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MOLECULAR IDENTIFICATION OF AIRBORNE BACTERIA THROUGH PROTEIN PROFILING IN DENSE GROUND-LEVEL ATMOSPHERE

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Abstract: Airborne bacteria in bioaerosols can inflict diseases to humans. In the Philippines where air quality in terms of bioaerosol content is poorly addressed, the number and frequency of airborne-related infections could escalate in the upcoming years. This prompted us to seek reliable, cheap and fast technology that could characterize airborne bacteria in dense ground-level bioaerosols.

Although, Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has been used abroad to identify microbes of clinical origin, the applicability of this technology in analyzing airborne bacteria from local atmosphere has yet to be tested. In this seminal study, MALDI-TOF MS has been used to identify airborne bacteria of a ground-level atmosphere. Bioaerosol sampling was conducted at De La Salle University (DLSU) Zaide Canteen using a bioaerosol sampler fitted with petri dishes. MALDI-TOF MS technology, which relies on ribosomal protein profile, was able to identify several bacteria genera such as *Lactobacillus*, *Bacillus*, *Micrococcus*, and *Staphylococcus*. The *Staphylococcus* species were that of *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus saprophyticus* and *Staphylococcus lentus*. We further investigated the protein profiles of *Staphylococcus* genus using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Unlike MALDI-TOF MS, SDS-PAGE depends on the overall protein profile of the cell lysates. Comparison of the protein profiles of *Staphylococcus* isolates derived from MALDI-TOF MS and SDS-PAGE have revealed phylogenetic relationships among these microbial species. In addition, our data suggest that MALDI-TOF MS could be used for local routine monitoring of airborne bacteria in bioaerosols.

Key Words: bioaerosols, airborne bacteria, MALDI-TOF MS, SDS-PAGE*, Staphylococcus*

1. INTRODUCTION

Bioaerosols are air suspensions which contain viable organisms and their by-products (Griffiths and DeCosemo, 1994). Bacteria, fungi viruses, spores and pollen are among the constituents of bioaerosols. Since bioaerosols are airborne in nature, they could be transferred with ease and are a threat to public health. Bioaerosols are also mixtures of different biological

contaminants thus translate to labor-intensive isolation and characterization. In order to reduce the inherent complexity of bioaerosol sampling, we limit our focus on the analysis of indoor airborne bacteria.

Indoor air is a good representation of a dense ground-level atmosphere. It is an ideal starting point for bioaerosol sampling because its atmospheric parameters are controlled, its inorganic components are minimal, and its components are frequently inhaled by humans. Studies have shown that indoor airborne bacteria can inflict a wide range of health effects. For example, some airborne bacteria can cause meningitis (Ilina et al., 2005), tuberculosis (Shigeto, 2012) and severe skin infections (Rocha, 2012). With current concerns on approaching pandemics (Tang et al., 2006), the identification of airborne bacteria in dense ground-level atmosphere is crucial in mitigating future disease transmission.

Traditional methods for bacterial identification involve morphology characterization in tandem with biochemical assays. Unfortunately, these methods are culture-dependent and are prone to misidentification. Recently, genotypic identification method through 16sRNA sequencing was found to be more superior for bacterial identification. However, this method is expensive, laborious and requires expertise. To overcome these drawbacks, MALDI-TOF MS was developed for culture-independent and reliable bacterial identification (Krishnamurthy and Ross, 1996; Maier, 2007). MALDI-TOF MS and 16sRNA sequencing identify the microorganism at the molecular level (i.e nucleic acids, proteins and peptides) and therefore their misidentifications are minimal. To the best of our knowledge, our study is the first effort on identifying airborne bacteria in the Philippines using MALDI-TOF MS.

2. METHODOLOGY

Bioaerosol Sampling

Air samples were collected at the main cafeteria (Zaide College Canteen) of DLSU-Manila using a Staplex® MBS- 6 6 Stage Microbial Air Sampler shown in Figure 1.

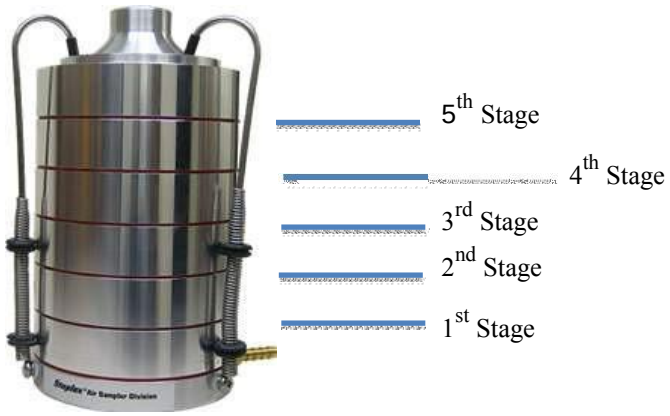


Figure 1. Staplex® MBS-6 Stages

PASPort® anemometer sensor was used to measure temperature, relative humidity, and barometric pressure. Samples were collected for 30 minutes each, every other Wednesdays from January-February 2012. Samples were collected at 12 noon where the canteen is occupied by students, faculty, and non-academic personnel of DLSU (dense ground-level atmosphere).

Table 1. Atmospheric Conditions in DLSU cafeteria

Atmospheric Conditions	**Air Sampling**		
	1	***2***	***3***
Temperature (°C)	31.3	29.5	29.3
Relative Humidity (%)	44.3	60.1	52.0
Wind speed (m/s)	0.0	0.0	0.0

Five Brain Heart Infusion Agar (BHIA) plates were used with the Staplex Stage Air Sampler. The five agar BHIA plates were subsequently incubated at 37 °C for 24-48 hours. Bacterial colonies were then picked and re-streaked several times until pure culture were isolated.

MALDI-TOF MS

All bacteria were analyzed by MALDI TOF MS using a Microflex LT™ instrument, Flexcontrol 3.0 software and the Biotyper 2.0 database (Bruker Daltonics, Bremen, Germany). Extraction of proteins and MALDI target preparation were done by the standard formic acid extraction protocol. Mass spectra within the mass range 2 000- 20 000 m/z were acquired. The identification of bacteria by MALDI TOF MS protein profiling was based on log score. A score of greater than 1.99 secures species identification while a score of indicates 1.70– 1.99 genus identification. No identification will be given if the score is less than 1.7.

SDS-PAGE

Bacterial cell lysate was loaded into the gel and electrophoresis was performed at 140V for 1 hour using SDS Running Buffer 1x. The gel was fixed with methanol-acetic acid-water mixture (5:1:4) and with stained with Coomasie blue cocktail. The gel was subsequently destained for 6 hours with methanol-acetic acid-water mixture (1.5:1:3). The gel images were taken using a Brother scanner (Model: DCP-115C). Finally, the gel images were analyzed using GelAnalyzer 2010 Software with Sigma™ Wide Range Ladder as reference.

3. RESULT AND DISCUSSION

All 64 isolates were successfully identified by MALDI TOF MS. Forty one (41) isolates

were identified up to genus level while 23 isolates were identified up to species level. Although the database of MALDI Biotyper is optimized against medical isolates, these results show the potential of MALDI in identifying environmental isolates. Interestingly, *Staphylococci* are all present in the three sampling (Figure 2). Our result is consistent with previous studies on the microbial composition of indoor air. Independent analyses of indoor air from Europe and from the United States revealed that *Staphylococci* are the most commonly found bacteria in indoor air environments (Gormy et al., 2002; Fox et al., 2010).

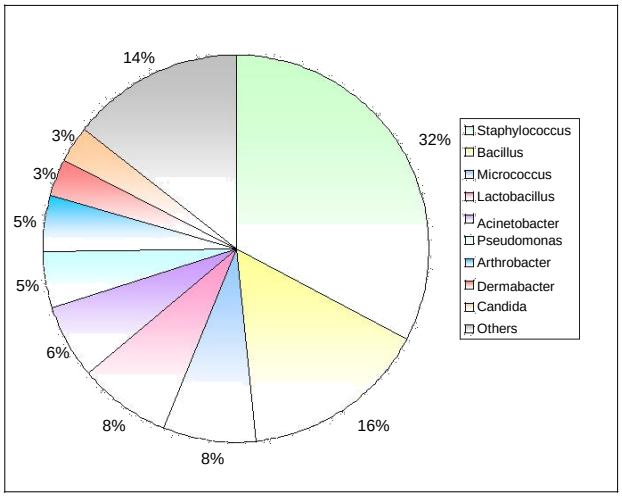


Figure 2. Microbial Genus of 64 isolates derived from three samplings.

The prevalence of *Staphylococci* in three air samplings prompted us to look closer on these airborne bacteria (Table 2). Most *Staphylococci* isolates are typical commensals of human skin and mucosa. *Staphylococci*, however, are also opportunistic pathogens. For example, *Staphylococcus epidermidis* is the most common cause of infections on catheters and implants.

Staphylococcus hominis, *Staphylococcus haemolyticus* and *Staphylococcus lentus* can cause infections on humans whose immune systems are compromised. Among *Staphylococcus* species isolated, *Staphylococcus saprophyticus* may pose potential health risk to humans since it is often implicated in urinary tract infections. Like most *Staphylococcus* species, *Micrococcus luteus* can also be found in human skin and also an opportunistic pathogen. The high occurrences of *Staphylococcus* species and *Micrococcus luteus* at the DLSU cafeteria could be attributed to the presence of skin debris in the bioaerosols. *Bacillus megaterium* are widely used as soil inoculants while some strains *Bacillus cereus* can cause food borne illness. These bacteria can be found on common surfaces that are frequently touched (e.g. tables and chairs). In DLSU cafeteria which is frequented by students and faculty, these common surfaces are easy to envision.

Table 2. Bacterial species in the 64 isolates with log score > 2.

Species		Sampling		
	No. of isolates	*1*	*2*	*3*
Staphylococcus epidermidis	5	+ + +		+ +
Staphylococcus hominis	3		+ + +	
Staphylococcus haemolyticus	2	+		+
Staphylococcus lentus	1			+
Staphylococcus saprophyticus	1		+	
Micrococcus luteus	2		+ +	
Bacillus megaterium	2	+		+
Bacillus cereus	1	+		

MALDI-TOF protein profile of *Staphylococcus* species

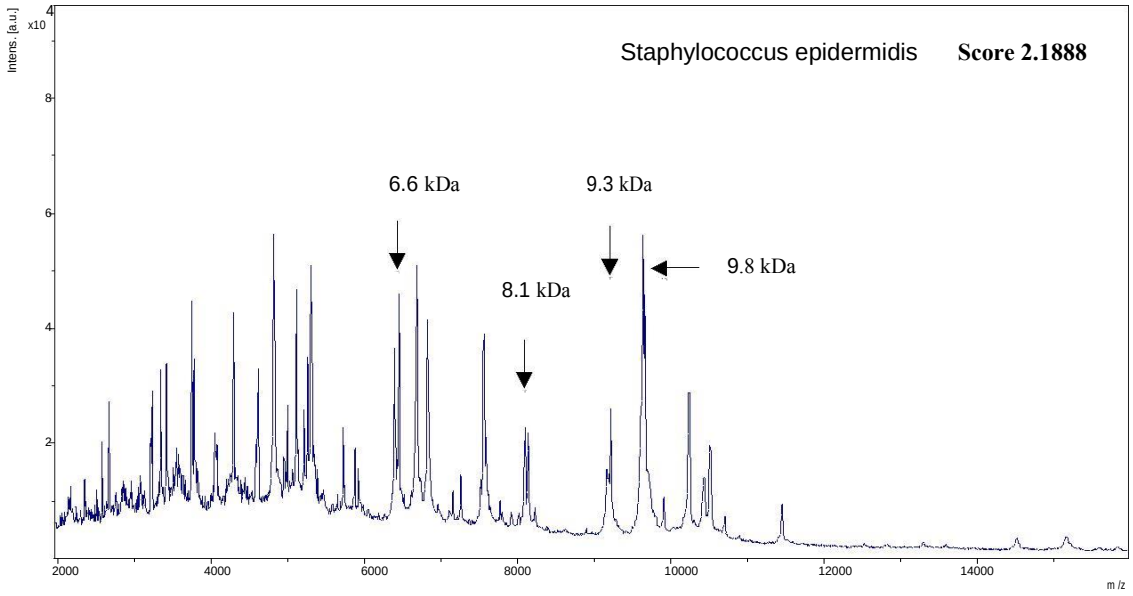
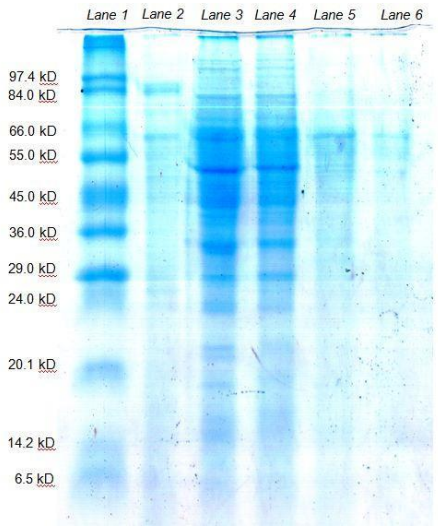


Figure 3. MALDI-TOF MS Spectra of *Staphylococcus epidermidis*.

One of the isolates was identified as *Staphylococcus epidermidis* (Figure 3). We observed four recurring peaks among *Staphylococci*: 6.6 kDa, 8.1 kDa, 9.3 kDa and 9.8 kDa. These four masses are examples of ribosomal proteins which are highly conserved between different species of bacteria. Indeed, the features of ribosomal proteins become the basis for the MALDI-TOF characterization of bacteria.

SDS-PAGE protein profile of *Staphylococcus* species



Lane 1- SigmaMarker™ Wide Range (MW. 6, 500 – 205, 000)
Lane 2- *Staphylococcus epidermidis*
Lane 3- *Staphylococcus haemolyticus*
Lane 4- *Staphylococcus hominis*
Lane 5- *Staphylococcus saphrophyticus*
Lane 5- *Staphylococcus lentus*

Figure 3. Protein bands of bacterial cell lysate.

Despite having distrinct protein profiles, we observed that the *Staphylococcus* species share common protein bands: 62.95-kDa ± 2.00 and 42.18-kDa ± 2.00 (Smith et al., 1991; Modun et al., 1994). These characteristics may be attributed to their common ancestry under the *Staphylococcus* genus. Through mapping a phylogenetic tree, we observed that *Staphylococcus haemolyticus* and *Staphylococcus hominis* show a similarity in the appearance and characteristic of the protein bands. This striking resemblance is presumably due to the high fidelity of ribosomal protein expression. It is also obvious in the context of phylogenetic tree that *Staphylococcus lentus* has branched out from the cluster earlier in evolution. *Staphylococcus lentus* might have altered its protein expression to cope with the pressing demands of evolution

4. CONCLUSIONS

MALDI-TOF MS was robust and efficient in method for molecular identification of bacteria. *Staphylococci*, which are prevalent in dense ground-level atmosphere, were subjected

for SDS PAGE protein profiling. SDS-PAGE protein bands 63.95 kDa (heat-shock protein) and 42.18 kDa (transferring-binding protein) were common among five *Staphylococcus* isolates. Our findings suggest that protein profiling of airborne bacteria through MALDI-TOF MS and SDS-PAGE is effective in mapping phylogenetic relations of *Staphylococcus* species. Indeed, MALDI-TOF MS is an emerging technology for microbial identification. We recommend that bioaerosol sampling should be done in more densely-populated areas (evacuation centers) and in institutions where occupants have compromised immunities (hospitals).

5. ACKNOWLEDGEMENTS

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