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Short communication

TiO_2 nanoparticle assisted mass spectrometry as biosensor of *Staphylococcus aureus*, key pathogen in nosocomial infections from air, skin surface and human nasal passage

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ABSTRACT

For the first time, we have demonstrated the use of mass spectrometry as a biosensor for detecting a clinically important bacterium: *Staphylococcus aureus* in air, nasal passage and skin samples using culture-free, rapid, direct analysis via TiO_2 nanoparticles (NPs) assisted MALDI-MS. When this bacterium is predominating, the nasal passage of an individual is observed to lead to wound infections especially when the individual has a surgery or some wounds. This study indicates that even at very low concentrations of an individual bacterium can be directly detected from a mixture of bacteria using the MALDI-MS analysis without the requirement of any culturing steps or any other sample pretreatment techniques. The current approach is extremely simple, rapid, straightforward and sensitive which could be widely applied for the detection of this deadly pathogen in clinical as well as environmental samples.

1. Introduction

Staphylococcus aureus (*S. aureus* or "*Staph*") has long been recognized as one of the most important bacteria that can cause severe morbidity and often rapidly fatal infections in humans. It is the main cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis. Although most Staph infections are not serious, *S. aureus* can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infections (Mulligen et al., 1993; Waldvogel, 1995). This organism is often resistant to multiple antimicrobial agents, including β-lactams, clindamycin, erythromycin, sulfonamides, tetracycline, chloramphenicol, macrolides, aminoglycosides and quinolones. The incidence of *Staphylococcous aureus* related infections has increased dramatically since the emergence of methicillin resistant strains and high rates of mortality and morbidity are occurring world-wide (Oliveira et al., 2002). Rapid differentiation of MRSA from methicillin-sensitive *S. aureus* (MSSA) is essential for appropriate therapy and timely intervention for cross infection control. Isolates sent to diagnostic laboratories have long been identified by traditional biochemical tests and then subtyped by a variety of methods (Ip et al., 2003; Fang and Hedin, 2003; Jury et al., 2006,). However, criteria for the identification of species are still equivocal. Some strains have been misidentified with closely related species. Given the clinical importance of nosocomial infections, failure to unequivocally identify between species can have far reaching consequences clinically and epidemiologically.

Most current techniques for the epidemiological identification of microorganisms are laborious and time consuming. Currently, the most popular methods for bacterial identification are based on microbiological procedures, antibody recognition, and PCR amplification. Traditionally, microbiological methods are culture-based assays that examine the presence of bacterial species. These methods provide high sensitivity and specificity, but their efficiency is limited by the complexity of the procedures, including culture, selection, isolation, and morphologic and biochemical characterization, which usually take 48 h or longer. Serologic methods are presumptive and confined to the availability of antibodies and to bacteria that are included ahead in the assays. Molecular biology techniques, particularly PCR, have been regarded as non-culture based methods with high efficiency and specificity (Dworzanski and Snyder, 2005). However, they are completely dependent on the known genetic sequences of the target bacteria. Molecular subtyping methods, e.g., pulsed field gel electrophoresis (PFGE), provide better discrimination than the traditional phage-typing technique, but PFGE takes several days to be performed. Mass spectrometry (MS) with its capability of de novo protein/peptide

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sequencing (such as electrospray ionization or matrix assisted laser desorption/ionization (MALDI)MS for Tandem MS) or its high efficiency for proteome profiling (particularly MALDI-TOF MS) has been suggested as an alternative for microbial identification (Pershing et al., 2003; Holland et al., 1996; Krishnamurthy and Ross, 1996). Therefore, rapid, identification of this bacterium using MALDI MS can be an important application in the diagnosis of infections caused by this bacterium, which would help in early detection and control. Early reports involved solvent extraction and chemical modification before analysis, but some methods use simple specimen preparation and direct analysis via appropriate ionization methods. Recent studies have indicated the possibility for direct analysis of bacteria using MALDI-MS, by picking up bacteria grown in agar plates and placing them on MALDI target plates for analysis (Lakshani et al., 2009; Katussevani et al., 2002).

Usually as described above, samples collected from various sources are grown on agar plates and after 48 h the colonies are spotted on target plates and analyzed; so far this was what is being called as 'direct analysis' of bacteria using MALDI-MS. The present work takes the realm of MALDI-MS based direct analysis and identification of bacteria to a higher dimension, in that we report the direct analysis of *S. aureus* from samples collected from nasal passage without preliminary culturing. This technique would give real meaning to rapid analysis using MALDI; since samples are taken as such and after a simple two-step preparation procedure, are directly used for MALDI-MS analysis assisted by TiO_2 nanoparticles (NPs). Additional experiments were also conducted to confirm the presence of *S. aureus* using direct analysis technique as reported in earlier papers. This protocol involved isolating this bacterium from air, human skin surface and nasal passage and growing the bacteria in agar plates and then analyzing the individual colonies using MALDI-MS. Moreover, this paper also reports the sensitivity of TiO_2 assisted MALDI–MS to selectively detect/distinguish a particular bacterial species (in this case *S. aureus*) from a mixture of unknown bacteria collected from air, skin and the nasal passage.

2. Experimental

2.1. Requisites

All the glassware and media used for the studies were autoclaved at 15 lb pressure for 15 min. All aqueous solutions were prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All the chemicals used were of analytical grade.

2.2. Procedure for direct analysis

The bacterial swabs were collected from forehand surface and nares of a female human volunteer. The air sample was collected by exposing a swab wetted in sterile water which was exposed to air for 2 h. Using sterile cotton, swabs were collected from nares and forearm skin surface of the volunteer. The respective swabs were washed into 200 μL of sterile water. These samples were vortexed (VM 2000, Digi System Laboratory, Taipei, Taiwan) for 5 min and spotted for direct analysis on the MALDI target plates. Each spot was overlaid with 5 μL of matrix (3, 5-dimethoxy-4-hydroxycinnamic acid 0.05 M in acetonitrile: water (3:1, v/v) containing 0.1% TFA) and air dried; all experiments were performed in triplicates.

2.3. TiO_2 synthesis and characterization methods

Indigenously prepared TiO_2 NPs following standard protocols reported in literature (Qourzal et al., 2006), were used to enhance the bacterial signals in the real samples. The freshly synthesized TiO_2 NPs were calcined in a Thermolyne furnace (model FB 1315 M, Thermo Scientific, Dubuque, IA, USA) for 2 h at 400 °C to obtain anatase form of TiO_2. The TiO_2 nanoparticles were characterized using HR-TEM (TEM-3010, JEOL, Tokyo, Japan) to confirm the particle size and shape. The UV–vis spectrum was acquired from the UV–vis spectrophotometer (U3501, Hitachi, Tokyo, Japan). X-ray diffraction (XRD) was used to confirm the presence of anatase form of TiO_2 NPs.

We had recently evaluated the threshold concentration of these indigenously prepared TiO_2 nanoparticles (NPs) for bacterial analysis with respect to *S. aureus* (unpublished data). 1 mg/mL concentration of TiO_2 was observed to work well for enhancement of *S. aureus* signals. All the experiments conducted in this paper used 1 mg/mL of concentration for assisting the real sample analysis. 5 μL TiO_2 (1 mg/mL) was mixed with 5 μL of the real sample and incubated at room temperature for 1 h. 10 μL of SA matrix was added to this and hand incubated (by shaking back and forth) and 5 μL of this sample solution was spotted on the target plates for MALDI analysis.

2.4. Procedure for bacteria analysis after culture

100 μL of the swab samples from air, skin and nose were suspended in 200 μL sterile water and plated on sterile Luria Bertani agar plates. After an incubation period of 48 h at 37 °C (Firstek, Orbital Shaking incubator, Firstek Scientific Co., Ltd.), the colonies developed were counted to obtain the total viable count (TVC) which is the standard method to quantify bacteria in an unknown sample. The bacterial count is expressed as cfu/mL. These colonies were then scraped up into 500 μL sterile water, vortexed and then spotted (5 μL) on the MALDI plates. The MALDI-MS mass spectra of a standard *S. aureus* subsp. aureus BCRC 1045, purchased from the culture collection of the Bioresource collection and research center (BCRC), Hsin-Chu, Taiwan was used as the standards to identify this bacterium in the unknown samples. Fig. S1(a) of supporting information displays the schematic representation of the methodology applied in this study.

2.5. MALDI-MS analysis

All mass spectra were obtained in the positive ion mode using a MALDI-TOF MS (Microflex, Bruker Daltonics, Bremen, Germany). The MALDI source was equipped with a nitrogen laser (337 nm) for irradiation of analyte and the accelerating voltage was obtained at +20 kV. All experiments were performed in the linear mode with 200 laser shots at the laser energy of 63.2 μJ.

3. Results and discussion

3.1. Isolation and direct analysis of bacteria

Fig. S1(b) shows the yellow colonies predominating the plate grown from nasal swab. Although similar yellow colonies were also obtained from air and skin swabs, maximum colonies were observed from nasal samples compared to those from the surfaces of the skin and air. This is understandable because compared to the air and skin surfaces which are dry environments, the nares are moist and serve as better environments for bacterial colonization. Supporting Fig. S2(a–c) gives the mass spectra obtained from direct analysis of the air, skin and nasal passage. As can be observed from these spectra, it is evident that the bacterial concentration was below the MALDI-MS detection limit and so very less peaks in the case of the nasal samples (FigS2(b)) were observed; in the case of the air and skin samples, no signals were detected (FigS2(a and b)). The total bacteria in these samples were enumerated by the plate counting method. The bacterial count in the air sample was 9×10^2 cfu/mL, in skin swab; 7×10^2 cfu/mL and nasal