Biosensors and Bioelectronics 35 (2012) 235–242

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Biofunctionalization of nanoparticle assisted mass spectrometry as biosensors for rapid detection of plant associated bacteria

Faheem Ahmad [a], Mansoor A. Siddiqui [b], Olubukola O. Babalola [a], Hui-Fen Wu [c,d,e,*]

[a] Department of Biological Sciences, Faculty of Agriculture, Science and Technology, North-West University, Mafikeng Campus, Private Bag X2046, Mmabatho 2735, South Africa
[b] Department of Botany, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India
[c] Department of Chemistry, National Sun Yat-Sen University, Kaohsiung, 70, Lien-Hai Road, Kaohsiung, 80424, Taiwan
[d] Center for Nanoscience and Nanotechnology, National Sun Yat-Sen University, 70, Lien-Hai Road, Kaohsiung, 80424, Taiwan
[e] Doctoral Degree Program in Marine Biotechnology, National Sun Yat - Sen University, Kaohsiung, 80424, Taiwan

ARTICLE INFO

Article history:
Received 25 January 2012
Accepted 26 February 2012
Available online 3 March 2012

Keywords:
Rhizospheric soil
Root
PAB
Nanoparticle
MALDI-TOF MS
Detection

ABSTRACT

This study is based on the application of matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) as biosensor to detect the plant associated bacteria (PAB) isolates from rhizospheric soil and root. The rapid bacterial detection via on particle ionization/enrichment technique using IgG functionalized Pt NPs (IgG-Pt NPs) assisted MALDI-TOF MS was successfully used to explore two PAB isolates, namely, *Bacillus thuringiensis* and *B. subtilis* from rhizospheric soil and roots of carrot plant. When these bacteria are used as bioformulations in agricultural as well as biotechnological applications, the plant growth promotion of economic crops was observed especially when the crops grow in less fertilize soil regions. This study proved that even at low concentrations, bacteria can also be directly detected without morphological, molecular and biochemical test. The current applied technique is simple, rapid and highly sensitive. Besides, it could be widely used for the detection of beneficially important PAB isolates in environmental samples.

1. Introduction

Microorganisms represent the largest reservoir of biodiversity yet to be described, and consequently offer great potential for the discovery of new natural products. It is estimated that Earth sustains between 3 and 30 million species of organisms. Of these, approximately 1.4 million species have been described by science, including virtually all the bird and mammal species. In contrast, only close to 200,000 of the possible 1.0–1.5 million species of fungi have been characterized. This percentage is even lower for bacteria, with estimates that between 1 and 10% of the probable species have been described (Bull et al., 2000; Babalola et al., 2009). Among the about 7100 classified bacterial species, much less species is reported to promote plant growth promotions by more or less specialized mechanisms (Babalola, 2010). Due to the above fact, the interest in biotechnological and agricultural field have increased recently, fuelled by public concerns, and need to find eco-friendly bacterial strains for plant growth promotion to gain high crops yield. Plant associated bacteria (PAB) may benefit plant performance by inducing systemic defense responses that confer broad-spectrum resistance to plant pathogens and even insect herbivores. Plant growth activity has been reported in strains belonging to several genera, such as *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Burkholderia* and *Bacillus* (Parka et al., 2005; Babalola et al., 2007; Babalola, 2010; Babalola and Akindolire, 2011) for enhancing the growth productivity of plants. Some beneficial microbe-associated molecular patterns (MAMPs) are well recognized in plant system by Wees et al. (2008). Irrespective of mode of action, a key feature of PAB is that an effective biological control strain isolated from one region may not perform in the same way in other soil and climatic conditions (Duffy et al., 1997; Johnsson et al., 1998). Identification of native strains adapted to the environment and their study may contribute to the formulation of inoculants to be used in region crops. Rapid identification is essential for appropriate plant growth promotion and timely invention for effective strains in the environment. 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.

Most current techniques for the identification of microorganisms are laborious as well as time consuming. Currently, the most popular techniques for bacterial identification are based on microbiological procedures, antibody recognition, and PCR amplification followed by sequencing, hybridization, pyrosequencing or single-stranded conformation polymorphism. These methods provide high sensitivity and specificity, but their efficiency is limited by the

* Corresponding author. Tel.: +886 7 5252000x3955; fax: +886 7 5253908.
E-mail address: hwu@faculty.nsysu.edu (H.-F. Wu).

0956-5663/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2012.02.055

complexity of the procedures, including culture, selection, isolation, morphologic and biochemical characterization, which usually take 2–3 days or longer (Wei et al., 2008). 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 (Jock and Geider, 2004). Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) allows rapid identification of bacteria grown on solid medium, by its high efficiency for proteome profiling from isolated colonies (Pershing et al., 2003; Dare, 2006; Seng et al., 2009). The importance of biosensor as innovative tools has also been well described by Arora et al. (2011). The adaptation of this technology to the identification of PAB isolates grown in biological media would provide immediate species identification. The advantages of this technique, in addition to its rapidity, are the moderate cost and the ease of implementation. The challenge in analyzing the whole bacterial cells lies in the need to improve the sensitivity of the MALDI-MS technique for bacterial detection. This brought the idea of nanoparticle based affinity probes for enhanced detection of bacteria. Unlike bulk materials, nanomaterials offer a tremendous potential due to their large surface area, high surface activity, and strong adsorption ability (Cui et al., 2001; Feldheim, 2007; Chen et al., 2008a). Nanomaterials are the nucleus for the design of the detection devices and they are of great aid to improve the detection limit. Chiang et al. (2010) investigated six nanomaterials (Au NPs, TiO_2 NPs, Se NPs, CdTe QDs, Fe_3O_4 NPs, and Pt NPs) for their applicability as surfaces for MS of small analytes, peptides, and proteins. Among these nanomaterials, a platinum nanoparticle (Pt NPs) was the most efficient nanomaterials for proteins, with an upper detectable mass limit of 25 kDa. In addition, platinum nanoparticles are of great interest due to their exceptional catalytic activity, which is affected by the ligands at their external surface. Pt NPs may display multiple functional groups at the surface, which can be hydrophilic, lipophilic, and chemically reactive (Nel et al., 2006). Pan et al. (2007) observed that particles of 1–2 nm were highly toxic and that nanoparticles larger than 15 nm were not toxic. Metal NPs with large diameter exhibit strong light-scattering in the visible region, which could be used directly for light-scattering labels in biochemical assay. However, small NPs, which apparently do not feature light-scattering, can also be used to sense chemical interactions (e.g., antigen–antibody, avidin-biotin, DNA hybridization, and electrostatic attraction), since enhanced light-scattering signals would be produced if these NPs were to aggregate during the interactions. Because the enhanced light-scattering signals from the aggregated species are sufficiently sensitive to monitor NP aggregation, in a simple procedure, biochemical assay based on such light-scattering signals has been widely used in the determination of DNA, proteins, and drugs (Ling et al., 2009). Immunoassay is important in basic research and clinical diagnostics. Sandwich-type immunoassay, using a primary antibody to capture the analyte, and a labeled, secondary antibody to detect antigen binding, is widely accepted procedure. The aggregation of nanomaterials, especially platinum (Pt), induced by the immunoreactions offers a new approach for immunoassay, using light-scattering detection to obtain high sensitivity (Lina et al., 2010). The study reported a potentially rapid and effective immunoassay using antibody-platinum nanoparticles (Antibody-Pt NPs) conjugates as a message molecule (Lina et al., 2010). This excellent method has several advantages: (1) it is easy to complete the assay, (2) a small amount of reagent is used, (3) it gives a faster reaction and (4) it allows effective and low-cost detection. Yonezawa et al. (2009) investigated the systematic considerations of the feasibility of using various stabilizer free inorganic metal (Cu, Ag, Au, and Pt) NPs for MS of peptides. Although all metal NPs absorbed N_2 laser light (337 nm) energy in MS, the performance of desorption/ionization of a representative peptide strongly depended on the metal element. Among these metal NPs, Pt NPs showed the highest performance in MS, owing to their smaller heat conductivity and higher melting temperature. Several functionalization procedures have been proposed for rendering nanorods suitable for assembly and use as transducers in biosensing and as tracers for in vivo imaging and targeting. Examples of biosensing strategies using nanorods as label have been proposed. In some recent work, gold nanorods were used as labels for enhancing the SPR signal in the detection of *Escherichia coli* (Eum et al., 2010). In parallel with studies performed to develop new nanomaterials and systems for sensing purposes, much research effort has been focused on adapting the nanomaterials for diagnostic and biotechnological applications.

The excellent catalytic, optical and biological properties of platinum nanomaterials motivated us to apply the application of Pt NPs in the field of biotechnologically as well as agriculturally importance microorganisms. Therefore, the present work based on MALDI-MS analysis by connecting biological receptor molecules (IgG antibody) to activate platinum nanoparticle in detection of PAB isolates (*Bacillus* spp.) to higher dimensions without biochemical testing. This technique would give real meaning to rapid analysis using MALDI; since samples are taken as such and after a simple few-step preparation procedure, are directly used for MALDI-MS analysis assisted by biofunctionalized Pt NPs. The direct analysis techniques as reported in earlier papers were also performed to prove the presence of *Bacillus* spp. (Ryzhov et al., 2000; Wahl et al., 2002). The current method involved isolating these bacteria from soil, roots and growing the bacteria on biological media and then analyzing the bacterial colonies using MALDI-TOF MS. In addition to improve mass peak profiling, the current study reports the biosensing activity of functionalized Pt NPs and combinatorial approach to selectively detect PAB isolates from the microbes rich regions using MALDI-TOF MS. The current method could be applied for rapid detection of PAB isolates for plant growth promotion and their application in modern bioscience.

2. Materials and methods

2.1. Standard solutions and reagents

Sodium borohydride, hydrogen hexachloroplatinate hexahydrate (H_2PtCl_6), antibody immunoglobulin G (h-IgG) from human serum, 3,5-dimethoxy-4-hydroxycinnamic acid (Sinapinic Acid, SA), trifluoroacetic acid (TFA; HPLC grade) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The nutrient agar (NA, pH 7.1) and Petri-plates were purchased from Biolab-Merck (Frankfurter Str., Darmstadt, Germany). The standard bacterial samples viz., *Bacillus thuringiensis* ATCC10792 and *B. subtilis* ATCC11774 were purchased from Microbiologics Inc., South Africa. Water was purified with a Destilador de agua water system (BOE-8707500, Boeco, Germany) and used for all the experiments.

2.2. Collection and description of soil and root sample

The two different samples of carrot plant were collected from Molelwane farm-Mafikeng, one is the rhizoshperic soil and other is the roots. Its geographical coordinates are latitude 25°47′S and longitude 25°32′E, having 1281 meter altitude. The average annual precipitation is 464 mm and average temperature range from 16 °C to 34 °C. The samples were collected carefully by uprooting the root system and placing in the labeled sterilized polyethylene bags for transport and maintained at 4 °C. One set of experiment was conducted on the rhizospheric soil (Fig. S1) of carrot plant and another experiment was conducted on the endophytic bacteria

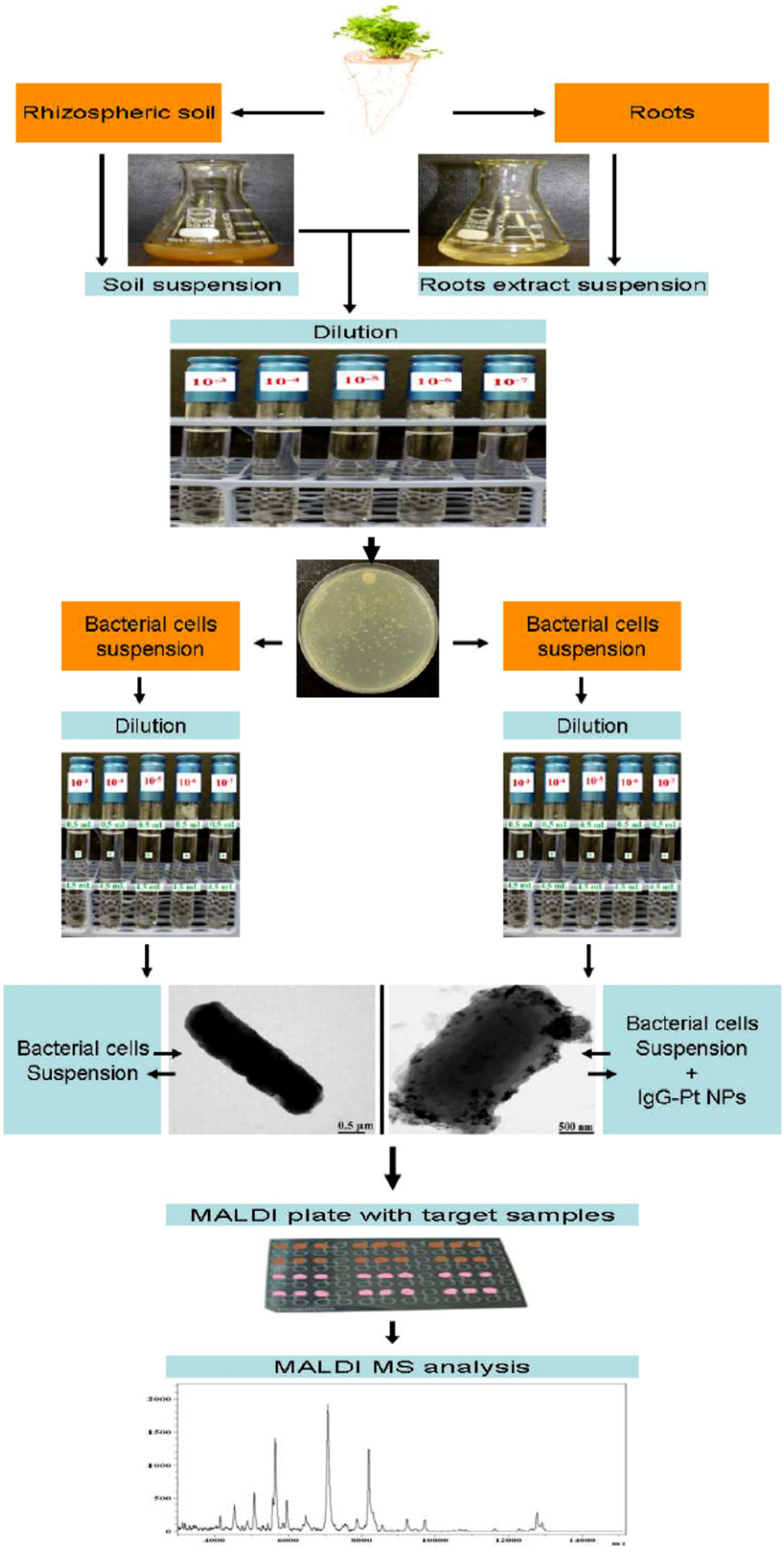

Fig. 1. Flowchart representation of the process employed to detect PAB isolates from soil and root samples.

of the root. Pure culture was obtained as described in supporting material (Fig. S2). The pure cultures were maintained at −80 °C with glycerol (25%) for MALDI-TOF MS analysis.

2.3. Rhizospheric soil and root sample isolates for direct analysis

The developed respective bacterial isolates as described above were diluted separately in sterilized water. The desired bacterial concentration in suspensions was checked by measuring the plate counting methods, which is the standard method to quantify the bacteria. The bacterial count is expressed as cfu/mL. The samples were shaken well by hand for 5 min and spotted on wells of MALDI plate. Each spot was overlaid with 5 μL of matrix solution (3,5-dimethoxy-4-hydroxycinnamic acid 0.05 M in acetonitrile:water (3:1, v/v) containing 0.1% TFA) and kept at room temperature to dry. The dried MALDI plate containing the isolated sample was loaded in MALDI instrument for analysis. Fig. 1 represents the methodology employed to detect the PAB isolates from target samples.

2.4. Platinum nanoparticle synthesis and characterization

The indigenous platinum nanoparticles (Pt NPs) were synthesized according to a slightly modified protocol reported in literature (Chen and Kimura, 2001). Five mL aqueous solution of $NaBH_4$ (0.02 mM) was added drop-wise into the mixture (10 mL) of aqueous solution of H_2PtCl_6 (0.02 mM) under vigorous stirring. The solution turned immediately from yellow to light brown, then gradually to deep brown resulting in the formation of Pt NPs. The PtNPs were characterized using HR-TEM (Philips CM 200) at an accelerating voltage of 200 kV to confirm the particle size. The UV–vis spectrum was acquired from the UV–vis spectrophotometer (Hitachi, Tokyo, Japan) with scan wavelength of 250–450 nm to characterize the Pt NPs (Fig. S3). The average size of the Pt NPs was 50 nm as expressed in Fig. S4. The indigenous prepared Pt NPs were used with antibody IgG for enhancing the protein profiling in the soil and root samples.

2.5. Procedure for biofunctionalization of Pt NPs and its attachment to bacteria

The Pt NPs were concentrated by using centrifugation (15,000 rpm for 25 min), while the supernatant was discarded. The yielded Pt NPs were again resuspended into distilled water (0.5 mL) and vortexed for proper mixing. The above aqueous suspension of Pt NPs (0.5 mL) was mixed with aqueous suspension of human serum immunoglobulin G, h-IgG (30 μg/mL, 0.5 mL) and incubated at room temperature for 30 min to obtain antibody functionalized nanoparticles. Functionalization of Pt NPs involves the use of bifunctional ligands in which a moiety is used for anchorage to the particle while the other is directed to the outer-surface for specific interaction with biomolecules. The above incubated antibody IgG-Pt NPs suspension (1.0 mL) was added with 0.5 mL pure bacterial cell suspension (4×10^5 cfu/mL) of selected isolates separately for interaction/attachment with cell membrane. The mixtures suspension were incubated at −20 °C for 10 min, and then at room temperature for 25 min. The bacterial cell surface attachment of antibody functionalized Pt NPs was confirmed by transmission electron microscopy (TEM) imaging.

2.6. Sample preparation for TEM imaging

The conjugated cells solution was concentrated by centrifugation (15,000 rpm for 10 min), and obtained concentrated dense conjugated bacterial cells. This conjugated suspension was washed with deionized water under gentle vortex for 5 min. The conjugated cell suspension was again centrifuged, while supernatant was discarded. The obtained sample was again mixed with deionized water and gently vortexed to get homogeneous suspension. This suspension was used to determine the TEM images. The 0.5 μL of above suspension was deposited on the copper grid with the help of a micropipette, and kept overnight in vacuum oven. The dried copper grid containing the target samples was subjected for TEM analysis.

2.7. MALDI-TOF MS instrument and target sample analysis

All protein mass spectra were analyzed using a Microflex MALDI time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany). The MALDI-TOF MS source was equipped with a nitrogen laser (337 nm) for irradiation of analyte and the accelerating voltage was obtained at +20 kV. Each experiment was performed under similar conditions in the linear mode with laser energy of 63.2 μJ and 150 laser shots. The instrument was calibrated by using a mixture of Protein Standards I and Peptide Standards II (Bruker Daltonics). One μL of the target suspension was deposited on MALDI target well, overlaid with 0.5 μL of SA matrix solution and air-dried before MALDI-MS analysis. Shown (Fig. 1) is the process employed to detect the PAB isolates from target samples. The spectra were collected by using the Reflex Control, and processed with the Bruker Flex Analysis software.

3. Results and discussion

3.1. Target isolates and direct MALDI-TOF analysis

The two isolates were grown predominantly on Petri-plates containing nutrient agar. The isolates were obtained from soil and roots of carrot plant. The standard isolates viz., *B. thuringiensis* ATCC10792 and *B. subtilis* ATCC11774 were also grown on nutrient agar, which show similar pattern of colonies as a soil and root samples. Although similar color colonies were obtained from soil and root isolates, slightly more colonies observed from standard samples compared to those from soil and root isolates. This is understandable because compared to soil and root isolates which are new strains; the standard samples are processed from nutrient media by suppler company as better conditions for bacterial colonization. Supporting Figs. S7 and 8(a–c) show the protein mass spectra produced from direct analysis of bacteria which are obtained from the rhizospheric soil, roots and standard samples. As can be observed from these spectra, it is evident that the bacterial concentrations was below the MALDI-MS detection limit and so very less peaks were observed (panel a–c of Figs. S7 and 8). The total bacteria in these samples were enumerated by the plate counting method. The bacterial count in the target sample was 4×10^5 cfu/mL. Since, the lowest detectable concentration of MALDI-TOF MS for bacteria analysis is more than 10^7 cfu/mL (Gantt et al., 1999), it is reasonable that direct analysis did not yield good bacterial signals.

3.2. Characterization of biofunctionalized Pt NPs

Functional groups on the surface of NPs allow conjugation with ionic ligands. The surfaces of platinum nanoparticles possess anions, which bound strongly through ionic interactions (Chen and Kimura, 2001). The value of p*I* of IgG is close to 7.0, and therefore, in a solution at pH 6.0, IgG should bear a net positive charge. Thus, when the IgG suspension was mixed with the platinum nanoparticle suspension, IgG gets attached onto the surfaces of platinum nanoparticles through ionic interactions. We used UV-vis absorption spectroscopy to confirm whether the biomolecule (IgG) units had become interacted to the platinum nanoparticle surfaces. The results clearly indicated that absorption intensity of

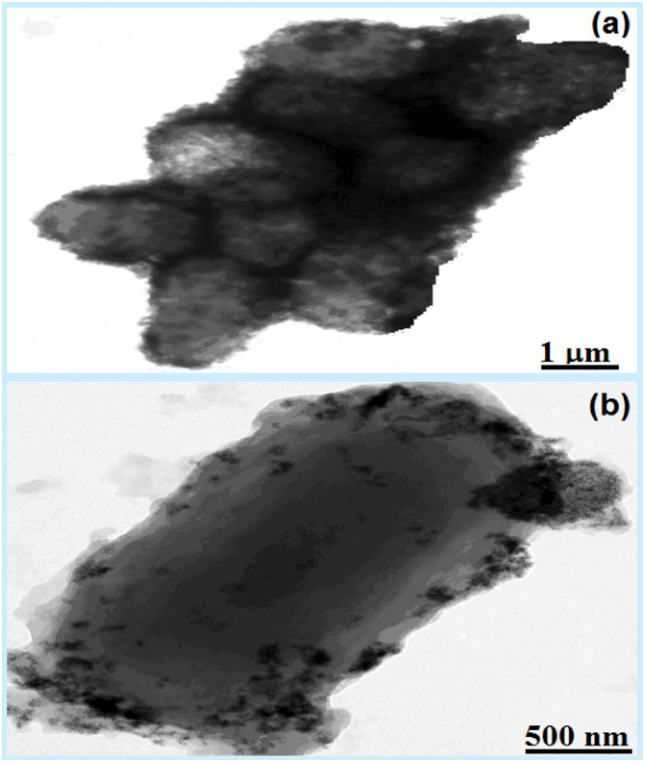

Fig. 2. TEM images obtained after incubation of *B. thuringiensis* with IgG-Pt NPs. (a) IgG-biofunctionalized Pt NPs interacted with surface of bacterial cells showing the attachment around the group of cells and (b) IgG-biofunctionalized Pt NPs interacted with outer surface of bacterium showing the high resolution image of cell.

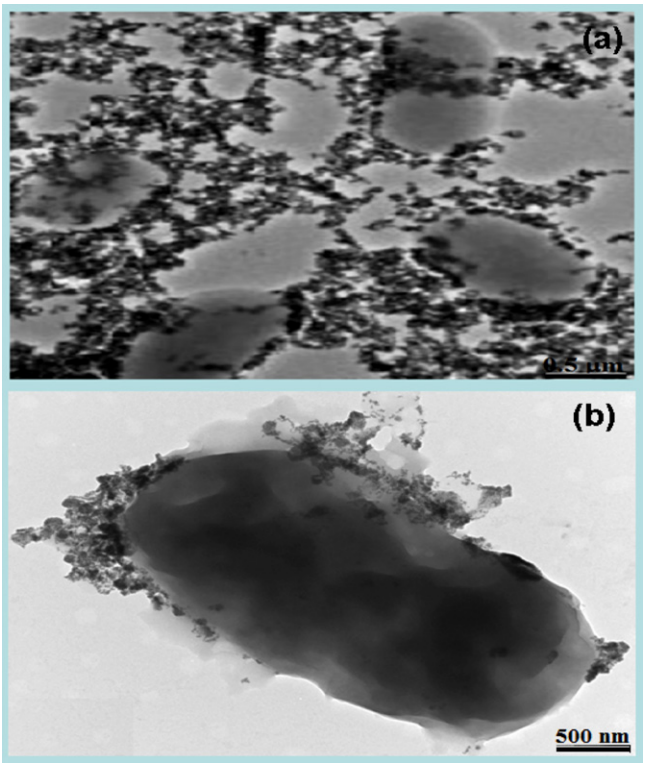

Fig. 3. TEM images obtained after incubation of *B. subtilis* with IgG-Pt NPs. (a) IgG-biofunctionalized Pt NPs interacted with surface of bacterial cells showing the attachment around the group of cells and (b) IgG-biofunctionalized Pt NPs interacted with outer surface of bacterium showing the high resolution image of cell.

antibody IgG functionalized platinum nanoparticle was obviously lower than that of the platinum nanoparticles suspension (Fig. S3). This result proved that biomolecule had interacted/attached successfully to the surface of Pt NPs. The present functionalization technique has also advantage to lead stabilization and prevents uncontrolled growth as well as aggregation of the nanoparticles. With the molecule bound to the nanoparticle, the charge resides on the outside of particle giving way to coulombic repulsion and thus dispersion of the nanoparticles. Coulombic repulsion is also useful as it can protect ligands on the NPs from exchanging with biomolecules as reported by Chompoosor et al. (2008).

3.3. TEM analysis for recognition of IgG-Pt NPs attachment on bacterial surface

Distinct surface attachments were observed to found in case of target isolates tested in present study for enhancing the protein profiling. This is clearly shown in the TEM images which are represented as Figs. 2 and 3. Fig. 2 showing the biofunctionalized nanoparticle attachment on the cell surface of *B. thuringiensis*. The group of cells were clearly indicated attachment in Fig. 2a, while Fig. 2b representing the magnified image of cell attached with functionalized biomolecules. The other bacterium, *B. subtilis* was also tested to confirm surface attachment with above used functionalized nanoparticle. A similar trend was also observed in this case (Fig. 3). From the results shown in Figs. 2 and 3, it is apparent that a surface based with functionalized NPs is effective in capturing bacterial cells either specifically or non-specifically. Specific capture is a necessary condition for detection of a target organism by an antibody previously placed on the surface. Non-specific capture is useful if detection or quantification of bacteria regardless of type is the goal. Huang et al. (2003) reported that the gram positive bacteria exhibit strong adsorption on the surface with hydrophobic properties when compared to gram negative bacteria. The current study is also showing the attachment on the surface of gram positive bacteria viz., *B. thuringiensis* and *B. subtilis* captured by IgG functionalized Pt NPs which could be used to detect the different type of isolates. The experiments were carried out at least three times in order to check the reproducibility of cells surface attachment of biofunctionalized Pt NPs. The TEM images in supporting information as Fig. S6(a and b) clearly showing the reproducibility of current approach. While, TEM images in supporting information as Fig. S5 indicated that the cells of *Bacillus* spp. treated with unmodified Pt NPs were not shown any attachment on surface of bacteria. In this study, obtained results indicated that current biofunctionalized technique could capture successfully the target bacterial cells from unknown biological samples.

3.4. IgG functionalized Pt NPs assisted MALDI-TOF MS for selective bio-sensing of PAB isolates

In order to obtain significant bacterial protein profiling, functionalized Pt NPs were used to enhance the bacterial detection from direct MALDI-MS analysis. Figs. 4 and 5 give the comparative mass spectra where addition of NPs resulted in significant enhancement of bacterial peaks. Fig. 4a shows the mass spectra of *B. thuringiensis* obtained from direct analysis of the soil samples after incubation with IgG-Pt NPs. Addition of functionalized Pt NPs in addition to the SA matrix resulted in significant enhancement of the bacterial peaks in the soil (Fig. 4a) and endophytic/roots (Fig. 4b) samples. Fig. 4c belongs to the mass spectra obtained from the standard *B. thuringiensis* ATCC10792 purchased from Microbiologics Inc., Davies Diagnostics (Pty) Ltd., South Africa. As we can clearly observe, many protein peaks from the soil samples and a couple of peaks from the roots samples could be matched with the standard *B. thuringiensis* peaks. This confirms the presence of

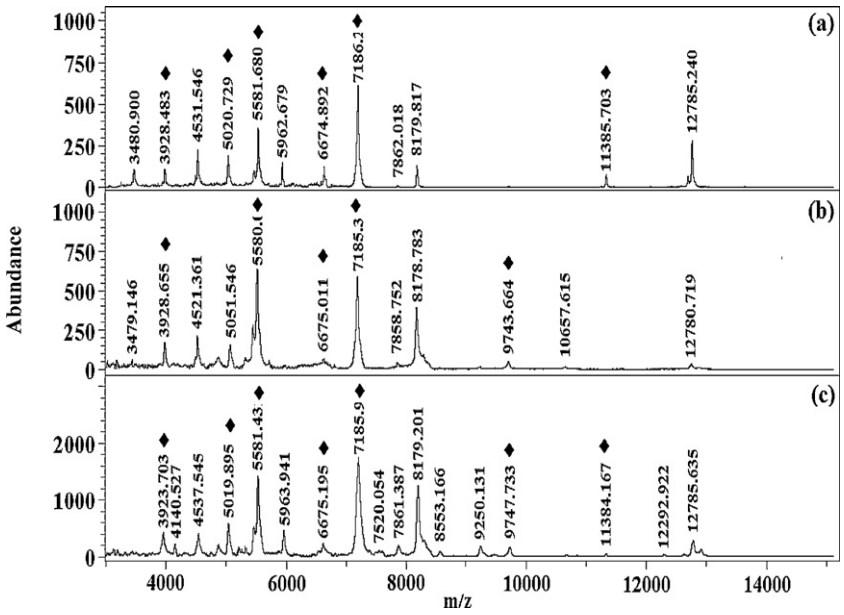

Fig. 4. MALDI-TOF MS spectra showing antibody IgG biofunctionalized Pt NPs assisted protein analysis. (a) Rhizosheric soil, (b) endophytic samples compared with the (c) standard *B. thuringiensis*. The biofunctionalize Pt NPs resulted in selective enrichment of bacterial protein peaks and lead to identification of *B. thuringiensis* protein peaks in the rhizosheric soil and endophytic samples. The marker protein mass peaks matching the standard sample are represented with symbol ♦.

B. thuringiensis in these samples and the efficacy of IgG functionalized Pt NPs to selectively enrich the bacterial peaks in soil and root samples. The following marker mass peaks such as *m*/*z* 3923, 5019, 5581, 6675, 7185, 9747 and 11,384 is successfully produced in this study using IgG functionalized Pt NPs biosensing technique toward PAB isolates (Table 1). The protein peaks observed in the spectra within *m*/*z* differences ±10 were considered reproducible. The similar trends were also observed in case of *B. subtilis* when using the same functionalized NPs as biosensor (Fig. 5). Fig. 5a and b showing the proteins mass peaks of *B. subtilis*, which was obtained from soil and root samples viz. rhizospheric soil and roots respectively. While Fig. 5c shows the MALDI spectra generated from the standard sample of *B. subtilis* ATCC11774 obtained from same source as described above. The marker protein ions *m*/*z* 3889, 5086, 6289, 7724, 7962, 9165 and 9889 were produced in *B. subtilis* sample (Table 1). These protein peaks are also reported previously by various researchers as biomarker peaks. Thus, the current results proved that our biosensing approach provides a new path in microbiology to detect different types of PAB isolates from various regions of the world. In fact, it is possible that soil and root samples obtained from soil and roots would contain some other proteins, but IgG functionalized Pt NPs were able to selectively

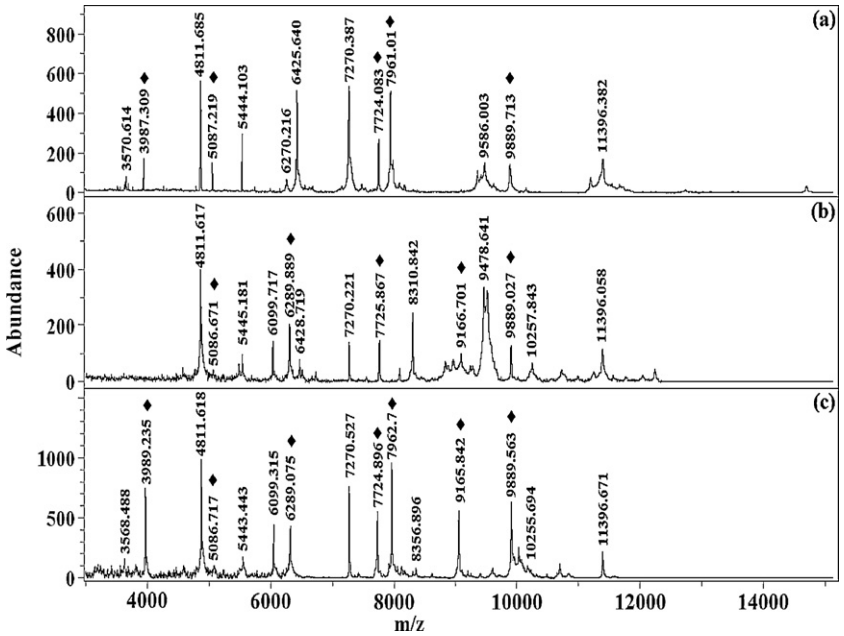

Fig. 5. MALDI-TOF MS spectra showing antibody IgG biofunctionalized Pt NPs assisted protein analysis. (a) Rhizosheric soil, (b) endophytic samples compared with the (c) standard *B. subtilis*. The biofunctionalize Pt NPs resulted in selective enrichment of bacterial protein peaks and lead to identification of *B. subtilis* protein peaks in the rhizosheric soil and endophytic samples. The marker protein mass peaks matching the standard sample are represented with symbol ♦.

Table 1
The marker protein peaks of PAB isolates was demonstrated from soil and root sample using IgG functionalized Pt NPs assisted MALDI-TOF MS.

Marker protein peaks (m/z value)	PAB isolates	
	B. thuringiensis	*B. subtilis*
3923	√	
3989	–	√
5019	√	–
5086	–	√
5581	√	–
6289	–	√
6675	√	–
7185	√	–
7724	–	√
7962	–	√
9165	–	√
9747	√	–
9889	–	√
11,384	√	–

√ denotes the presence of diagnostic marker peaks of PAB isolates.

enhance the bacterial proteins. The role of functionalized Pt NPs as affinity probes for MALDI-MS studies of PAB isolates from soil and root samples is never reported and that is the uniqueness of this study. However, detection limit of bacterial cells varies with type of instrument, target biological samples and properties of nanomaterials. The variation in detection limit has been observed in earlier research (Ho et al., 2004; Gao et al., 2006; Chen et al., 2008b).

The experiment was also extended to verify whether the marker peaks obtained were indeed from conjugated bacteria sample was made by functionalized NPs and analyzing those using MALDI-TOF MS. To make sure that the peaks in the direct sample analysis without biosensing technique are not belonging to significant markers as obtained from treated bacterial samples with IgG-Pt NPs. Fig. S7(a–c) reconfirms that only few protein peaks of *B. thuringiensis* were produced, while Fig. S8(a–c) also showed similar trend and produced few protein peaks of *B. subtilis*. Considering the results from this experiment, we have confirmed that IgG functionalized Pt NPs can be used successfully to explore biosensing properties between IgG-Pt NPs and the cell surface of target bacterial isolates to leading the enrichment of protein mass peaks determined by MALDI-TOF MS. The interactions of biomolecules with specific high affinity exist prevailingly in nature. If one of the biomolecular entities conjugates with the nanoparticles, the resulting biofunctional nanoparticles can specifically bind to the other different biomolecular entity. Then, the most direct consequence is that the interaction force can control the position of the biological entity. Based on this mechanism, several applications using biofunctional nanoparticles, such as bacterial detection, protein purification, and toxin decorporation have been determined in clinical and environmental research. Advances in nanotechnology and biological sciences are having a significant impact on the field of diagnostics, where a number of nanoparticle-based approaches have been implemented for detection of bacterial isolates. Nanoparticles (NPs) provide a high surface to volume ratio which can increase the binding/capture efficiency of bacteria. A combination of enriching technique and MALDI-TOF MS has been demonstrated as an efficient strategy for accurate identification of target bacteria (Jiang et al., 2007; Gao et al., 2009; Riddin et al., 2010). Therefore, it is no doubt that the role of IgG functionalized Pt NPs in this study is straightforward and has lead to the enhancement of the bacterial protein signals. The *B. thuringiensis* and *B. subtilis* are reported to be present in both target sources of carrot plant analyzed in this current study viz., rhizospheric soil and roots. They can also be found in the rhizospheric soil and roots of other plants in different regions as effective PAB strains, which would be used in the biotechnological as well agricultural applications.

4. Conclusions

We have reported that the two PAB isolates namely, *B. thuringiensis* and *B. subtilis* from soil and root samples can be directly detected by MALDI-TOF MS using biosensor application. The current method focuses on the proteomic characteristics to detect the presence of specific PAB isolates. Not only does this method increase the rapidity of analysis, but they are also able to achieve a high degree of sensitivity and specificity without the need for complex cultivation and additional confirmation steps. The obtained results indicated that the present experimental approach could be applied to explore new path for rapid detection of PAB. This technique is easy and rapid to determine bacterial isolates without morphological, molecular and biochemical application. For biotechnological and agricultural applications, the effective and beneficially important PAB isolates in the environment are highly considered. Therefore, it is necessary to further study and to explore the current technique for developing the successful PAB isolates as byproduct in biotechnological and agricultural field.

Acknowledgments

Faheem Ahmad highly acknowledged the postdoctoral fellowship from North-West University. We are very thankful to the Director, National Botanical Research Institute, Lucknow, India, for their assistance in MALDI-TOF MS analysis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2012.02.055.

References

Arora, P., Sindhu, A., Dilbaghi, N., Chaudhury, A., 2011. Biosens. Bioelectron. 28, 1–12.
Babalola, O.O., Akindolire, A.M., 2011. Biol. Agric. Hort. 27, 294–309.
Babalola, O.O., 2010. Biotechnol. Lett. 32, 1559–1570.
Babalola, O.O., Kirby, B.M., Le Roes-Hill, M., Cook, A.E., Cary, S.C., Burton, S.G., Cowan, D.A., 2009. Environ. Microbiol. 11, 566–576.
Babalola, O.O., Sanni, A.A.I., Odhiambo, G.D., Torto, B., 2007. World J. Microbiol. Biotechnol. 23, 747–752.
Bull, A.T., Ward, A.C., Goodfellow, M., 2000. Microbiol. Mol. Biol. Rev. 46, 573–606.
Chen, M.H., Chuang, Y.J., Tseng, F.G., 2008a. Nanotechnology 19, 505301.
Chen, S., Kimura, K., 2001. J. Phys. Chem. B 105, 5397–5403.
Chen, W.J., Tsai, P.J., Chen, Y.C.C., 2008b. Anal. Chem. 80, 9612–9621.
Chiang, C.K., Chiang, N.C., Lin, Z.H., Lan, G.Y., Lin, Y.W., Chang, H.T., 2010. J Am. Soc. Mass Spectrom. 21, 1204.
Chompoosor, A., Han, G., Rotello, V.M., 2008. Bioconjug. Chem. 19, 1342–1345.
Cui, Y., Wei, Q., Park, H., Lieber, C.M., 2001. Science 293, 1289–1292.
Dare, D., 2006. In: Tang, Y.W., Stratton, C.W. (Eds.), Advanced Techniques in Diagnostic Microbiology. Springer, New York, US, pp. 117–133.
Duffy, B.K., Ownley, B.H., Weller, D.M., 1997. Phytopathology 87, 1118–1124.
Eum, N., Yeom, S., Kwon, D., Kim, H., Kang, S., 2010. Sens. Actuators B 143, 784–788.
Fang, H., Hedin, G., 2003. J. Clin. Microbiol. 41, 2894–2899.
Feldheim, D.L., 2007. Science 316, 699.
Gantt, S.L., Valentine, N.B., Saenz, A.J., Kingsley, M.T., Wahl, K.L., 1999. J. Am. Soc. Mass Spectrom. 10, 1131–1137.
Gao, J.H., Li, L., Ho, P.L., Mak, G.C., Gu, H.W., Xu, B., 2006. Adv. Mater. 18, 3145–3148.
Gao, J., Gu, H., Xu, B., 2009. Acc. Chem. Res. 42, 1097–1107.
Ho, K.C., Tsai, P.J., Lin, Y.S., Chen, Y.C., 2004. Anal. Chem. 76, 7162–7168.
Huang, T.T., Geng, T., Sturgis, J., Li, H., Gomez, R., Bashir, R., Bhunia, A.K., Robinson, J.P., Ladisch, M.R., 2003. Enzyme Microb. Technol. 33, 958–966.
Ip, M., Lyon, D.J., Chio, F., Enright, M.C., Cheng, A.F., 2003. J. Clin. Microbiol. 41, 4980–4985.
Jiang, J., Parker, C.E., Fuller, J.R., Kawula, T.H., Borchers, C.H., 2007. Anal. Chim. Acta 605, 70–79.
Jock, S., Geider, K., 2004. Environ. Microbiol. 6, 480–490.
Johnsson, L., Hokeberg, M., Gerhardson, B., 1998. Eur. J. Plant Pathol. 104, 701–711.
Jury, F., Al-Mahrous, M., Apostolou, M., Sandiford, S., Fox, A., Ollier, B.W., Upton, M., 2006. J. Med. Microbiol. 55, 1053–1060.
Lina, H.C., Wanga, I.L., Linb, H.P., Chang, T.C., Lin, Y.C., 2010. Sens. Actuators B: Chem., doi:10.1016/j.snb.2010.05.055.
Ling, J., Cheng, Z.H., Yuan, F.L., et al., 2009. Trends Anal. Chem. 28, 4.
Nel, A., Xia, T., Mädler, L., Li, N., 2006. Science 311, 622–627.
Pan, Y., Neuss, S., Leifert, A., et al., 2007. Small 3, 1941–1949.

Parka, M., Kima, C., Yanga, J., Leea, H., Shina, W., Kimb, S., Saa, T., 2005. Microbiol. Res. 160, 127–133.
Pershing, D.H., Tenover, F.C., Versalovic, J., Tang, Y.W., Unger, E.R., Relman, D.A., White, T., 2003. American Society for Microbiology, Washington, D.C.
Riddin, T., Gerickeb, M., Whiteleya, C.G., 2010. Enzyme Microb. Technol. 46, 501–505.
Ryzhov, V., Hathout, Y., Fenselau, C., 2000. Appl. Environ. Microbiol. 66, 3828–3834.
Seng, P., Drancourt, M., Gouriet, F., Scola, B.L., Fournier, P., Rolain, J.M., Raoult, D., 2009. Clin. Infect. Dis. 49, 543–551.
Wahl, K.L., Wunschel, S.C., Jarman, K.H., Valentine, N.B., Petersen, C.E., Kingsley, M.T., Zartolas, K.A., Saenz, A.J., 2002. Anal. Chem. 74, 6191–6199.
Wees, S.C.M.V., Ent, S.V., Pieterse, C.M.J., 2008. Curr. Opin. Plant Biol. 11, 443–448.
Wei, G.H., Zhang, Z.X., Chen, C., Chen, W.M., Ju, W.T., 2008. Microbiol. Res. 163, 651–662.
Yonezawa, T., Kawasaki, H., Tarui, A., Watanabe, T., Arakawa, R., Shimada, T., Mafune, F., 2009. Anal. Sci. 25, 339.