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REVIEW PAPER

Toxicoproteomic approaches for analysis of microbial community inhabiting Asian dust particles

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Abstract In this review, we provide an overview of the bio-analytical approaches for proteomic analysis of Asian dust storms. Asian dust has been known to travel with high-speed eastward winds and affects air quality over Japan and even the western coast of the U.S. during severe occurrences. Several reports have shown that Asian dust particles have a negative effect on a wide range of industries and human health activities. Here we give a short overview of Asian dust feature and its proteomic analysis approaches including 2-DE PAGE, metaproteomic analysis of large environmental samples, and automatic techniques for dust microbial analysis. Following this, we discuss the detection system of Asian dust particles that can be integrated with biosensor platform.

Keywords Asian dust particles, Toxicoproteomics, 2-DE PAGE, Metaproteomic analysis, Biosensors

The "Asian dust" which generally originate from the desert regions of western or central China and Mongolia every year in the spring season moves to the eastern part of China, over the Yellow Sea to Korea, Japan, and in some cases even upto Pacific Ocean[1-3] and adversely affects the neighboring countries in many ways. Asian dust storms introduce a large amount of dust particles into the atmosphere which contributes to the large aerosol load globally. Jeong *et al*.[4] suggested that giant particles (>10 μm) can be transported over long distance that may play important roles in regional circulation of materials. These dust particles can also affect the radiative balance[5] as well as the presence of ice and cloud condensation nuclei present in the atmosphere[6,7]. A recent Asian dust study shows that the dust particles can significantly reduce the concentration of ozone during Asian dust period. Particularly, since Asian dust particles reaches Korea after passing over highly-industrialized areas in China[8], it might also include various aerosols, microorganism and hazardous air pollutants (HAPs) such as heavy metals and other chemicals (Figure 1)[9-14].

Asian dust not only is responsible for the environment such as climate change and desertification, but also it poses a major threat to human health. The mineral dust particles can provide reactive surfaces for heterogeneous reactions with trace gases in the atmosphere and result in a change in the atmospheric chemical balance and photochemical cycle due to the chemical modification of the dust particles[11,15]. A change in the physicochemical properties of dust particles can also alter their optical properties by modifying their light scattering and absorption properties[16,17]. The dust particles can also affect the human health which can be estimated from the increase in admissions to hospitals and visits to clinics for a wide range of allergy related and chronic respiratory problems[18,19]. The adverse effect of dust storms on health has been studied experimentally through studies on animals and biomarkers[18,20-22]. Some studies conducted on the relations between dust storms and morbidity or mortality has shown positive associations but the results are not consistent.

The Asian desert dust is also responsible for the eastward transport of microbial fractions and pollen to neighboring countries Korea and Japan. Dust particles can carry a large amount of microorganisms, such as bacteria, fungi and many viruses like particles. Accord-

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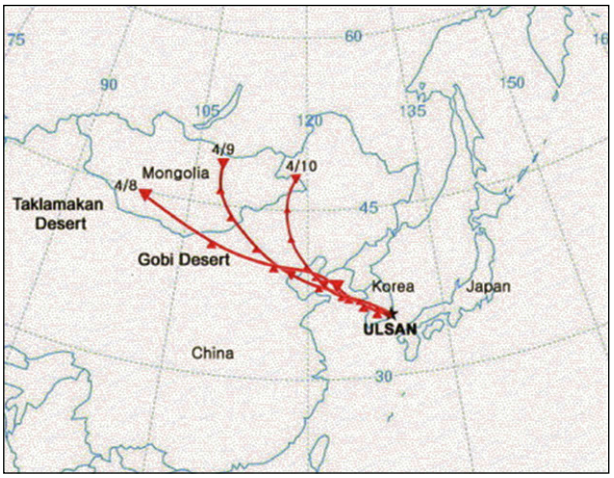

Figure 1. Transport paths of the Asian dusts identified by an analysis of a two-day backward isentropic air trajectory from Ulsan, Korea. Chemosphere, 2006, 63, 1106-1115, Copyright (2006), with permission from Elservier[8].

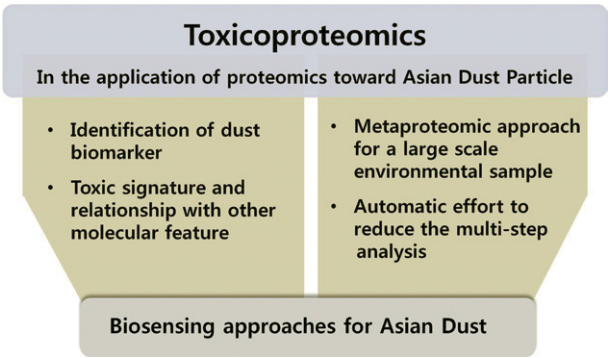

Figure 2. Toxicoproteomic approaches for Asian Dust particles.

ing to a rough estimate, 10^4 bacteria per gram of soil and 1 million tons of airborne soil moving around the atmosphere each year amounts to 10^{16} dustborne bacteria and these have been found to survive transport in air through large distances[23]. From the results of culture-based or molecular techniques used conventionally for detecting DNA sequences it has been observed that bacteria and fungi present in the Asian dust are the main causes of such diseases such as asthma (due to allergens) or various other illnesses[24-27]. Although this approach is still restricted to a small number of studies, yet it is clear that proteomic approach has wide potential in the field of environmental microbiology by focusing on limited number of highly expressed proteins[6,28,29]. Moreover, although various techniques are readily available for the analysis of microbial consortia, the diversity of its composition and other microbial ecosystems still poses challenges to many researches. It is therefore important to assess the biological risk and potential toxicity of Asian dust particles.

Here we give a short overview of Asian dust and its toxic components for human activity. The current achievements in Asian dust research through the proteomic approaches including 2-DE PAGE, Metaproteomic analysis of large environmental samples, and Automatic analysis for dust microbial analysis are discussed. Following this, we review bioanalytic approaches for Asian dust particle that can be integrated with biosensing platform.

Toxicoproteomic analysis of Asian dust particles

The metagenomic techniques provide a deep understanding of the functional dimensions of environmental genomic datasets and may help to link individual microbial species to their complex functions. However, the metagenomics approaches related with the environment have been limited to the correlation between microbial communities and Asian dust components[28]. The toxicoproteomic analyses can detect physiological toxic-responses to changes in various environmental conditions (Figure 2). The generation of de novo peptide sequences has resulted in proteomic studies being more specific to protein identity and phylogenetic origin. Proteomics has great potential for the functional analysis of microbial communities with dust particles as protein expression is a reflection of the specific microbial activities in a given ecosystem.

2D-electrophoresis (2-DE) and identification of protein

Proteomics is the analysis of gene expression at the protein level. One of the most common methods for quantitative proteome analysis is the protein separation by two-dimensional gel, and this separation is followed by in-gel digestion of selected spots and protein identification by mass spectrometry (MS). The identification of proteins from a fully sequenced organism is obtained by the data comparison of peptide mass fingerprinting (PMF), matrix assisted laser desorption ionization time of flight MS (MALDI-ToF-MS), fragmentation data of the peptides (electrospray ionization source tandem mass spectrometry (ESI-MS/MS)) or MALDI-ToF/ToF-MS). Identification of peptides from unsequenced species can be achieved in two ways, either the peptides or their MS/MS fragments resemble those of known proteins, or the peptides sequenced by MS exhibit sufficient homology to the already published sequences. An essential requirement for this technique is obtaining high quality MS data which require

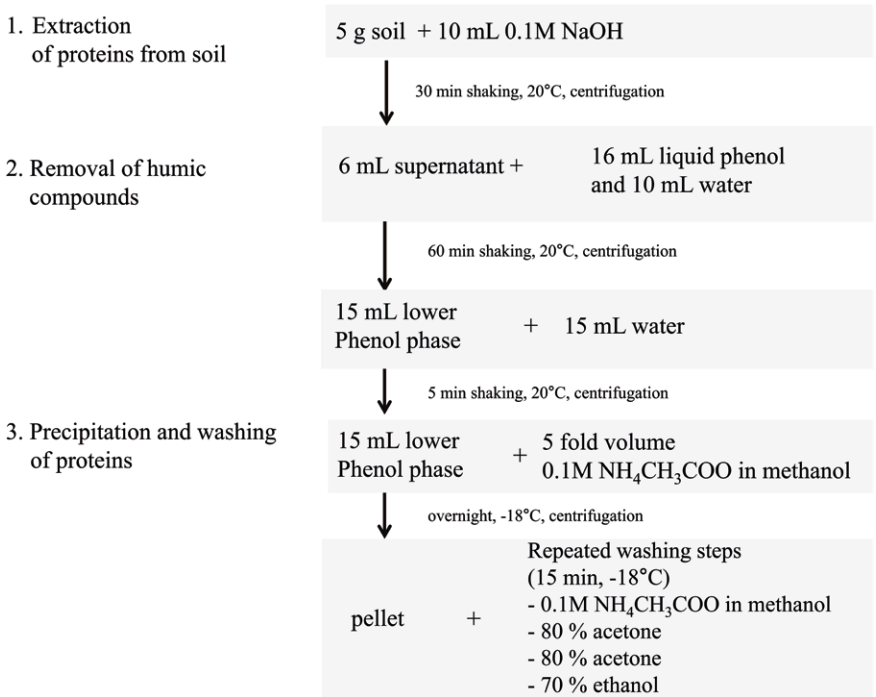

Figure 3. Scheme for extraction and purification of proteins from soil. Reproduced from Ref. 28 with permission from The Nature publishing group.

pure samples containing enough copies of every protein. Proper sample treatment is must to obtain optimum high resolution separation for the detection of maximum number of spots, avoiding presence of multiple proteins per spot and cross contamination of spots on 2-D gel. Proteomics was restricted mostly to microbial isolates in culture mainly due to this reason. Its application to soil requires considerably improved protocols of protein extraction and sample preparation since these are very critical for obtaining high resolution and sensitivity of 2-DE. The extraction of water-soluble proteins from soil has been proposed recently[28] (see Figure 3) where MS coupled to liquid chromatography was used to identify proteins extracted form minerals dissolved by organic solvents. Methods based on freeze-thawing or bead beating of soil for extracting intra- or extracellular proteins have been also proposed[30]. The greatest difficulty lies in the separation of proteins from humic acids. Groundwater is another environmental medium of importance due to the increasing need for high quality drinking water. Until now, metaproteome data has been reported from different environmental systems: soil particle[31], activated sludge[32], biofilms[33] and seawater[34] and in addition metaproteomics may be used for indication of biodegradation processes.

The proteomic approach based on 2D-PAGE has a clear advantage as protein spot intensities and sizes on 2D gels reflect the expression levels more accurately than the abundant data obtained from MS. 2-D electrophoresis is most common and convenient approach that can reveal thousands of protein at a time. The diversity of microbial ecosystems still poses enormous challenges, although the number of proteins identified and separated using available proteomic analysis methods has increased.

Metaproteomic analysis of large environmental samples

The proteomic approach to obtain functional components of microbial ecosystems has huge potential in environmental microbiology. The proteins exist in many different biological and physical conformations and there is no universal extraction method available. Schulze *et al*.[31] used mass spectrometry (MS)-based proteomics to study the protein complement of water that containing high levels of dissolved organic matter. The samples from four different environments (a peat bog lake, soil from an unmanaged deciduous forest, soil from a managed evergreen spruce forest and acidic soil from beneath a spruce) were collected, phylogenetic analysis and the potential catalytic function of these proteins in the sampled ecosystems was deter-

mined. The majority of proteins in the soil protein composition were of bacterial origin, and the proteins originating from plants, fungi and vertebrates were approximately twice the number obtained in the sampled lake water. A comparison of detected enzymes with known functions showed that the proteins from the lake and the forest are different. This study though detected only a small fraction of the proteins present in the sampled environments, yet it reflects the presence and activity of different taxonomic groups and demonstrates the potential of proteomic fingerprints by indicating that the overall changes in ecosystem biology are not limited to archaeal and bacterial constituents. Metaproteomic investigations of environmental samples will be very helpful when coupled with information about the diversity of species and richness within the ecosystem. It shows the large potential of environmental fingerprinting approaches based on either DNA[35] or protein[31,36] for the determination of functional details that are crucial to investigations of microbial assemblages although the application of metaproteomics to more-complex microbial communities presents a considerable challenge. The power of using multidimensional protein separation systems followed by MS for environmental proteomics has been highlighted[33]. The extraction of proteins from complex environments such as seawater and soil is very challenging[37]. Another important aspect is the activity of isolated proteins in dust samples.

Automated proteomic analysis using ProteomeLab PF2D platform

Obtaining a high level of reproducible fractionation of the protein samples is considered one of the main challenges in proteomics. Automated two-dimensional liquid phase fractionation (PF2D) system (Beckman Coulter) provides a fairly reliable process for proteome studies. However, the protein recovery efficiency of such system for metaproteome profiling of environmental samples is quite low. An alternative method that can overcome existing limitations is replacing the manufacturer's buffer with Triton X-100 in different concentrations during the PF2D evaluation. Varying the Triton X-100 concentration affects the protein recovery, 0.15% Triton X-100 concentration results in almost tenfold increase in the protein recovery without having adverse effects on samples. This novel use of 0.15% Triton X-100 for PF2D can lead to greater research possibilities in the field of proteomics.

The development of analytical tools for rapid analysis and identification of expressed protein profiles in cell, tissue or organism is currently an important area in biological research[34,38-40]. Although two-dimensional gel electrophoresis (2DE) is a powerful, sensitive, and mature, doubts remain concerning its ability to characterize all the elements of a proteome, particularly proteins of extreme mass (e.g., >200 kDa or <10 kDa) or *pI* values[41,42]. In addition, 2DE is not readily responsive to automation. Liquid-phase separation methods such as size-exclusion chromatography, affinity chromatography, and ion-exchange chromatography exhibit practical difficulties due to the lack of the isoelectric (*pI*) information and limited labelling efficiency[43-46]. Alternatively, the ProteomeLab PF2D platform (Beckman Coulter, USA) used for the quantitative comparisons and separation/fractionation of various biological and clinical samples, works in full automation combining chromatofocusing separation and hydrophobic fractionation[47]. During the first-dimension chromatofocusing of PF2D, proteins are first separated by their *pI* and then separated proteins with a pH gradient are collected using a fraction collector[48,49]. Subsequently, in the second dimension, the collected fractions from the first dimension are separated using reversed phase chromatography which works on the basis of hydrophobicity[49]. Separated fractions are then monitored with UV detection to observe changes in the proteome[50,51]. The selected peak is identified by mass spectrometry. Although PF2D system offers high loading capacity and improved detection limit with lower abundance proteins[45,52], variations are required in standard protocol as the protein recovery efficiency is low during the chromatofocusing step using the standard protocol. Sheng *et al*.[53] reported increase in the number of proteins identified in the serum with the addition of 20% isopropanol in the isoelectric focusing (IEF) buffer.

Perspectives

Proteomic approaches for the Asian dust can be used (a) for large scale microbial proteome profiling, (b) for comparative 2-DE analysis of two or more dust samples in different area, (c) for the identification of dust-biomarker, and (d) for the study of microbial interactions with dust particles in automatic operation system. However, the major limitation of proteomic analysis remains a very challenging task due to the complexity of the dust sample, the wide dynamic range of microbial protein concentrations and requirement of multistep processes. Biosensing approach, therefore, can be injectable to dust proteomic analysis.

Several efforts have been made to develop the sensing platforms which couple bioparameters for detecting microorganisms, heavy metals, allergens and harmful chemicals[54]. The comprehensive discussion on sensing mechanisms for biotargets such as phathogens

is provided in recently published papers. For example, pathogenic bacterial and fungal spores represent a great threat to safety through contamination of soil, water and other environment. The recent reports have shown that Asian dust particle include lipopolysaccharides and β-glucan, which are components of the bacterial cell membrane and fungal cell wall, respectively[55]. In addition, these pathogens can adhere to the outer surface of the dust particles that can stimulate the biological immune-responses in human body. The pathogenic bacteria such as *Bacillus cereus*, *Bacillus anthracis*, *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Shigella* spp., *Staphylococcus aureus*, and *Staphylococcal enteritis* are dominantly detectable within 10^3 to 10^5 cell/m^3 in Asian dust subsided, which are able to infect and harm humans[56].

A large number of antibody based biosensing methods were examined in "Affinity" biosensors with excellent sensitivity and selectivity for pathogenic bacteria and other harmful elements. These biosensors basically rely on the antibody-antigen interactions in direct and indirect methods of detection. The field of biosensors is exponentially expanding by using the novel bioreactive materials. Aptamers as affinity biorecognition factors have been applied to pathogen sensing devices. According to the stability test, apamers were chemically stable and applicable to the variety of environmental complex samples. In addition, it should be pointed out that aptamer can be actively selected against whole-bacterial cells by cell-SELEX process. Aptamer based biosensor has been significantly advanced as a nanomaterial that enable to detect a wide variety of pathogens in portable devices[57]. "Reactive" biosensors which include the interaction between the target and enzyme active site have been also developed for several different targets. Reactivity between the dust particles and enzyme can be found on the surface of solid supports and in solution, producing a color change and fluorescent aggregates *etc*[58]. These approaches are providing a wide range of applications that enable increased detection performance of Asian dust particles. A good example expected properties of an affinity biosensor is narrated from Tsci *et al*.[59] who indicated that efficiency, accuracy and high sensitive performance for dust mite allergen detection are some of the advantages conferred by the use of biosensors over other type of biomonitoring. Recently Lee *et al*.[60] have investigated aptamer biosensor for whole live bacteria, allowing for a "quantitative" detection using a specif-

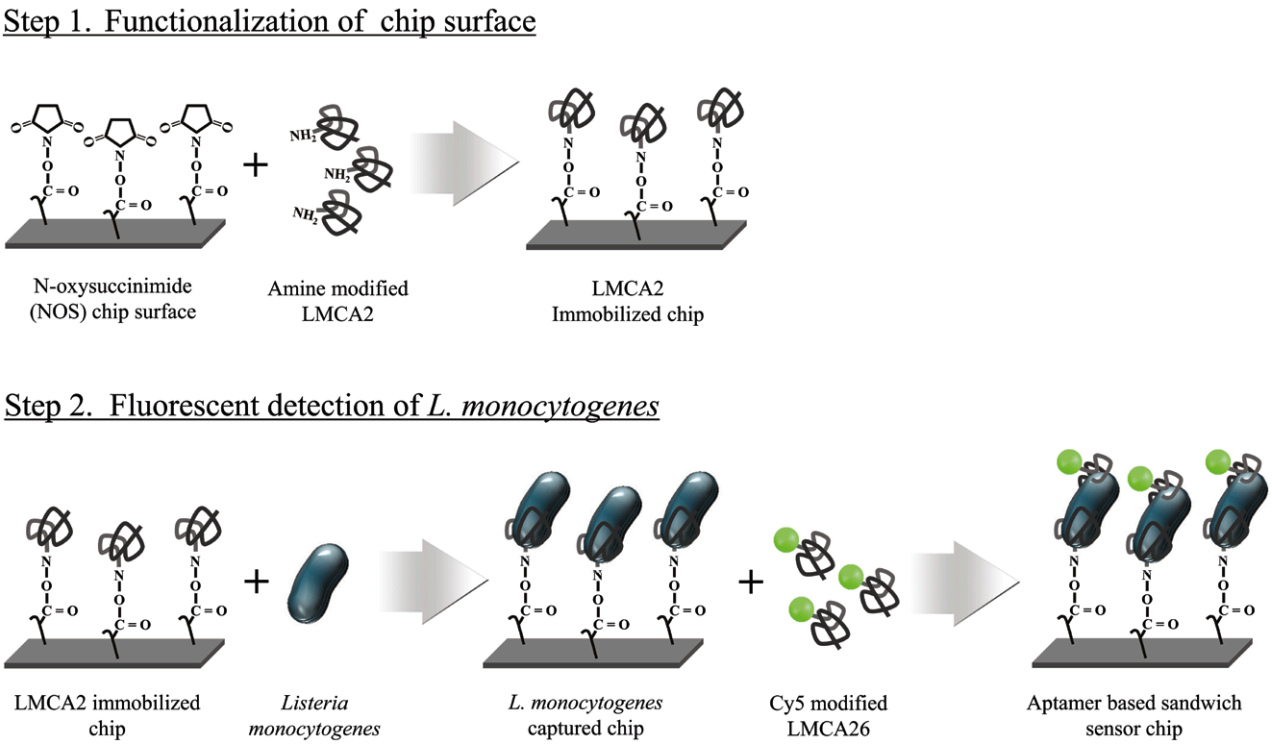

Figure 4. Illustrations of the aptamer-based sandwich assay (ABSA). Sandwich binding activity to *L. monocytogenes*. Fluorescent intensityfrom the ABSA platform was plotted with three controls (signals of plate surface, signals without capture aptamer in sandwichcomplex, signals without target cells in sandwich complex). Reprinted with permission from Elservier[60].

ic aptamer-sandwich mode. High degree of aptamer binding was found to *Listeria monocytogenes* cells, enhancing the capability to use in dust particle analysis (Figure 4).

Despite the limited number of environmental proteomic investigations, proteomic approaches have huge potential in the field of environmental microbiology and the development of biosensing platform. The availability of images from various satellites have also improved our understanding of the dust particles present in the atmosphere at the global level. These bioanalytic approaches can be further used to monitor the formation and movement of dust storms. This will help in understanding the harmful effect of soil-associated toxins and pathogenic microorganisms present in dust storms on the ocean and human health.

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Conflict of Interest The authors declare no conflict of interest.

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