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Quantitative sampling of indoor air biomass by signature lipid biomarker analysis

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Exposure to airborne biocontaminants may result in a multitude of health effects and is related to a pronounced increase in adult-onset asthma. Established culture-based procedures for quantifying microbial biomass from airborne environments have severe limitations. Assay of the phospholipid fatty acid (PLFA) components of airborne microorganisms provides a quantitative method to define biomass, community composition and nutritional/physiological activity of the microbial community. By collecting airborne particulate matter from a high volume via filtration, we collected sufficient biomass for quantitative PLFA analysis. Comparing high (filtration) and low (impaction) volume air sampling techniques at 26 locations within the Eastern United States, we determined that PLFA analysis provided a viable alternative to the established but flawed culture-based techniques for measuring airborne microbial biomass and community composition. Compared to the PLFA analysis, the culture techniques underestimated the actual viable airborne biomass present by between one to three orders of magnitude. A case study of a manufacturing plant at which there had been complaints regarding the indoor air quality is presented. Phospholipid fatty acid characterization of the biomass enabled contamination point source determination. In comparison with samples taken outdoors, increases in the relative proportion of *trans* PLFA, reflecting shifts in the physiological status of viable airborne Gram-negative bacteria, were detected in the indoor air samples at a majority of sampling sites.

Keywords: airborne bacteria; phospholipid fatty acids; human health

Introduction

Airborne microbial contaminants within indoor environments are an ever-increasing source of concern in public health and industrial hygiene. Exposure to such biocontaminants can give rise to numerous health effects including infectious disease [15,20,30], allergenic responses [5,9] and respiratory problems [8,20]. Microbial contaminants typically found in indoor air environments include bacteria, fungi, algae, protozoa and viruses. Additional immunoactive biocontaminants such as house dust mites or danders are often present.

Traditionally, methods employed for monitoring microorganism concentrations in indoor air environments have utilized culture-based techniques. However, it has been documented repeatedly that such culture techniques are less efficient than direct analytical techniques and account for only 0.1–10% of the total community [3,32,34,37,40,42]. Moreover, although conventional low-volume bioaerosol samplers are designed to damage microorganisms as little as possible [8], microbial stress has been shown to result from air sampling, aerosolization and microbial collection [7,11,21,28,33,36]. These procedures result in cell damage and non-culturability. Air filtration samplers can collect particulates at the highest efficiency (~100%), but captured microorganisms become dehydrated and damaged, again resulting in high levels of non-culturability [11,21]. A quantitative sampling/detection/identification technique independent of culturability, that assays both culturable and non-culturable (but still viable) biomass, is critical in defining risks from indoor air biocontamination.

Sensitive and direct chemical methods utilizing gas chromatography-mass spectrometry (GC-MS) have been developed for detection of both muramic acid, present in bacterial peptidoglycan (PG) [12–14], and 3-hydroxylated fatty acids. present in Gram-negative lipopolysaccharide (LPS) [13,16,35]. Both LPS and PG are toxic cell wall components [14,35], the direct detection of which bypasses the disadvantages of bacterial culture and provides assessment of non-culturable allergens. However, although providing very useful information regarding the presence of organic toxins, neither PG nor LPS is sufficiently labile to be used for reliable detection of viable biomass [14,34]. Moreover, neither material has the structural diversity among different bacterial species to provide detailed information regarding bacterial community composition.

Extractable phospholipid fatty acids (PLFA) are found in all cell membranes and cell walls of microorganisms, are actively metabolized during growth and have a relatively rapid turnover [34,39]. As PLFA degrade rapidly following cell death, they are a measure of viable biomass. Specific groups of microorganisms often contain characteristic lipid patterns, and lipid extraction, purification and subsequent analysis by GC-MS has enabled determination of the microbial biomass, nutritional status, and community composition from different environments including soil, sediment, and water [34,37,38,40]. The feasibility of using PLFA for the quantitative analysis of airborne biomass has

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been confirmed in laboratory-based studies [26]. The air filtration sampling device and lipid biomarker assays described herein are highly efficient and do not rely on cell culture. As lipid analysis does not require culturability for quantification, air filtration is the most appropriate method for high-volume sample collection.

Here we describe a comparison between biomarker (PLFA) data and culturable bacterial data obtained following the sampling of airborne particulate matter at 26 commercial and residential sites within the eastern United States (principally located in East Tennessee). To demonstrate the versatility of PLFA analysis for the assessment of total microbial biomass, community structure and physiological status, the results of our analyses following sampling at a manufacturing plant which had received complaints regarding indoor air quality, are described in detail. Outdoor air samples were taken concurrently at each site, enabling determination of whether outdoor air was a contaminant source and providing a microbial community for comparison with that collected indoors.

Materials and methods

Sampling sites

Sites that were occupied during the day were selected for inclusion within the study. Site names are not presented for confidentiality reasons. Sampling sites were assigned letters of the alphabet. Included in our sampling sites were office buildings, industrial sites (including a manufacturing plant), school buildings and residential sites. Details of the sampling sites are presented in Table 1. For purposes of confidentiality, two sites (N and S) were not assigned a category. Complaints regarding the indoor air quality were reported at a number of sites (Table 1, *sample sites). Of these, locations within site Z (manufacturing plant) had been subject to complaints regarding air quality and were in close proximity to air sampling locations within the same plant which had not received any complaints. This was, therefore, an ideal site at which to carry out contamination point source determinations. At this site, samples were taken from: (a) air that had been subject to complaints; (b) 'clean' air; and (c) a possible source of contamination, the aqueous-based washing fluid utilized on two of the manufacturing lines.

High-volume air sampling

High-volume air sampling was done using a portable air sampler designed in conjunction with Graseby GMW (Atlanta, GA, USA; Figure 1). The sampler was 1.6 meters in diameter, and was able to pump air at more than one cubic meter per minute over a glass fiber filter (GF/D; 2.7 μm; Whatman International, Maidstone, UK). The sampler operated with 115 volt AC current at 60 Hertz. To avoid 'air stripping' ie double-sampling of air, samples were only obtained from sites with indoor air volumes in excess of the air sample collected. Also, sampling sites were selected only if they had heating, ventilation and air conditioning (HVAC) systems providing air circulation. Dependent on indoor air volume, samples taken comprised approximately 500–1500 m^3 of air. Prior to lipid analysis all sample filters were stored at –20°C. Concurrent outdoor air samples were obtained at all sites.

Low-volume air sampling

Total culturable heterotrophs were sampled using single-stage Andersen viable particle samplers (Graseby Andersen, Atlanta, GA, USA) with an air flow rate of 0.028.3 cubic meters min^{-1} (28.3 L min^{-1}). Collection plates for the Andersen sampler were prepared as described in the manufacturer's instructions [1]. The sterile Petri dishes contained R2A agar (R2A; Becton Dickinson and Co, Cockeysville, MD, USA) for enumeration of bacterial populations. The R2A agar contained cycloheximide (50 μg ml^{-1}) to inhibit fungal growth. Air samples were collected for either 2 or 5 min over the single Petri dish placed within the Andersen sampler. Five replicate air samples were taken at each of the indoor sample locations with an extra set of samples collected for 30 s at Site Z. Outdoor samples (five replicates per sample) were collected for 2 and 5 min throughout the winter and 1 and 3 min throughout the summer. Plates were incubated at room temperature for up to 14 days. Colony counts were made on days 2, 5, 8, 10 and 14. Numbers of culturable heterotrophic bacteria were adjusted using the 'positive hole' correction [25].

Liquid sampling

Samples of aqueous-based machine line washing fluid were collected from site Z (a manufacturing plant). Triplicate fluid samples (1000 ml) were collected when the lines were running. Samples were filtered sequentially through glass fiber filters (GF/D, 2.7-μm pore size; GF/B, 1-μm pore size; GF/F, 0.7-μm pore size; Whatman International) and finally through anodisc filters (inorganic alumina filters, 0.2-μm pore size, Whatman International). The filter-collected residues were then extracted for PLFA as described below.

Lipid biomarker analysis

All solvents were of GC grade and were obtained from Baxter Scientific Products (McGaw Park, IL, USA). All glassware was washed in a 10% (v/v) 'Micro' cleaner solution (Baxter Diagnostics, Deerfield, IL, USA), rinsed five times in tap water and then five times in deionized water. The glassware was then heated overnight in a muffle furnace at 450°C.

All sample filters and negative controls (a field blank filter, a glass fiber filter onto which no bacteria had been deposited and a buffer blank) were extracted for lipids using the modified Bligh and Dyer extraction [39]. The total lipids obtained were then fractionated into neutral-, glyco-, and polar lipids on a silicic acid column [17]. The phospholipid-containing polar lipid fraction was subjected to a mild alkaline methanolysis, transesterifying the fatty acids into methyl esters [17]. The PLFA were separated, quantified and identified by gas chromatography-mass spectrometry (GC-MS) [33]. Fatty acids were identified both by relative retention times compared with authentic standards (Matreya, Pleasant Gap, PA, USA) and by mass spectra [31]. Fatty acid nomenclature is in the form of 'A:BωC' where 'A' designates the total number of carbons, 'B' the number of double bonds, and 'C' the distance of the closest unsaturation from the aliphatic end of the molecule. The suffixes 'c' for *cis* and 't' for *trans* refer to geometric isomers. The prefixes 'i' and 'a' refer to iso and anteiso methyl

Table 1 Sampling site descriptions and results

Site	Type[a]	Sample number	Sampling date	BCA m^{-3} [c]	CFU m^{-3} [d]	Ratio *t/c* indoors[e]	Ratio *t/c* outdoors[f]
*A	S	9	Oct/Nov 96	$9.6 \pm 4 \times 10^4$	352 ± 57	0.3 (0.04)	No *trans*
*B	S	18	Nov 96	$1.9 \pm 0.4 \times 10^5$	$1.24 \pm 0.25 \times 10^3$	0.33 (0.04)	0.16 (0.06)
*C	S	9	Nov, Dec 96	$1.2 \pm 0.3 \times 10^5$	629 ± 67	0.37 (0.08)	0.24 (0.1)
D	S	6	Aug 97	$6.7 \pm 3 \times 10^3$	475 ± 164	0.39 (0.3)	No *trans*
E	R	2	Oct 97	$3.5 \pm 0.5 \times 10^3$	335 ± 65	No *trans*	No *trans*
F	R	3	Nov 97	$2.3 \pm 0.3 \times 10^4$	246 ± 88	No *trans*	No *trans*
*G	O	4	Aug 97	$7.2 \pm 1.8 \times 10^4$	ND	0.33 (0.07)	No *trans*
*H	R	2	Sep 97	$6.1 \pm 5.9 \times 10^5$	598 ± 120	1.45 (0.7)	No *trans*
I	O	2	Sep 97	$1.15 \pm 0.7 \times 10^4$	460 ± 45	No *trans*	No *trans*
J	O	4	Aug 97	$2.4 \pm 0.96 \times 10^3$	180 ± 16	No *trans*	No *trans*
K	O	2	Jul 97	$3.5 \pm 0.2 \times 10^3$	273 ± 5.5	0.4 (0)	No *trans*
*L	R	3	Jun 97	$7.7 \pm 6.4 \times 10^4$	124 ± 9.5	0.36 (0.36)	No *trans*
M	O	3	Jun 97	$4.4 \pm 0.4 \times 10^3$	297 ± 60	No *trans*	No *trans*
*N	C	2	Apr 97	$5.5 \pm 1.8 \times 10^5$	ND	0.05 (0.02)	No *trans*
O	I	15	Apr 97	$5.7 \pm 1.0 \times 10^4$	574 ± 127	No *trans*	No *trans*
P	O	6	Apr 97	$4.4 \pm 1.6 \times 10^3$	87 ± 18	0.12 (0.04)	No *trans*
Q	O	6	Apr 97	$4.25 \pm 0.8 \times 10^3$	177 ± 127	0.25 (0.05)	No *trans*
*R	R	1	Mar 97	4.76×10^5	726 ± 643	No *trans*	No *trans*
*S	C	4	Mar 97	$2.7 \pm 1.5 \times 10^4$	340 ± 83	0.21 (0.08)	0.05 (0.05)*
T	O	6	Jan 97	$4.83 \pm 1.2 \times 10^3$	256 ± 98	0.35 (0.06)	0.27 (0.07)*
*U	O	1	Oct 96	1.07×10^4	141 ± 60	No *trans*	No *trans*
V	O	2	Oct 96	$2.2 \pm 1.4 \times 10^4$	218 ± 22	0.25 (0.05)	No *trans*
W	O	3	Dec 95, Aug 96	$6.5 \pm 0.3 \times 10^3$	1900 ± 1.200	No *trans*	No *trans*
*X	O	8	Apr, Aug 96	$5.6 \pm 0.7 \times 10^3$	201 ± 62	No *trans*	No *trans*
*Y	O	3	Jul 96	$6.5 \pm 1.0 \times 10^4$	105 ± 25	0.53 (0.01)	0.08 (0.02)
*Z[f]	I	14	Aug 96	$*2.0 \pm 0.3 \times 10^6$	$*1.5 \pm 0.05 \times 10^4$	0.24 (0.01)	No *trans*
				$3.1 \pm 0.9 \times 10^4$	308 ± 79		

[a]S = School, R = Residential, O = Office, I = Industrial, C = confidential.
[b]BCA = bacterial cell abundance (calculated from PLFA [4] m^{-3}).
[c]CFU = colony forming units m^{-3}.
[d,e]Sites at which *trans* fatty acids were not detected are labeled 'No *trans*'. For indoor samples, *n* is as indicated in the site column, for outdoor samples *n* = 3 unless noted (*indicates *n* = 2).
[f]Air at site Z was sampled at locations from which the air had received complaints regarding its 'quality' (*n* = 6) as well as from air which had received no complaints regarding air quality (*n* = 8). For these sites BCA and CFUs are listed separately.
Errors represent standard errors of the means. ND = Not Determined. NA = Not Applicable.
*Identifies sites at which complaints had been received regarding the indoor air quality.

branching respectively, and cyclopropyl rings are indicated by 'cy' [22].

Statistical analysis

Statistical analysis (standard deviations, standard errors of the mean and Student's *t*-test) were performed using an Excel spreadsheet (Microsoft Office 97, Microsoft Corp). Principal components analysis of the square root transformed PLFA mole percent data were performed using the Einsight Statistical Package, version 4.04 (InfoMetrix, Woodinville, WA 98072-1528, USA).

Results

Viable biomass

Phospholipid and culturable heterotroph data obtained comprised 135 samples from 26 sites. For each site, details of biomass content per cubic meter of air measured as both bacterial cell abundance (obtained from PLFA content [4]) and culturable bacteria (CFU m^{-3}) are presented in Table 1. Bacterial cell abundance was calculated using a conversion factor (2.0×10^4 cells per picomole PLFA; [4]). It is important to remember that, as with any PLFA conversion factor, the number of cells can vary by up to 0.5 orders of magnitude either side of the mean. Culturable counts per cubic meter of air were generally between one to three orders of magnitude lower than was the PLFA-determined bacterial cell abundance.

At the manufacturing plant (site Z), sample analysis is shown for the air taken from locations adjacent to reservoirs of: (a) aqueous-based machining fluid (low 'quality' air); and (b) oil-based machining fluid (higher 'quality' air) (Table 1). The samples located adjacent to the aqueous-based fluid contained approximately two orders of magnitude more biomass than did those taken adjacent to the oil-based fluid. At each sampling site there was an approximately 10–1000 fold discrepancy between culturable bacterial counts and PLFA-based bacterial cell abundance measurements (Table 1).

Community structure

In general, the lipid profiles of both indoor and outdoor air samples were dominated by the presence of PLFA characteristic of eukaryote biomass (16:0, 18:0, 18:2ω6 and 18:1ω9c). Lipids typical of bacteria (monoenoic and terminally branched saturate PLFA [43]) were detected in the majority of all indoor and outdoor air samples. Due to the relatively large amount of 16:0 and 18:0 in human skin,

Figure 1 The portable air sampling device (PASD).

these two normal saturate PLFA have been included as eukaryote-type PLFA; however, they are also common in bacteria [44], which may have resulted in a slight underestimation in bacterial numbers. Air sampled from outdoors frequently contained visible micro- (fungal spores) and macro-eukaryote (pollen, and insects) biomass.

Figure 2 shows the function-specific groupings of the PLFA sampled from site Z. Such groupings are defined according to PLFA molecular structure which is related to fatty acid biosynthesis [41]. Phospholipid fatty acid profiles sampled from the air adjacent to the oil-based machine lines and from the air sampled when the machine lines were down were similar in structure to the majority of those sampled at the remainder of the sites throughout the study. However, air sampled adjacent to the aqueous-based lines contained a 100-fold greater relative proportion of PLFA typical of bacteria than was usually detected, indicating the presence of a substantially higher concentration of airborne bacteria.

The PLFA data sets obtained from different sites throughout this study were invariably complex with at least 25–60 different PLFA per sample; therefore, further statistical analysis was required to obtain information regarding the community structures of the samples. Principal component analyses (PCA) were carried out enabling the major relationships between the samples to be visualized. Generally, concurrently-collected outdoor air samples contained different microbial communities from those collected indoors. An example of a typical PCA from this study is shown in Figure 3a (site Y). This PCA shows the separate clustering of the indoor and outdoor air with principal component 1 most heavily influenced by the presence of 16:1ω7t. For the manufacturing plant (site Z), the airborne bacteria sampled adjacent to the aqueous- and oil-based machine washing fluid clustered separately from one another (Figure 3b). Two principal components were derived which accounted for 84% and 7% of the variance inherent in the data, respectively. The first principal component was most strongly influenced by 18:1ω7c and 16:1ω7c, both of which are primarily associated with the presence of Gram-negative bacteria [43]. These were most prevalent in samples obtained from aerosols located around the machine lines utilizing the aqueous-based cooling fluid. Moreover, the lipid profiles of the biomass filtered from the aqueous-based tool washing fluid were similar to those from the airborne bacteria from near the machining line (aqueous 1 and 2). In contrast, the lipid profiles from outdoors clustered with those sampled from the air next to the machine lines utilizing the oil-based fluid (oil 1 and 2).

Physiological status

Gram-negative bacteria make *trans* fatty acids which modify their cell membranes as protection against environmental stresses [18,23] with the higher ratio of *trans/cis* fatty acids indicating greater levels of environmental stress. As such, the physiological status of Gram-negative communities can be assessed from the ratios of specific PLFA. Combined ratios for the 16:1ω7 and 18:1ω7 *trans* and *cis* PLFA of 0.1 or less are taken to be representative of healthy non-stressed communities [41]. Independent of data set, the summed ratios of the *trans/cis* PLFA indoors were generally higher for the indoor samples than they were for equivalent samples taken concurrently outdoors (Table 1). Site Y provided the most compelling evidence of this lipid stress response. Figure 4 shows the ratios of the *trans* to *cis* fatty acids in the indoor and concurrently-taken outdoor samples from site Y. In the majority of sample sets, *trans* PLFA were not detected in the outdoor air samples, demonstrating that air sampling *per se* had not induced the physiological stress.

Discussion

Previous studies in different environments have demonstrated that culturable bacterial counts generally account for between 0.1–10% of the total viable community detectable by other more direct techniques such as microscopic counting or lipid analysis [3,32,34,37,42]. A possible explanation for the non-culturability of otherwise viable airborne bacteria is that the cells may be damaged. The aerosolized state poses multiple stresses on a bacterium including dehydration, radiation, oxygen concentration and temperature [19]. Previous studies have shown that the outer lipids of Gram-negative bacteria can be damaged by dehydration making the cells no longer culturable [8]. However, within physiological limits, the effects of dehydration on bacteria are reversible [2] and the bacteria are still viable.

Further complicating the comparison between viable and

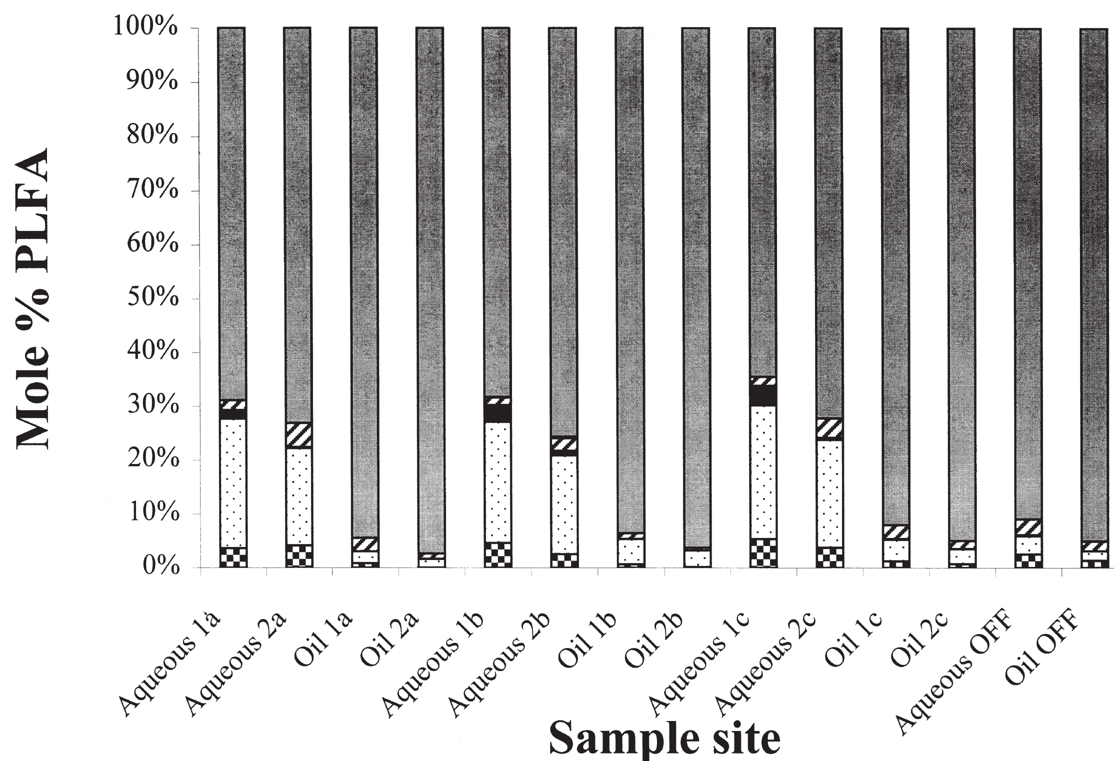

Figure 2 A comparison of the relative percentages of total PLFA functional groups in the samples from site Z. Functional groups are defined according to molecular structure. Terminally branched saturates (▚) are attributed to Gram-positive/anaerobic Gram-negative bacteria; monounsaturates (⊡) to Gram-negative bacteria; branched monounsaturates (□) and mid-chain branched saturates (■) to sulfate-reducing bacteria and actinomycetes; normal saturates (▨) are ubiquitous; and eukaryote-type PLFA (⊠) comprise polyunsaturates and 16:0 and 18:0 normal saturates.

culturable cells were the different sampling procedures necessitated for the lipid and the culture analysis. Sampling devices used in culture-based studies, eg Andersen, all glass impinger and Mattson–Garvin slit-agar samplers, are designed to damage the microorganisms as little as possible so as to maximize culturability. As a result, sampling efficiency is generally not as high as it is for filtration devices [10]. Macnaughton *et al* [26] determined that counts of test bacteria sampled using an all glass impinger accounted for approximately 25% of those organisms sampled onto the filter and analyzed using PLFA. We were comparing a highly efficient filtration device with a far less efficient device, an Andersen sampler, and were not comparing like with like. Given that the difference between cell abundance measured using the air filtration and the Andersen sampler techniques was one to three orders of magnitude, the four-fold difference in sampling efficiency does not prevent major conclusions being drawn. Indeed, the greater sampling efficiency of the filtration device resulted in the PLFA analysis providing more quantitative bioaerosol data than did the culture analysis.

Because PLFA can be microorganism- or group-specific, in addition to providing data as regards total biomass, lipid analysis also enables community structure assessment. At site Z the differences between the airborne bacterial populations adjacent to either the aqueous- or oil-based fluids were such that PCA analysis of the PLFA profiles from these samples was instrumental in determining the source of contamination (Figure 3b). Analysis of the PLFA profiles of the samples taken from the aqueous-based washing fluid showed these samples contained similar PLFA profiles to those of the airborne biomass, indicating that the fluid was the primary source of the airborne contamination. The PLFA profiles obtained for airborne bacteria sampled from next to the oil-based fluids were similar to those obtained from the outdoors. This was in direct contrast to the results obtained at site Y where the PLFA profiles for the indoor and outdoor air clustered separately (Figure 3a). The most likely reason for the unusual results obtained at site Z was that the factory doors remained open during the working day, negating the effect of the HVAC system in circulating the indoor air.

A further advantage of the PLFA over other methodologies is that it enables determination of the nutritional/physiological status of Gram-negative bacteria. Gram-negative bacteria make *trans* fatty acids which modify their cell membranes as protection against environmental stresses [18,23], with the increase in *trans* fatty acid indicative of increased environmental stress. Compared to the concurrently sampled outdoor air bacteria, in the majority of the indoor air bacterial PLFA profiles, the *trans* fatty acids comprised a larger relative proportion of the monoenoic 16:1ω 7 and 18:1ω7 PLFA. A possible reason for this increase in *trans* fatty acids may be the low relative humidity indoors caused by the use of HVAC systems. The samples taken from outside were collected using identical high volume sampling devices to those used indoors. As such, the high volume sampling procedure itself was eliminated as a possible cause of the environmental stress that resulted in the increase in *trans* fatty acids.

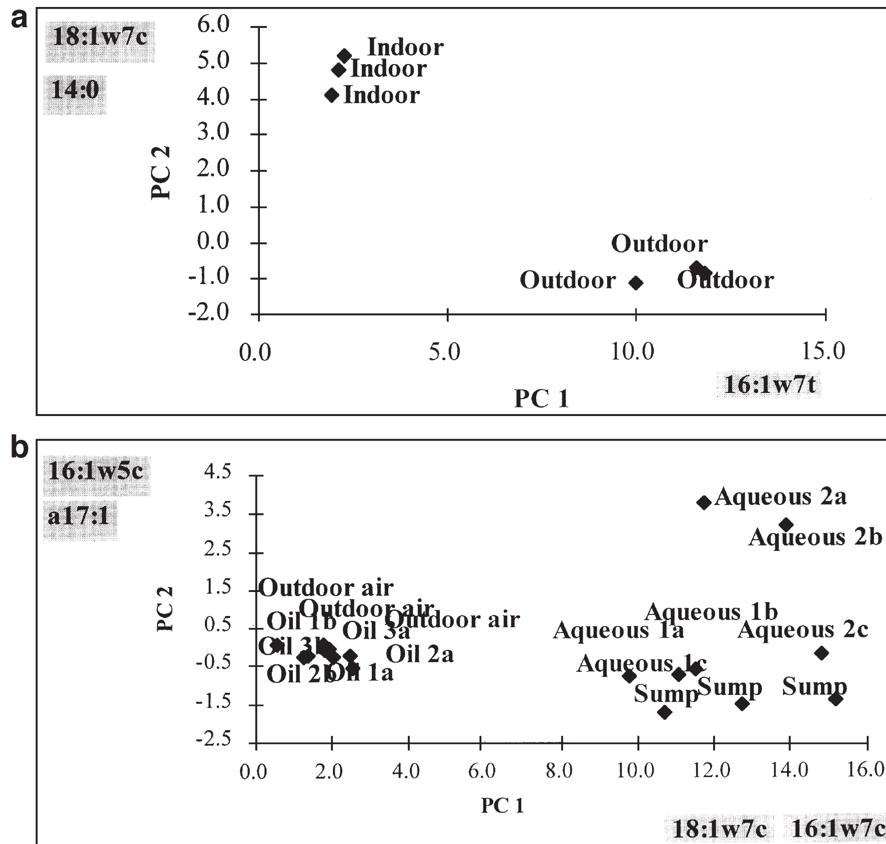

Figure 3 (a) Scatter plot of the PCA from the bacterial PLFA profiles sampled from Site Y. 'Indoor' represents samples from the indoor air, 'outdoor' represents samples taken concurrently from the outdoor air. PLFA representative of eubacterial cells have been removed from the profiles. The major PLFA influencing the PCA are listed adjacent to the *x* and *y* axes. (b) Scatter plot of the PCA from the bacterial PLFA profiles sampled from Site Z. 'Aqueous' represents air sampled near to machine lines containing the aqueous-based fluid; 'Oil' represents air sampled from near to machine lines containing an oil-based fluid; 'Sump' represents PLFA profiles obtained from the aqueous-based fluid reservoirs; 'Outdoor air' represents air concurrently sampled from outdoors. PLFA representative of eubacterial cells have been removed from the profiles. The major PLFA influencing the PCA are listed adjacent to the *x* and *y* axes.

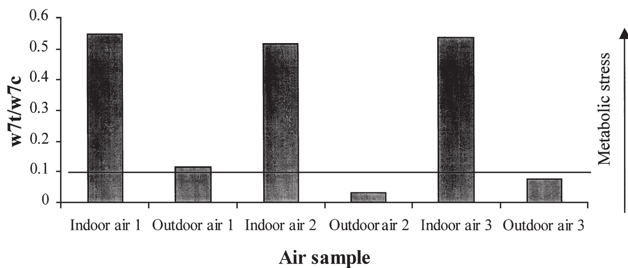

Figure 4 Metabolic status of the indoor and outdoor Gram-negative bacteria at Site Y. Locations 1, 2 and 3 were all within office environments. The dotted line indicates a *trans/cis* ratio of 0.1 and is considered the cutoff for non-stressed *vs* stressed communities.

One disadvantage of PLFA analysis for indoor air is that it does not as yet provide useful data regarding fungal biomass. The phospholipids of eukaryotes tend to be very similar, with fatty acid data frequently too limited for differentiation between fungal species, especially among higher fungi [24]. Moreover, like fungal cells, human cells contain substantial proportions of 16:0, 18:0, 18:1ω9c and 18:2ω6. Of those PLFA also found in substantial proportions in fungi, only 18:3ω3 does not occur in human skin. Therefore, it was impossible to infer any information regarding fungal biomass in indoor air from the PLFA analysis, although the lower proportion of 18:2ω6 and the higher relative proportion of 16:0 and 18:0 in human skin compared to fungi made it easy to distinguish between the predominantly human skin of the indoor air and the microeukaryote biomass of the outdoor air (data not shown). Direct microscopic counting and quantification of ergosterol by GC/MS/HPLC are useful techniques with which to quantify total fungal biomass [29]. However, such analysis was beyond the scope of this study. Moreover, as specific populations of fungi rather than total fungal counts are associated with adverse health effects, fungal culture with identification is still the method of choice [6,27]. For many dry-walled fungi, which have evolved various mechanisms that enhance survival and dispersal in air, culturing remains an accurate assay of biomass [28].

Conclusions

Filtration collection of airborne biomass followed by subsequent quantitative PLFA analysis enabled detection of one to three orders of magnitude more biomass than did the conventional 'viable counting' technique. Using PLFA, a community structure analysis of the indoor air biomass

was possible, which in turn led to successful contamination point source determination at a manufacturing plant. From analysis of the indoor air PLFA data, evidence was detected of Gram-negative bacterial cell wall modification associated with protection against environmental stresses. This information was obtained from single filter analysis. Future research will concentrate on increasing the sensitivity of the lipid analysis through eg HPLC/electrospray/MS which should enable a reduction in sample size requirements making this a viable technique for widespread application.

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