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Monitoring the heat stress response of *Escherichia coli* via NiO nanoparticle assisted MALDI–TOF mass spectrometry

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ABSTRACT

The heat stress response of *Escherichia coli* at various temperatures has been investigated using NiO nanoparticles assisted MALDI–TOF-MS. Significant numbers of protein peaks were obtained in the presence of NiO NPs when the samples were incubated at various temperatures in comparison with the control *E. coli* suspension (10^7 cfu/mL). The 10 kDa chaperonin (groES) is the principal protein operating both for the protection of proteins from denaturation and in the assembly of newly synthesized proteins. During the heat stress response with NiO NPs, 10 kDa chaperonin (grosES) proteins were detected using MALDI–TOF MS. The viability of *E. coli* was checked on LB agar plates at different temperatures and time treatments. In the presence of NiO NPs, viability decreases drastically; this has been explored and correlated with the MALDI–TOF MS results. Further, surface morphological changes of *E. coli* at different temperatures were investigated with NiO NPs by transmission electron microscopy (TEM). The response of heat stress toward *E. coli* for generating more stable protein ions can be applied for bacterial detection under high temperature conditions from biological, clinical and environmental samples.

1. Introduction

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI–TOF MS) has been widely applied as promising tool for rapid characterization and highly throughput analysis of proteins, DNA, oligonucleotides, oligosaccharides, pathogenic bacteria and bacterial proteins [1–4]. The *m*/*z* values in MALDI–TOF MS for mass spectral peaks with unique patterns of analytes provide very specific and unbiased analysis for microorganism especially for bacteria. Anhalt for the first time used gently heat treated gram-negative bacteria for small molecular weight phospholipid and ubiquinone analysis by pyrolysis based mass spectrometry [5]. Williams et al. investigated the influence of experimental parameters which affect the analysis of whole bacteria biomarker proteins with MALDI–TOF MS [6]. Verena Horneffer et al. have applied wet heat treatment as a soft releasing technique for high molecular weight biomarker proteins from bacteria spores and directly analyze by MALDI–TOF MS. In their wet heat treatment setup, they applied 120 °C heat shock on three different *Bacillus* species spores in 20, 15 and 3 min time zones. Further, characteristic biomarker proteins from wet heat treatment were analyzed by MALDI–TOF MS [7]. These biomarker proteins, generated from the bacterial cell wall, try to maintain the space vicinity stress effects. Bacterial cell consists of two compartments called the cytoplasm and envelope, which includes the inner membrane, periplasm and outer membrane. Various cellular stress responses are generated from each of these compartments. Cytoplasmic stress due to heat shock is responsible for protein unfolding in the cytoplasm, shown by the σ^{32} family [8]. In gram-negative and in gram-positive bacteria σ^S (RpoS) and σ^B (SigB) have been found as simple stress responsive sigma factors. *Escherichia coli* shows σ^S (RpoS) factor which activates the balance expression of numerous genes that are required for viability as the cell leaves the exponential growth conditions and moves into the stationary phase [9,10]. The envelope stress response and the extracytoplasmic function (EOF) are responsible for transcription factors that direct the envelope environmental stress responses through the cytoplasm and signal transaction [10]. Heat or other environmental stresses damage cellular constituents, and in response, cells induce the expression of genes with other products that counteract the damage and the cells withstand external environmental stress. The ability of microorganisms to sense and respond to changes in its environment could influence the potentially lethal threats for human beings. Bacteria pass

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through various physical and chemical stresses when residing within human hosts either as commensals or pathogens. In this regard, *E. coli* is a prime pathogen and is mostly responsible for food-born diseases, which cause hemorrhagic colitis and hemolytic ureamic syndrome [11]. It has been studied as a model microorganism to explore the environmental stresses on specialized systems [12,13]. The identification of specific mechanisms, which contribute to the survival of microorganism under rapidly changing conditions, provides the recognition within stress response processes. Various bacterial strains deserve special attention regarding acid tolerance also, because it is likely to play an important role in the survival of the bacteria at low pH. Arginine and glutamate-dependent systems play a significant role in the acid tolerance at lower pH. This system increases bacteria ability to maintain the intercellular pH as well as survival [12,14]. Nano-structured materials have attracted considerable interest as they exhibit the small size effect, quantum size and high surface to volume ratio effects. NiO nanoparticles have attracted extensive interest due to their novel optical, electronic, magnetic, thermal, and mechanical properties for their applications in sensors, as catalyst, as battery electrodes, as photoelectronic devices, etc. [15,16]. These nanoparticles access high surface-to-volume ratio, positive zeta potential at low pH ($pH < 10.8$) and have high affinity for enrichment of polyoxy anions like phosphoproteins and carboxylate moieties [17]. Besides this, NiO NPs small sizes attribute weak ferromagnetism or superparamagnetism properties [16,18]. Due to the weak magnetic characteristics of NiO NPs, they exhibit good heat absorption capability which could enhance the heating rate resulting in unfolding of the peptide/protein chains for enrichment onto their surfaces. These properties of NiO nanoparticles were exploited for the heat absorption and high molecular weight heat stable proteins enrichment during the heat stress on *E. coli* cell at different temperature conditions and times. *E. coli* suspensions (1.53×10^7 cfu/mL, 2 mL in each) were prepared in different glass tubes and subjected to three sterilized water bath systems for heat stress applications. We applied 40, 60 and 80 °C heat stresses on *E. coli* with different time zones 10, 20, 30, 60 and 90 min separately. Different heat shocked responses on *E. coli* were further checked by the plate counting method and bacterial marker proteins profiles were investigated by NiO NPs assisted MALDI–TOF MS. The 10 kDa chaperonin (groES) proteins, which carry protection nature to maintain other proteins denaturation and gave assembly to new synthesized proteins, were observed during the heat response with NiO NPs in MALDI–TOF MS. During different heat stresses in progressive time periods, *E. coli* viability was checked by the plate counting method on LB agar plates. Data shows that in the presence of NiO NPs the viability of cell decreases which was correlated and explored with MALDI–TOF MS data. Surface changes of *E. coli* without NPs/with NiO NPs, at different heat stresses, were checked and investigated by transmission electron microscopy (TEM). This study is on *E. coli* heat stable proteins analyses which provide easy and simple characterization of bacteria at different stages of temperatures by MALDI–TOF MS. Therefore, we investigated the response of temperature stress toward *E. coli* for the purpose of generating more stable protein ions which could assist in precise identification of bacterial species in clinical, biological or environmental samples.

2. Material and methods

2.1. Chemical and reagents

All chemicals used was of analytical grade without further purification. Ultra-pure deionized water from a Milli-Q purification system (Millipore, Milford, MA, USA) was used for all experiments. Nickel chloride ($NiCl_2$) was purchased from Aldrich Chemical (USA). Ethylene glycol, potassium chloride and ammonium bicarbonate were obtained from Riedel-de Haën (Sigma-Aldrich Laborchemikalien, Germany). 3,5-Dimethoxy-4-hydroxycinnamic acid (Sinapinic Acid, SA), sodium chloride and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ethanol and acetonitrile (MeCN) were purchased from J.T. Baker, USA. The nutrient broth Luria–Bertani (LB with formula per liter of 10 g tryptone, 5 g yeast extract, and 10 g sodium chloride) and agar were obtained from Bio Basic Inc (Taiwan). Disodium hydrogen phosphate ($Na_2HPO_4 \cdot 2H_2O$) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Merck, Germany. Phosphate buffer saline (PBS) was prepared using 8.0 g sodium chloride, 0.2 g potassium chloride, 1.44 g disodium hydrogen phosphate and 0.24 g potassium dihydrogen phosphate per liter of solution. The PBS pH (7.2) was adjusted by a pH meter (Istek model 720P, Korea). PBS and nanoparticles suspension solutions were sterilized by using a 0.45 μm size filter (Minisart, Sartorius Stedim Biotech GmbH, and Germany).

2.2. Bacterial strain and cells culture preparation

E. coli (BCRC12570) strains were preserved at −80 °C. Microbank Microbial Preservation System beads were purchased from Bioresource Collection and Research Center (BCRC), Hsinchu 30062, Taiwan. Bacterial cells were cultured on Luria–Bertani (LB) agar for production of fresh cells at the time of experiment. The inoculated LB agar plates with bacterial cells were incubated at 37 °C for 12 h [19]. The freshly harvested bacterial cells were used for the heat stress experiments. The *E. coli* cultured inside a biohazard safety level-2 cabinet (NuAire, Plymouth, MN, USA) equipped with UV light.

2.3. Synthesis of NiO nanoparticles

NiO nanoparticles were synthesized via the wet chemical method with some modification from the literature [20]. $NiCl_2$ (1.3 g) was dissolved in warm ethylene glycol (1.7 mL) and an ammonium bicarbonate aqueous solution was added slowly into the solution mixture resulting in immediate precipitation of Ni^{2+} ions and ethylene glycol. Precipitated suspension was then kept at 90 °C with constant stirring for 30 min. This product was filtered and washed with deionized water (3×30 mL) and ethanol (2×10 mL) respectively, to remove free nickel ions, chloride ions, carbonate ions and hydroxide ions. The sample was dried in vacuum at 100 °C for 3 h, and the green dry complex was finally heated at 400 °C for 2 h. A grey-black sintered product was obtained which was pulverized by a glass rod. The suspension of NiO NPs (5 mg/mL in H_2O) was then ultrasonicated for 5 min and sterilized by using a 0.45 μm size filter (Minisart, Sartorius Stedim Biotech GmbH, Germany), and was used to study the stress response of bacteria under various temperature treatments.

2.4. Procedure of heat treatment to E. coli bacterial cells

Fresh *E. coli* colonies were harvested from LB agar plates in an eppendorf tube containing 1.0 mL PBS solution (pH 7.2). Bacteria were suspended properly and centrifuged at 5000 rpm for 5 min at 4 °C. *E. coli* ($\sim 10^9$ cfu/mL) stocked suspension was prepared in PBS (1.0 mL). From stocked suspension sub-suspensions of *E. coli* (1.53×10^7 cfu/mL, 2 mL in each) were prepared in six different glass tubes. Three bacterial suspension tubes were used as control (without nanoparticles) while other three tubes were applied with NiO NPs (20 μg/mL) at different temperatures. Three consecutive temperatures (40, 60 and 80 °C) for 10, 20, 30, 60 and 90 min of incubation periods were used for heat stress treatment on *E. coli*. The required temperatures were maintained using a

water bath, which was set up inside the sterile laminar flow. The digital thermometer was connected to the water bath to check the appropriate application of temperature throughout the experiments. The systematic procedure scheme has been shown in Fig. S1 (Supporting information).

2.5. Measurement of cells growth at different temperatures treatment

The *E. coli* inoculated separately onto LB agar plates after the given heat treatment. Bacterial cells in the presence of NiO NPs were also inoculated onto the LB agar plates to confirm the influence of NiO NPs on cell growth. The viable cells' countings were performed the next day after inoculation to check the viability of cells, so that the cell growth conditions on temperature tolerance could be correlated.

2.6. Sample preparation for transmission electron microscopy

The size of the NiO NPs and the morphological changes of *E. coli* in the presence of NiO NPs were confirmed and analyzed by transmission electron microscopy (TEM-3010 microscope, JEOL, Tokyo Japan). After heat stress treatment on bacterial cell at 40, 60 and 80 °C, 20 μL samples were taken in 1.0 mL of autoclaved deionized water. Samples were centrifuged at 5000 rpm for 5 min. The supernatant was removed and the precipitate was dispersed in water. 1.0 μL suspension was deposited on a TEM copper grid. After drying, the samples were subjected to TEM analysis.

2.7. Heat treated bacterial cell samples for MALDI–TOF MS analysis

Before MALDI–TOF MS analysis, the MALDI target plates were washed with Milli-Q grade water, treated with methanol, and allowed to dry at room temperature. 0.5 μL of sample from the treated bacterial cells was immediately transferred to the wells of a MALDI target plate (96 wells). Each well was immediately overlaid with 0.5 μL matrix solution (50 mM, Sinapinic acid). The matrix (SA) was freshly prepared as a saturated solution in 3:1 acetonitrile:water containing 0.1% trifluoroacetic acid. The sample–matrix mixtures were allowed to dry at room temperature in air prior to MALDI–TOF MS analysis.

2.8. MALDI–TOF MS instrumentation and acquisition

A MALDI–TOF MS (Microflex, Bruker Daltonics, Bremen, Germany) was used for all mass spectral measurements which was equipped with a 337 nm nitrogen laser with a 1.25 m flight tube and a target plate having the capacity to load 96 samples simultaneously. The parameters for sample analysis were set at IS1, 20.09 kV; IS2, 18.23 kV; lens, 9.55 kV and linear detector 2.834 kV at positive ion extraction mode. The laser energy was adjusted to slightly above the threshold with 200 laser shots in order to obtain good resolution and signal-to-noise (S/N) ratios at a laser frequency of 60 Hz. All MALDI–TOF mass spectra were collected on flex control and flex analysis 3.0 software after baseline corrections of each mass spectrum. A thermocouple (Genechian Industrial, Taiwan) was used to measure the solution temperature each time before and after taking the bacterial suspension for MALDI–TOF-MS detection and cell growth measurements. The centrifuge (Hermle Z-233, M-2) was purchased from Hermle Labortechnik (Germany).

3. Results and discussion

The *E. coli* is a gram-negative bacteria comprising two membranes, a cytoplasmic inner and an outer membrane, with different lipids and protein compositions. When heat or other stresses are subjected on *E. coli*, they damage the cellular constituents. *E. coli* induce the expression of genes as products that proceed to sustain the cells and counteract the damage of stable proteins. Marvin-Guy et al. have applied low temperature stress (4 °C) for several days on *Bifidobacterium lactis* and analyze the whole cells' characteristic mass spectra by MALDI–TOF MS [21]. In their study, physiological status assessment of *B. lactis* was performed with minor differences in protein profile of MALDI MS signals in 0, 2 and 6 days studies. However, as they used different percent bile salts new proteins of m/z ion peaks at 12,064, 13,095, 16,060 and 16,278 were observed. Verena Horneffer et al. have applied 120 °C sudden stroke of high temperature for 20, 15 and 3 min time period on three different *Bacillus subtilis* strains. New high molecular weight biomarker proteins were observed by MALDI–TOF MS as heat stress duration was increased on *B. subtilis* spores [7]. In our experiment, we applied different high temperature stresses on *E. coli*. After heat stress, protein profiles of bacteria were checked by MALDI–TOF MS. The schematic procedure has been shown in Fig. S1 (Supporting information). Figs. 1(a), 2(a), 3(a) and S2(a)–S3(a) (Supporting information) are the mass spectra of proteins obtained from *E. coli* at different time intervals (10, 20, 30, 60 and 90 min) and various heat stresses (40, 60 and 80 °C) of control experiments (without NiO NPs).

These results demonstrated that a number of stable protein peaks of *E. coli* were observed at m/z 2504, 3553, 3636, 4712, 5037, 5382, 5550, 7192, 7782, 8231, 9147, and 9437. These observed ion peaks are the same as reported in literatures [22–27]; however, the above described protein ions were not reported as stable protein peaks after the treatment of heat stress. MALDI-MS spectra of *E. coli* obtained from direct analysis (without any physical stress at room temperature) generated very few protein peaks [28] which were not sufficient for the detection of bacterial species compared to those of heat treated *E. coli*. We observed significant number of protein ions in the present study. Note that, not all the mass peaks could be detected from direct MALDI-MS analysis at room temperature.

These investigations were further verified in the presence of metal oxide nanoparticles like NiO (20 μg/mL) which show very less toxicity on *E. coli* [29]. NiO NPs have amphoteric properties in which they can act either as a Lewis acid or a Lewis base. The zeta potential of NiO NPs (isoelectric point 10.8) indicates that at low pH (below 10.8) of the sample, NiO NPs possess positive surface charge and carry anion-exchange properties owing to unsatisfied valencies of both oxygen and nickel atoms on the upper surface layers of nanoparticles [30,31]. These NPs show high binding affinity for polyoxy anions such as phosphate, carboxylate, and sulfate at pH < 10.8. The TEM images of NiO NPs with particle size distribution are shown in Fig. S4 (Supporting information). During different heat stresses on *E. coli*, the multiple cellular moieties adsorb on NiO nanoparticle surface (please see the inset of Fig. 5(D) and (F)) due to anion-exchange properties. So, the above experiments were further extended to obtain significant bacterial protein signals by using NiO NPs in MALDI–TOF MS. NiO NPs were used to enhance the protein peaks detection from direct MALDI-MS analysis. Figs. 1(b), 2(b), 3(b) and S2(b)–S3(b) of supporting information display the mass spectra where addition of NiO NPs with bacterial suspension containing *E. coli* (10^7 cells mL^{-1}) resulted in significant enhancement of bacterial protein peaks.

We searched the protein profiles to identify the peaks between m/z 2000 and 16,000 derived from the target cells. The mass peaks at m/z 2137, 2504, 3553, 4712, 5037, 5382, 5550, 7192,

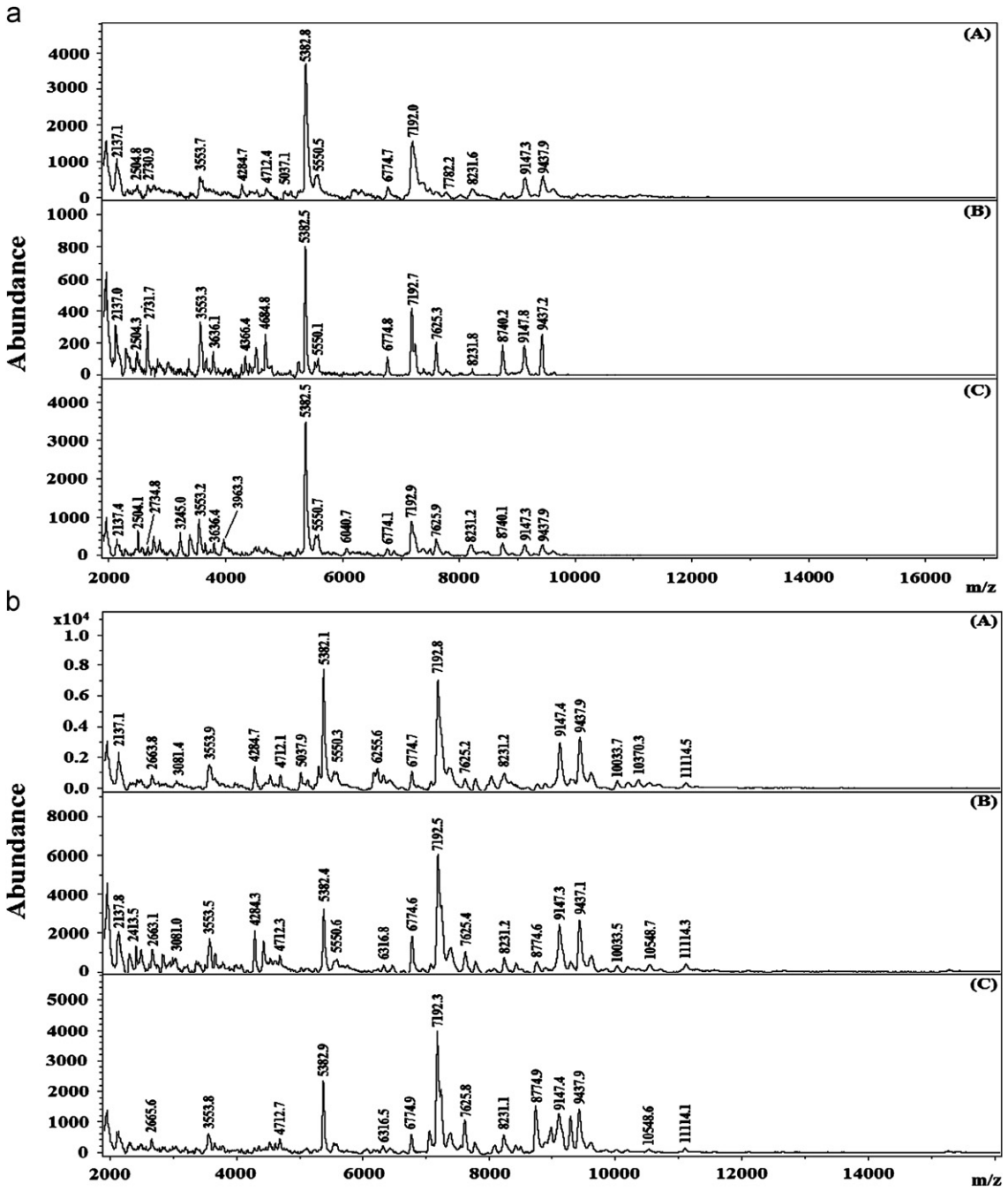

Fig. 1. MALDI mass spectra of stable protein ions of *E. coli* after different temperature treatments to the aqueous bacterial suspensions solution for 10 min (a) without NiO nanoparticles at (A) 40 °C, (B) 60 °C and (C) 80 °C and (b) With NiO nanoparticles at (A) 40 °C, (B) 60 °C and (C) 80 °C.

7782, 8231, 9147, 9437, and 10370 demonstrated significant signal enhancement (Figs. 1(b), 2(b), 3(b)). The chaperonin protein (groES) at *m*/*z* 10,370 (10 kDa) was constantly observed at temperatures 40, 60 and 80 °C with incubation from 10 to 60 min in MALDI-MS analysis. It was also found that during the stress response to NiO NP assisted MALDI-MS, the 10 kDa ion was significantly enhanced. The 10 kDa chaperonin (groES) is the principal protein operating both in the protection of proteins from denaturation and in the assembly of newly synthesized proteins. The property that keeps proteins in solution following heat treatment can be interpreted in three ways. First, the applied heat stress might not be sufficiently strong to break proteins folded structures due to proteins rigidity and stability which is the characteristic of thermophilic organisms' proteins. Second, heat treatment is sufficient to unfold the proteins and enhance their activities unless to reach denature state. Third, the protein may be unfolded after the application of heat stress but refolded to its normal structure when the temperature again dropped. In an attempt to investigate the dynamics of protein conformation on a proteomic scale, Park et al. [32] challenged the *E. coli* proteome with extensive proteolysis methodologies. They identified 22 survivors from digestion with trypsin and 34 survivors from digestion with thermolysin. It was suggested that many of these proteins might have evolved extreme proteolytic resistance because of their critical roles under heat stress. It seems that thermal stability of proteins and the property of proteolytic resistance are not related and might have evolved independently for their relevant functions. The obtained results also proved that the 10 kDa chaperonin protein

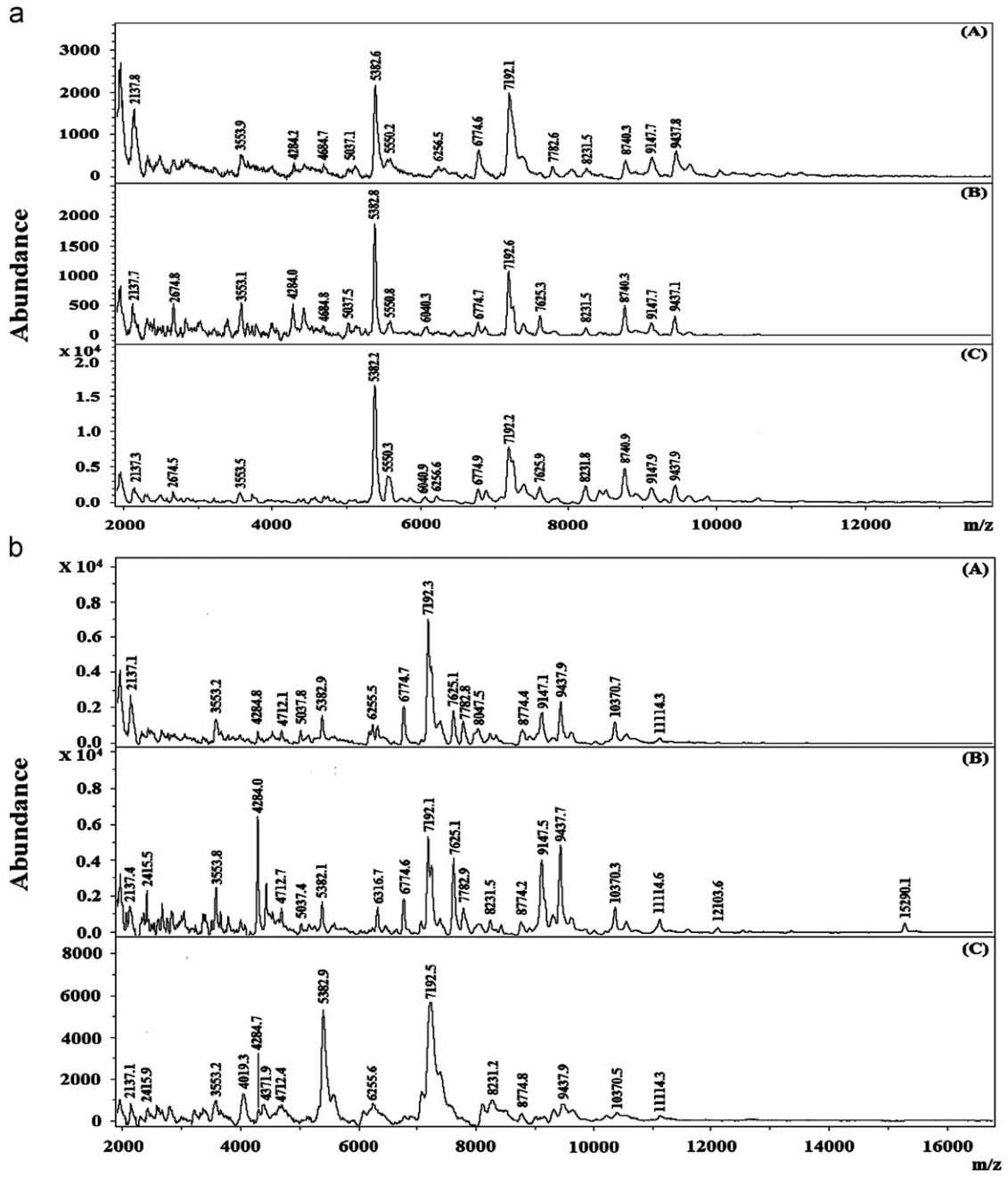

Fig. 2. MALDI mass spectra of stable protein ions of *E. coli* after different temperatures treatment to the aqueous bacterial suspensions for 30 min (a) without NiO nanoparticles at (A) 40 °C (B) 60 °C and (C) 80 °C and (b) with NiO nanoparticles at (A) 40 °C (B) 60 °C and (C) 80 °C.

(groES) disappeared at 40, 60 and 80 °C and with higher incubation of 90 min (Fig. S3(b), Supporting information). This revealed that some proteins can be destroyed at long incubation time. However, we observed good signals for protein ions after NiO NPs treatment in the MALDI–TOF MS. *E. coli* sustainabilities at different temperatures (40, 60 and 80 °C) with various incubation times (10, 20, 30, 60 and 90 min) were investigated by the plate counting method on LB agar plates. This has been shown in Fig. 4(a)–(e) in which graphs are plotted between log CFU/mL vs different temperatures.

The cell growth was not affected at 60 °C for 10, 20, 30 and 60 min of incubations but at the above described conditions of applied temperature for 90 min of incubation, the growth of *E. coli* was apparently suppressed on the LB plates. These cells mixed with NiO NPs showed inhibition at 60 °C for 60 min of incubation. The MALDI–TOF MS spectra results of *E. coli* also show significant differences with applied and nonapplied NiO NPs systems as shown in Fig. 3(a) and (b). However, the bacterial counts were not proportional to the expected MALDI–TOF MS results. This is due to the catalytic inhibitions of NiO NPs which are localized to membrane damage along with heat stress. Such damaged membranes could easily release huge amount of proteins and therefore in the MALDI–TOF MS spectra many more protein peaks were observed. But the damaged bacteria grown on the LB agar medium were counted after 60 min of incubation; since they were not able to recover at the specified incubation time, the cells' counts were suddenly suppressed (Fig. 4(d)). The cell colonies were also fully suppressed at higher incubation time (90 min) during analysis (Fig. 4(e)). This is due to complete lyses of bacterial cells at longer incubation periods. The MALDI–TOF MS results also showed minimal numbers of mass peaks. The obtained mass spectra as observed in Fig. S3(b)

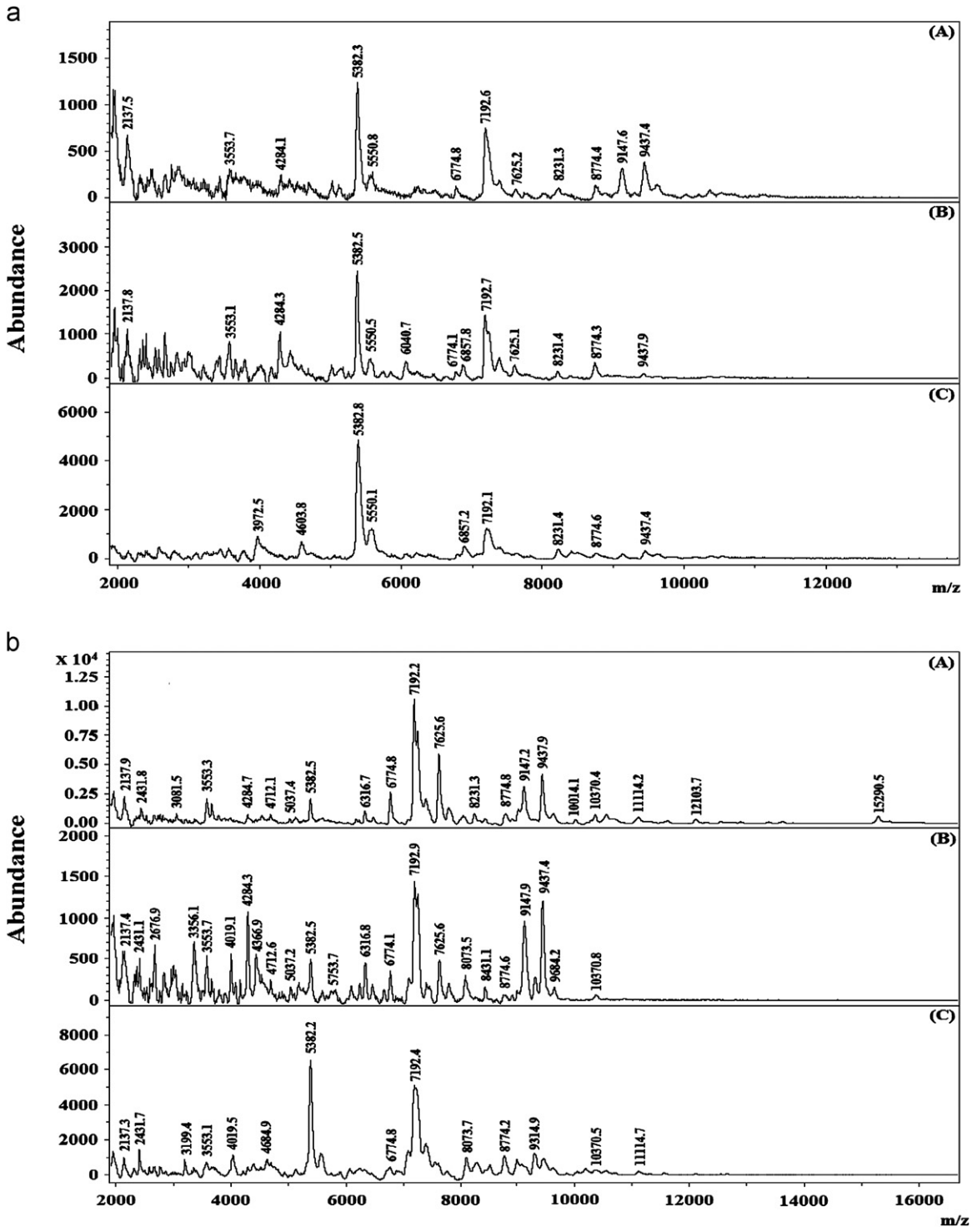

Fig. 3. MALDI mass spectra of stable protein ions of *E. coli* after different temperature treatments on the aqueous bacterial suspensions for 60 min (a) without NiO nanoparticles at (A) 40 °C (B) 60 °C and (C) 80 °C and (b) with NiO nanoparticles at (A) 40 °C (B) 60 °C and (C) 80 °C.

(Supporting information) indicated that it might be due to denaturation and loss of function of essential proteins. The rate of survival of bacteria was recorded in Fig. 4(a)–(e). *E. coli* is capable of growing (viable growth) in temperature range from 7.5 °C to 49 °C and optimum growth temperature is approximately 37 °C [33]. These results indicated that the exposure of *E. coli* to 40–60 °C for incubation periods of 10–60 min did not significantly affect their survival. At different heat stress conditions (40, 60 and 80 °C), *E. coli* cells were checked by transmission electron microscopy (TEM). The TEM images were determined to confirm the morphological changes on the surface of *E. coli* membrane as well as the NiO NPs attachment with bacteria and proteins. Fig. 5(A, C and E, without NiO NPs) and Fig. 5(B, D and F, with NiO NPs) indicate the TEM images of *E. coli* with different heat stresses at 40, 60 and 80 °C. The inset figure in Fig. 5(A) indicates the protection layer of the bacterial surface which was observed at 40 °C and 60 min of incubation. Similarly, at this stage NiO NPs show coagulation to the bacterial surface and sides. Consequently, higher temperature stressed cells excrete extracellular moieties in the presence of NiO NPs (please see Fig. 5(D) and (F)). It is found that the membrane clearly showed the lyses, when cells were

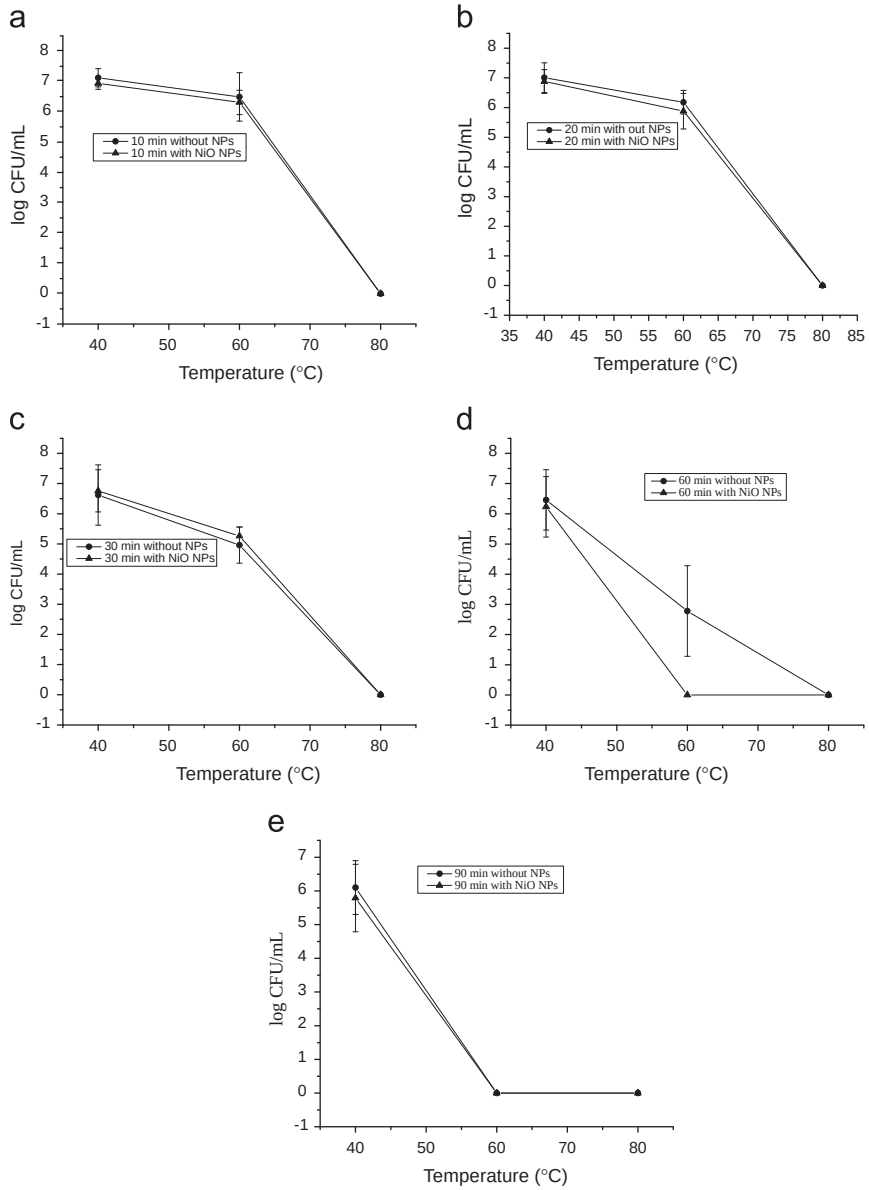

Fig. 4. Graphs showing *E. coli* cell survival after heat stress: the *E. coli* suspension alone (●) and mixed with NiO NPs (▲) were heat stressed at 40 °C, 60 °C and 80 °C for (a) 10 min, (b) 20 min, (c) 30 min, (d) 60 min and (e) 90 min. The cell colonies were counted on LB agar plate after 24 h incubation at 37 °C.

stressed at 80 °C (Fig. 5(E)) with incubation period of 60 min. This result supports the possibility of denaturation of proteins.

Thus, the MALDI mass spectra also produced fewer peaks compared to those of cells stressed at low temperatures for short periods of incubation. Note that, the cell surfaces of *E. coli* did not show significant changes at 40 and 60 °C (Figs. 5(A) and (C)). The NiO NPs attachment and enrichment onto the stress cell surfaces of *E. coli* and generated proteins were clearly observed. The detection sensitivity of NiO NPs toward membrane proteins was also confirmed from protein enrichment in the MALDI–TOF MS. These results indicate that application of heat stress with NiO NPs could be useful to detect the membrane proteins of bacteria with high degree of enrichment in bacterial and clinical research. The heat stress response in bacteria is affected by the following parameters: first, the alternative sigma factors (transcriptional activators) recognize specific heat shock promoters of upstream heat shock genes. Among these factors, σ^{32} and σ^{E} belongs to gram-negative bacteria and σ^{B} for gram-positive bacteria strain. Second, the system utilizes transcriptional repressors among them HrcA (Heat regulation at CIRCE) is most conserved and ubiquitous repressor. HrcA repressor binds to a conserved CIRCE and present upstream of the heat stress operons. These heat tress operons are controlled by HrcA-CIRCE are transcribed by the vegetative sigma factor σ^{A} ($=\sigma^{70}$) in gram-positive bacteria and by the heat stress sigma factor σ^{32} in gram-negative bacteria. The protein peaks in the *E. coli* were progressively reduced as the temperature increased and the number of protein peaks was minimal at 80 °C (Figs. 1(a), 2(a) and 3(a)). These results revealed that some proteins might be destroyed at the above conditions. NPs provide a high surface to volume ratio which can increase the capture efficiency of bacteria proteins. The NP affinity based separation techniques to selectively concentrate trace levels of bacteria from biological and food samples have been previously reported [34]. We also reported the use of various unmodified and modified NPs as affinity probes to increase the detection sensitivity of bacteria proteins and biomolecules in the MALDI–TOF MS [2,3,28,35,36]. NiO NPs can significantly enhance the detection sensitivity of biomolecules in the MALDI–TOF MS. These results are in agreement with the role of NiO NPs for significantly enhancing the detection sensitivity of bacterial proteins.

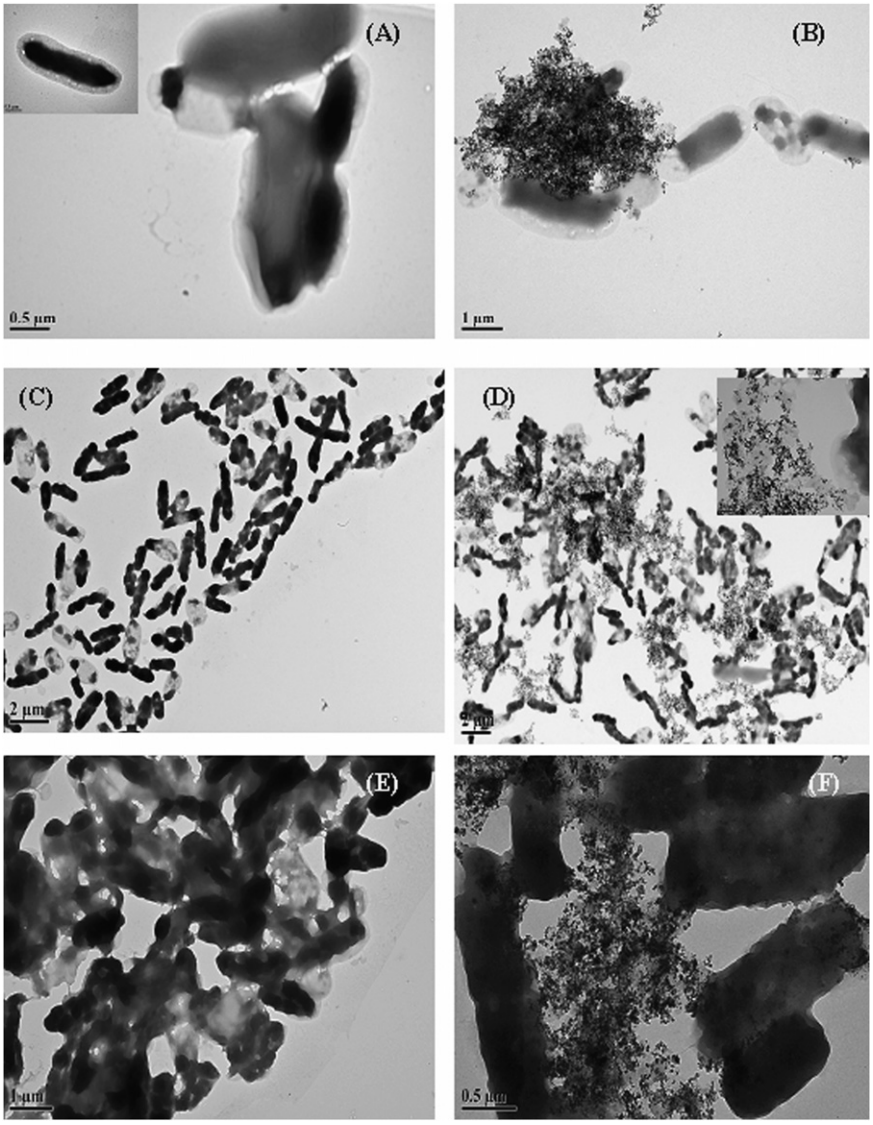

Fig. 5. TEM images of different heat treated *E. coli* cells at (A) 40 °C, (C) 60 °C and (E) 80 °C treated bacteria alone and (B) 40 °C, (D) 60 °C and (F) 80 °C treated bacteria in the presence of NiO nanoparticles.

4. Conclusion

The stress response applied to determine the stable protein peaks for rapid detection of *E. coli* using NiO assisted MALDI–TOF MS was demonstrated. In the presence of NiO NPs, *E. coli* on heat stress at different temperatures produced 10 kDa chaperonin (grosES) proteins which were successfully detected by MALDI–TOF MS. Further, the morphological changes of *E. coli* and NiO NPs interaction with bacterial proteins were explored using transmission electron microscopy. The current approach can greatly enhance the number and signal intensities of stable protein peaks with significantly higher resolution compared to those bacterial cells without using NiO NP treatment. It appears that the heat stability of these proteins may have evolved from their special functions, which allow them to maintain themselves in harsh conditions. Thus, we believe that this heat stress technique using NiO NP assisted MALDI-MS is a rapid, sensitive, straightforward and efficient approach to detect the stable proteins of bacteria in clinical and environmental samples under extremely harsh environmental conditions.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.10.003.

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