée et du bureau est principalement dominée par les champignons affiliés aux *Basidiomycota* (63,7% des séquences pour le musée et 66,5% des séquences pour le bureau) et aux *Ascomycota* (35,0% pour le musée et 32,3% pour le bureau), quelque soit le lieu étudié (Figure 50).

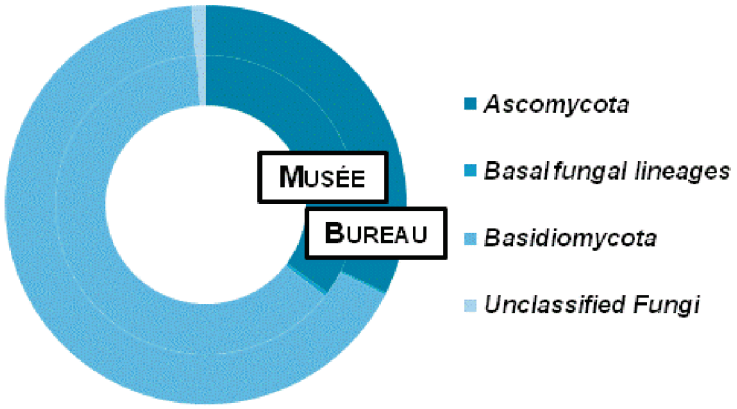


Figure 50 : Répartition des genres fongiques retrouvés dans les échantillons d'air du musée et du bureau. Les cercles représentent, en partant de l'intérieur, le musée et le bureau.

Comme précédemment, la diversité eucaryote des bioaérosols du musée et du bureau, a été évaluée à l'aide de plusieurs paramètres permettant de mesurer la diversité (Figure 51 et Tableau 24).

Tableau 24 : Analyse de la diversité eucaryote de l'air de deux environnements intérieurs (un musée et un bureau).

				Indices de diversité	
Echantillons	**Nombre de séquences**	**Nombre de phylotypes**	**Recouvrement C (%)**	**S_{chao1}**	**Simpson**
Musée	13064	722	67	1039	5.1
Bureau	10447	1029	56	1682	5.3

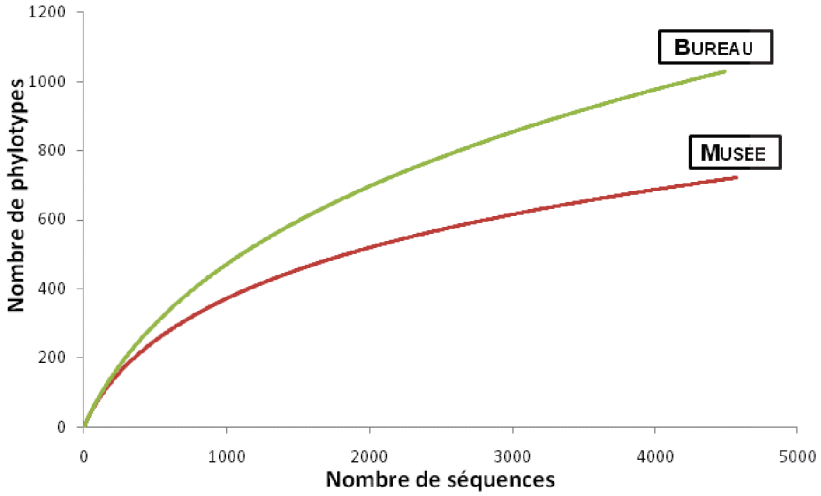


Figure 51 : Courbes de raréfaction déterminées à partir de bibliothèques ADNr 16S pour les échantillons d'air du musée et du bureau.

L'analyse des valeurs et des courbes de raréfaction estime qu'un total de 1 039 et 1 622 OTU distincts est retrouvé dans l'air du musée et du bureau, respectivement. Le recouvrement calculé est plus élevé pour le musée que pour le bureau. Les indices de Simpson et de S_{chao1}sont du même ordre de grandeur et montrent que la microflore eucaryote présente une diversité équivalente dans le musée et le bureau.

D'un point de vue spatial (deux sites de prélèvements différents), la diversité eucaryote moyennée sur quatre semaines apparaît plus stable.

II.3.C Discussion et conclusions

A l'heure actuelle, il n'existe que très peu de données disponibles permettant de caractériser la diversité des eucaryotes présents dans l'air des espaces clos (Chapitre 1, § IV.2.A.a).

Deux études traitant du sujet ont été publiées, l'une portant sur l'air d'une piscine thérapeutique et l'autre sur deux centres commerciaux. Les résultats de l'analyse de la diversité montrent comme dans notre étude la prédominance de séquences fongiques et notamment des phyla *Ascomycota* et *Basidiomycota* (Tringe *et al*., 2008 ; Angenent *et al*., 2005).

Récemment, quelques études se sont intéressées à la diversité fongique en aérosols de l'air extérieur par l'utilisation de méthodes moléculaires. Les auteurs suggèrent que les séquences d'ADN fongiques détectés dans l'air, proviennent de spores (connues pour résister aux stress environnementaux et pour leur capacité à survivre aux transports dans l'air) (Desprès *et al*., 2007 ; Fröhlich-Nowoisky *et al*., 2009). L'air extérieur apparait majoritairement dominé par les *Ascomycota* et les *Basidiomycota* quelque soit le lieu de prélèvement et le temps. Les différences observées sont au niveau de l'abondance de chaque phyla dans les différents échantillonnages (Boreson *et al*., 2004 ; Desprès *et al*., 2007 ; Fierer *et al*., 2008 ; Fröhlich-Nowoisky *et al*., 2009 ; Lee *et al*., 2010).

Pour les personnes souffrant d'allergies, l'exposition à des spores fongiques ou des pollens en environnements intérieurs est particulièrement préoccupante. L'ensemble des maladies allergiques (asthme, rhinite, conjonctivite, etc.) concerne 20% de la population dans les pays industrialisés. La concentration en allergènes dans les lieux clos résulte assez souvent d'une mauvaise aération ou ventilation conduisant à une augmentation des concentrations en polluants et ainsi à une dégradation de la QAI. Si les spores d'ascomycètes (*Clasdosporium* spp., *Aspergillus* spp. ou *Alternaria* spp.) sont fréquemment citées comme pouvant induire un certain nombre de réponses allergiques (Kurup *et al*., 2000), les basidiomycètes sont eux, peu incriminés malgré qu'ils apparaissent représenter une part importante de la diversité fongique de l'air. L'exposition des individus aux spores de basidiomycètes ainsi que leurs implications dans les phénomènes allergiques sont peu documentées. L'étude de Helbling *et al*. (1998) démontre l'existence d'une sensibilisation aux basidiomycètes fréquente chez des sujets souffrant d'allergies respiratoires. Par ailleurs, ils mirent en évidence que des spores de *Pleurotus pulmonalis* pouvaient certainement induire des allergies respiratoires chez des sujets sensibilisés. Une autre étude sur quelques 701 adultes vivant aux Etats Unis ou en Europe de l'Ouest, montre que les basidiomycètes peuvent être une importante source d'allergènes de l'air et ce, dans des régions géographiquement très disparates. En outre, ces allergènes pourraient représenter un réel risque sanitaire pour les personnes souffrant d'asthme (Lehrer *et al*., 1994).

Contrairement à la diversité bactérienne, qui affiche une certaine stabilité à la fois dans le temps et sur différents environnements, les données montrent une variabilité temporelle de la diversité eucaryote (Chapitre 2, § II.1 et § II.2). Cette observation est à relier à la valeur de stabilité obtenue à partir des profils de CE-SSCP. En effet, alors que la stabilité bactérienne était d'environ 55%, elle avoisinait les 30% pour la stabilité eucaryote.

D'un point de vue méthodologique, il n'y a pas congruence entre l'identification des champignons basée sur l'ADNr 18S et la classification encore essentiellement basée sur la région ITS, et sur les caractéristiques morphologiques et physiologiques. Ceci parce que l'ensemble des champignons n'ont aucune cohérence phylogénétique. Il existe des formes sexuées et non sexuées classées séparément. La région ITS habituellement utilisée est pertinente pour une classification intra-