

Comparison on the molecular response profiles between nano zinc oxide (ZnO) particles and free zinc ion using a genome-wide toxicogenomics approach

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Abstract Increasing production and applications of nano zinc oxide particles (nano-ZnO) enhances the probability of its exposure in occupational and environmental settings, but toxicity studies are still limited. Taking the free Zn ion (Zn^{2+}) as a control, cytotoxicity of a commercially available nano-ZnO was assessed with a 6-h exposure in *Escherichia coli* (*E. coli*). The fitted dose-cytotoxicity curve for $ZnCl_2$ was significantly sharper than that from nano-ZnO. Then, a genome-wide gene expression profile following exposure to nano-ZnO was conducted by use of a live cell reporter assay system with library of 1820 modified green fluorescent protein (GFP)-expressing promoter reporter vectors constructed from *E. coli* K12 strains, which resulted in 387 significantly altered genes in bacterial ($p < 0.001$). These altered genes were

enriched into ten biological processing and two cell components ($p < 0.05$) terms through statistical hypergeometric testing, strongly suggesting that exposure to nano-ZnO would result a great disturbance on the functional gene product synthesis processing, such as translation, gene expression, RNA modification, and structural constituent of ribosome. The pattern of expression of 37 genes altered by nano-ZnO (fold change > 2) was different from the profile following exposure to 6 mg/L of free zinc ion. The result indicates that these two Zn forms might cause toxicity to bacterial in different modes of action. Our results underscore the importance of understanding the adverse effects elicited by nano-ZnO after entering aquatic environment.

Keywords Nanoparticle · Cytotoxicity · Gene expression · Gene set enrichment analysis · Pathways

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Introduction

Nano zinc oxide particles (nano-ZnO) are widely used in many different industrial and consumer products including sunscreen products (Schilling et al. 2010), feed industry (Sunder et al. 2007), rubber (Vladuta et al. 2010), and anti-bacterial agents (Brayner et al. 2006). Increased production and application of nano-ZnO enhances the probability of its exposure in occupational and environmental settings. Several studies have demonstrated toxicity of nano-ZnO in a wide array of organisms including bacteria, algae, yeast, protozoa, and zebrafish (Franklin et al. 2007; Kasemets et al. 2009; Mortimer et al. 2010; Zhu et al. 2008). However, there are contradictory reports, where the toxicity has been attributed to the potential dissolvability of nano-ZnO into free zinc ions (Zn^{2+}) (Brunner et al. 2006; Deng et al. 2009; Nel et al. 2006), while others suggested that dissolution of particles into Zn^{2+} is

not a major mechanism of cytotoxicity (Franklin et al. 2007; Lin et al. 2009; Moos et al. 2010; Yang et al. 2009). A recent study found differential toxicity of nano-ZnO in various aqueous media and suggested that the toxicity of nano-ZnO is mainly due to the free zinc ions and labile zinc complexes (Zhu et al. 2011). This ambiguity on the role of zinc ions as a contributory factor for all the induced toxicity by nano-ZnO has to be resolved. In general, the generation of reactive oxygen species (ROS) is believed to be a major contributor for the nanoparticle's toxicity. Interaction of nanoparticles with cells stimulates the cellular defense mechanisms to minimize damage. However, if ROS production exceeds the antioxidative defensive capacity of the cell, it may result in oxidative damage of biomolecules, which can lead to membrane rupture and then cell death (Carmody and Cotter 2001; Rytter et al. 2007). Studies of toxicity of nano-ZnO are still limited and the mechanism of toxicity is not completely understood.

Genome-wide gene profiling is a powerful technique that can be used to characterize the molecular mechanisms of toxicity caused by toxicants. Transcriptional responses, especially the time-course response, can be used to capture the molecular signaling pathways disturbed either directly by the toxicant molecular itself or indirectly by the cellular damage caused by toxicants. Because of its long history of laboratory culture and ease of manipulation, *Escherichia coli* (*E. coli*) is frequently used as a prokaryotic model organism in microbiology studies and has the most well-understood genome (Blattner et al. 1997), where it could serve as one of the best cell models to study molecular mechanisms of toxicity. Fusion of stress promoters to fluorescent transcriptional reporter (Zaslaver et al. 2006) provided a very useful toxicogenomic approach to characterize the toxicity mechanism of chemicals or environment toxicants as demonstrated in our previous study, which can be utilized for the genome-wide transcriptional investigations through the green fluorescent protein monitoring (Su et al. 2014, 2012, 2013; Zhang et al. 2011). The developed bioassays based on *E. coli* can provide the basic concept of cellular signal detection, which also avoids complex protocols of pretreatment, high-cost experimental materials, have less interference, and can provide temporal resolution, compared with microarray technology. Because of its rapid generation, that all gene signals can be observed in living organisms within just a few hours, makes the live cell array a rapid, economical, high-throughput biosensor system for detecting toxicity, and elucidating specific signaling pathways. Furthermore, the generated data is not bacteria-specific and can represent responses of systems that are conserved in multiple organisms, including metazoans (Zhang et al. 2011).

Despite its large production and applications, toxicity information of nano-ZnO is still limited. In the present study, cytotoxic effects of a commercially available nano-ZnO were compared with the free Zn ion (Zn^{2+}) following exposure of

E. coli for 6 h. Mechanisms of modulation of gene expression by both nano-ZnO and Zn^{2+} were further assessed using a live cell reporter assay system with a library of 1820 modified green fluorescent protein (GFP)-expressing promoter reporter vectors constructed from *E. coli* K12 strains.

Methods and materials

Chemicals Nano-ZnO was purchased from Vive Nano (#15010L, Toronto, ON, Canada), which has a strong UV absorption and was usually used for applications in ultra-small, water-dispersible UV-stable additives. To characterize cytotoxicity of free Zn^{2+} , zinc chloride ($ZnCl_2$) was purchased from Sigma-Aldrich (#SZBA2880V, St. Louis, MO, USA).

Quantification of free zinc After the nano-ZnO was dissolved into LB medium, the free Zn in the medium was determined following the protocol proposed by manufacturers. In brief, 500 μ L of media was placed on top of filtration membrane (cat. no. UFC501024, Pall Corporation, NY, USA) (Reyes et al. 2012) and centrifuged at 14,000 \times g. Then, nanoparticle would be blocked by the filtration membrane and the collected nanoparticle-free medium was analyzed for free Zn concentration with atomic absorption spectroscopy (Varian AA240 Duo, Palo Alto, CA, USA). In this research, ZnO particle suspensions in bacterial medium (50,000, 16,700, 5560, 1850, 617, 206, and 68.6 mg/L) were incubated for 6 h at a same temperature of 37 °C. Then, the medium samples were collected for a future released Zn content analysis.

Live cell array The microbial live cell array collection was purchased from Thermo Fisher Scientific (Huntsville, AL, USA), which includes more than 1800 out of 2500 promoters in the entire genome of *E. coli* K12 strain MG1655 developed by researchers at the Weizmann Institute of Science (Rehovot, Israel) (Zaslaver et al. 2006). Each of the reporter strains is coupled with a bright, fast-folding GFP fused to a full-length copy of an *E. coli* promoter in a low-copy plasmid. This enables rapid, accurate and reproducible measurement of gene expression. All clones from either live cell array or knockout collections were grown at 37 °C in LB-Lennox media plus 25 mg/L kanamycin.

Cytotoxicity A stock solution of nano-ZnO (50,000 mg/L) was prepared in LB medium, and other working solutions were prepared by serial dilution with LB medium. Thirteen different concentrations of nano-ZnO (50,000, 16,700, 5560, 1850, 618, 206, 68.6, 23, 7.6, 2.5, 0.85, 0.28, and 0.94 mg/L, respectively) ($n=3$) were used in the *E. coli* cytotoxicity test.

Similarly, nine different concentrations of ZnCl_2 (25,000, 8330, 2780, 926, 309, 103, 34.3, 11.4, and 3.8 mg/L) ($n=3$) were used in the *E. coli* cytotoxicity test. The bacterial treated with fresh LB medium was regarded as positive control. The negative control was observed in LB medium without bacterial. After 5 h of incubation at 37 °C, 10 μL of Alamar blue (Beijing CellChip Biotechnology Inc., Beijing, China) was added to 150 μL LB medium for each well to assess cell viability. After 1-h incubation with Alamar blue, the blue-red fluorescence was detected by a Synergy H4 hybrid microplate reader (exCitation/emission 545/590 nm) (BioTek Instruments Inc., Winooski, VT).

Gene expression observation Gene expression was observed in two separate rounds. In the first round, strains of *E. coli* were inoculated into a fresh 96-well plate from a 96-well stock plate by use of disposable replicators (Genetix, San Jose, CA, USA). To minimize bias due to differential expression among different growth phase of bacterial (Beyer-Sehlmeyer et al. 2005; Bradley et al. 2007), cells were incubated at 37 °C in 300 μL LB medium for 3 h in 96-well plates. Then, 72 μL bacterial medium was transferred into each well of 384-well plate. In each 384-well plate, the eight positive control wells were inoculated with promoterless strains, and the four negative control wells were filled with fresh medium. Finally, 3.79 μL of pure water (solvent control) or chemical stock solutions were added into individual wells on the 384-well plate to make final concentration of 0, 1, 10, and 100 mg nano-ZnO/L. GFP intensity of each well was consecutively monitored every 10 min for 6 h by use of a Synergy H4 hybrid microplate reader (exCitation/emission 485/528 nm) (BioTek Instruments Inc., Winooski, VT, USA). Then, all the altered genes selected in the first round (fold change >2) were taken out from the stock plate to run for the second time. Only the genes, differently expressed in both of rounds, were considered to be responsible for the nano-ZnO's potential genotoxicology.

Data analysis For cytotoxicity, three technical replicates per concentration of nano-ZnO and ZnCl_2 were included. After correction for background, the blue-red fluorescence of each treatment was normalized to a percent response value expressed relative to the response elicited by the controls. Both S-curve visualization and ECx calculation were conducted using the “drc” package in R 3.0.2 version software. For gene expression analysis, a linear regression model was applied to select the promoter reporters of which expression was significantly differentiated relative to exposure to the chemicals. The response measured as GFP fluorescence was fitted to a function of time for each promoter reporter strain. Through the data analysis process, a p value less than 0.001 was considered significant. All the data analysis procedures

have been described previously (Su et al. 2012; Zhang et al. 2011). Gene set enrichment analysis (GSEA) was performed on R 3.0.2 version using “GOstats” package, and the R script had been tailored to the specific organism *E. coli*.

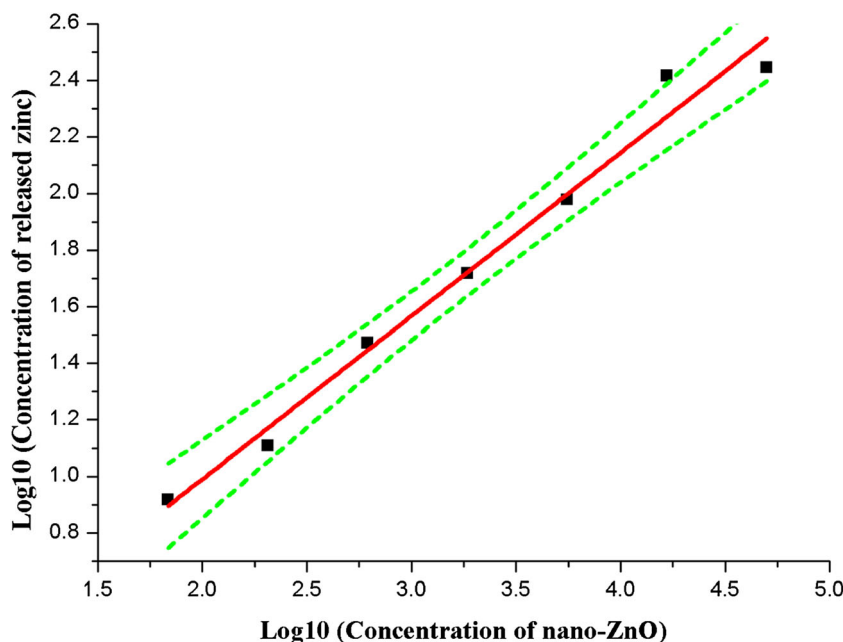
Results

Characteristics of nano-ZnO The commercially available nano-ZnO used in this study is being extensively used as UV-stable additives owing to their strong UV absorption. It is likely that they might be released into environment and cause exposure risk to organisms, even humans. These particles were water-dispersible and stabilized by polyacrylate sodium. Zinc content was determined to be 0.105 ± 0.002 g Zn/g nano-ZnO. All the concentration units in this manuscript were standardized as Zn. Particle size of nano-ZnO was determined to be 1–10 nm by transmission electron microscopy (TEM) (Fig. S1). After dissolving into LB medium, particle size of the nano-ZnO was also characterized at a concentration range of 10–10,000 mg/L, and a slight agglomeration was observed at concentrations of 1000 and 10,000 mg/L (Fig. S2). The zeta potential of particles (100 mg/L) in LB medium was determined to be -36.4 ± 1.65 mV, which indicated that the nano-ZnO had stable surface charge in solution phase. The released Zn content from nano-ZnO (concentrations 68.6–50,000 mg/L) were 8.26–279 mg/L after a 6-h incubation at 37 °C (Table S3). Most interestingly, the relationships between the released Zn and concentration of nano-ZnO can be fitted with either Freundlich ($R^2=0.9797$, Fig. 1) or Langmuir ($R^2=0.9557$, Fig. S3) equations very well.

Cytotoxicity ZnCl_2 and nano-ZnO exhibited different cytotoxicity profiles after a 6-h exposure in *E. coli* (Fig. 2). The EC_{20} to EC_{90} ranges for Zn in nano-ZnO and ZnCl_2 from 92.1 ± 7.1 to 2330 ± 398 mg Zn/L ($\text{EC}_{90}/\text{EC}_{20}$ ratio 25.3) and 23.7 ± 3.4 to 97.5 ± 14.4 mg Zn/L ($\text{EC}_{90}/\text{EC}_{20}$ ratio 4.1), respectively (Table S1). The median effect concentrations (EC_{50}) of nano-ZnO and ZnCl_2 were 321 ± 22.7 and 41.0 ± 2.9 mg Zn/L, respectively.

Gene expression profiles Three concentrations, 1, 10, and 100 mg/nano-ZnO/L (0.105, 1.05, and 10.5 mg Zn/L), were chosen to examine the transcriptional expression profiles of *E. coli* (Fig. 3). After exposure to nano-ZnO for 6 h, 387 genes in bacteria were altered significantly ($p < 0.001$). Regarding the biological process (BP), a statistical hypergeometric test was applied for these selected genes, and ten gene ontology (GO) terms were enriched relative to the full set of genes in the whole genome (Table 1). Translation, gene expression, and RNA modification were primary GO terms ($p < 0.005$) affected. While applied in cellular component (CC), two GO terms, structural molecule activity and structural

Fig. 1 Concentrations of zinc released from nano-ZnO solution. Before the Zn content determination, the nano-ZnO suspensions (50,000, 16,700, 5560, 1850, 617, 206, and 68.6 mg/L) were incubated for 6 h at a temperature of 37 °C. The fitted curve was based on Freundlich equation, which was expressed as $y=0.58x-0.16$, adjusted $R^2=0.9797$



constituent of ribosome, were considered to be enriched after exposure to nano-ZnO (Table S2). A total of 37 of 387 genes were selected by use of a twofold cutoff ($p<0.0001$). The number of genes upregulated by nano-ZnO was similar to that of the downregulated genes. Of the 37 genes selected using a twofold cutoff, exposure to nano-ZnO resulted in upregulation of 18 and downregulation of 19 genes, which had been classified into two clear expression groups by using

ToxClust. Interestingly, a subcluster of genes upregulated by nano-ZnO, including *b0671*, *yhbQ*, *yejK*, *ybgH*, *ynfA*, *fryB*, *rpmE*, were altered strongly in the first several hours, but returned to normal in late stages of exposure.

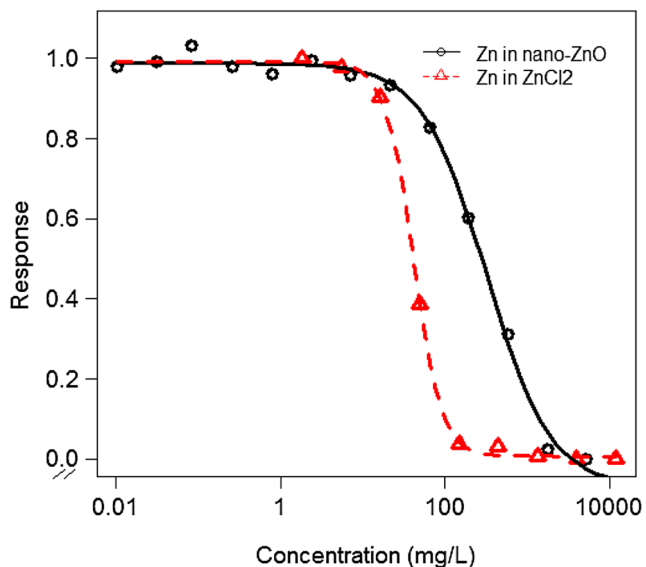


Fig. 2 Inhibition of cell division of *E. coli* by concentrations of Zn in nano-ZnO and ZnCl₂ after a 6-h exposure. The curve was fitted by use of a general model fitting function “drm (drc)” in R software, which relies on the general multipurpose optimizer function optim for the minimization of minus log likelihood function. Data points on the fitted curve represent the mean of three values

Gene expression profile comparison between nano-ZnO and Zn²⁺ Concentration of free Zn²⁺ was determined to be approximately 6 mg/L in nano-ZnO solution of 100 mg/L (Fig. S4). To describe the mechanistic differences between nano-ZnO and Zn²⁺, 37 genes, those were altered by nano-ZnO, were also examined following exposure to 0.06, 0.6, and 6 mg/L of Zn²⁺ ion (Figs. 4 and S5). Using a twofold change cutoff, 22 of 37 genes were altered significantly by free Zn²⁺. Ten genes, *b0671*, *dacB*, *efp*, *elaB*, *sieB*, *yccA*, *ycgF*, *ynfA*, *yohL*, and *ytfM*, showed same regulation directions following exposure to Zn²⁺ or nano-ZnO. However, the other 12 genes, *allS*, *ghrA*, *selD*, *slyA*, *tolB*, *yafD*, *ycjM*, *yejK*, *yggH*, *yhbQ*, *yibK*, and *yncE*, can also be dysregulated by these two types of zinc, but showed differential regulation directions. Specifically, *allS*, *ghrA*, *selD*, *slyA*, *tolB*, *ycjM*, and *yggH* were downregulated by free zinc ion, but upregulated by nano-ZnO. In contrast, *yafD*, *yejK*, *yhbQ*, *yibK*, and *yncE* were upregulated by free zinc ion, but downregulated by nano-ZnO. Overall, gene expression profiles in *E. coli* following exposure to nano-ZnO or free zinc ion were in two different patterns.

Discussion

The relationship between Zn released and concentration of nano-ZnO has been discussed in some previous publications,

Fig. 3 Real-time, quantitative determination of gene expression as measures of differentially expressed promoter activities in *E. coli*. Clustering of time-dependent expression of genes altered by nano-ZnO as selected by a twofold change cutoff ($p < 0.001$). Exposures to 1, 10, and 100 mg nano-ZnO/L were represented by the lower, middle, and upper bands in each gene column. Classification and visualization of gene expression were derived by using ToxClust. The dissimilarity between genes was calculated by the Manhattan distance between the gene expressions at all the concentration versus time combinations. The fold change of gene expression is indicated by color gradient, and the time course of expression changes is indicated from left to right

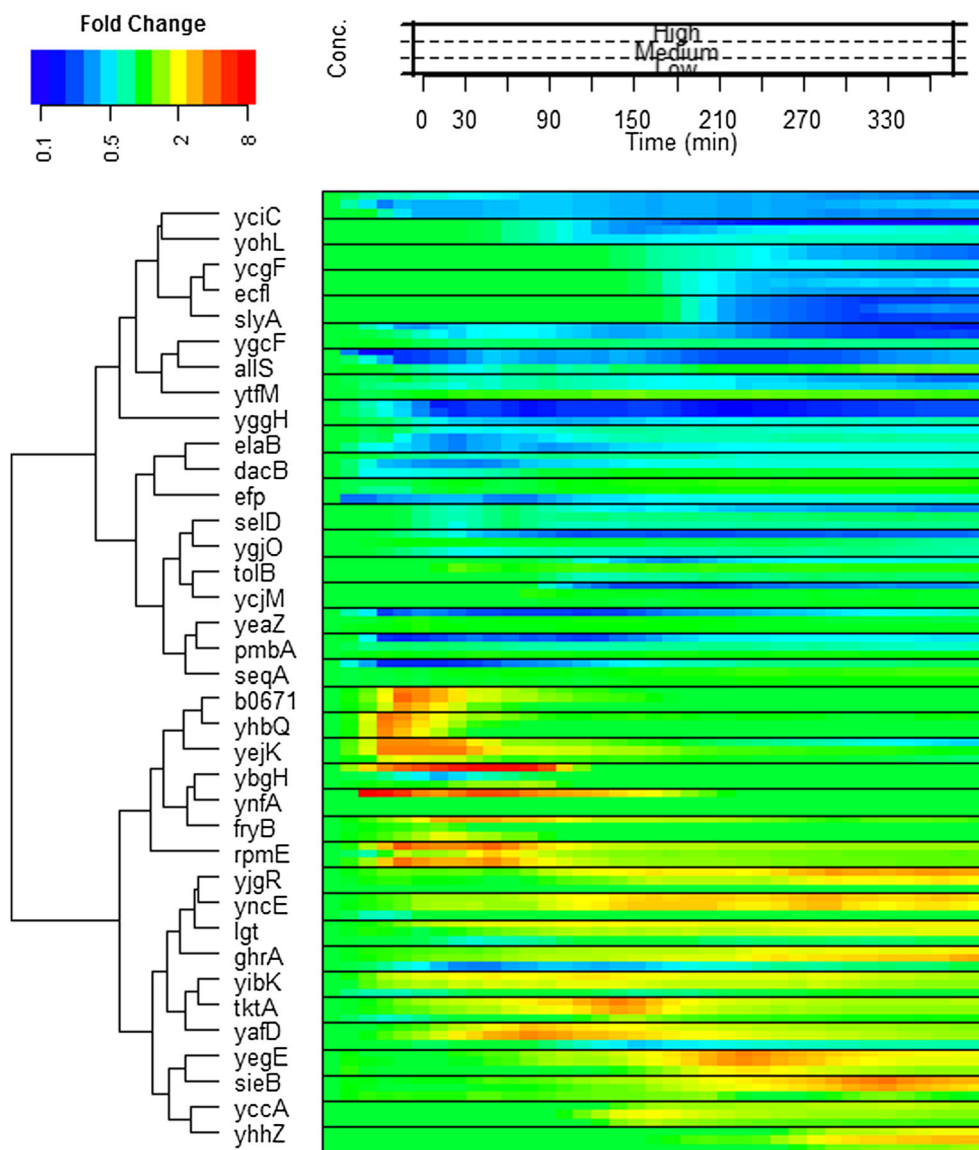
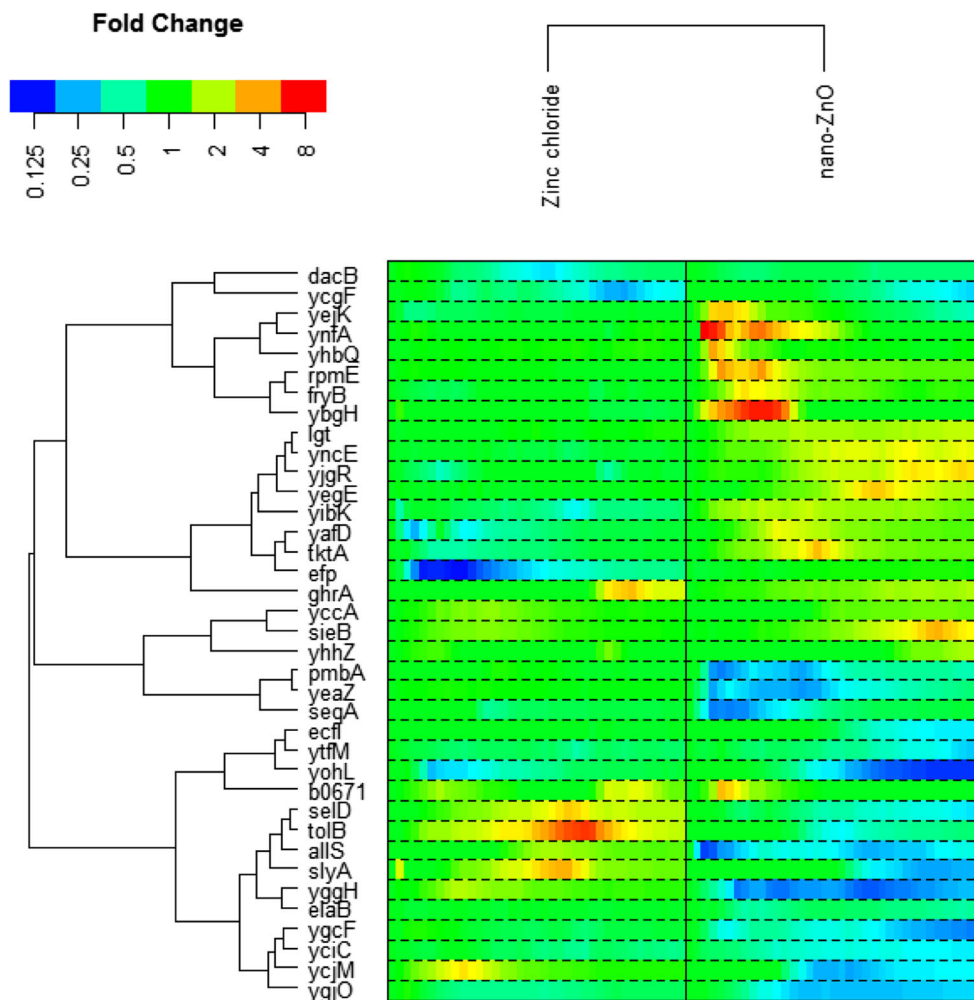


Table 1 Gene set enrichment analysis based on 387 genes altered by nano-ZnO against the live cell array library

	GO BP ID	<i>p</i> Value	Odds ratio	Exp count	Count	Size	Term
1	GO:0006412	0.001	2.918	9.558	19	46	Translation
2	GO:0010467	0.003	2.175	15.168	25	73	Gene expression
3	GO:0009451	0.004	3.222	5.610	12	27	RNA modification
4	GO:0019538	0.012	1.706	25.972	36	125	Protein metabolic process
5	GO:0043412	0.018	1.977	12.051	19	58	Macromolecule modification
6	GO:0044260	0.035	1.423	54.853	65	264	Cellular macromolecule metabolic process
7	GO:0034645	0.035	1.542	26.803	35	129	Cellular macromolecule biosynthetic process
8	GO:0044267	0.045	1.528	24.517	32	118	cellular protein metabolic process
9	GO:0043170	0.048	1.381	57.554	67	277	Macromolecule metabolic process
10	GO:0016070	0.049	1.659	14.960	21	72	RNA metabolic process

The association of gene ontology was performed by R 3.0.2 version using “GOstats” package. The universe and selected genes were defined according 1820 genes in the live cell array library and 387 selected genes, respectively. The analysis was conducted basing on the biological processing (BP) GO ontology

Fig. 4 Comparison of gene expression profiling in *E. coli* following exposure to 100 mg nano-ZnO/L (right) or 6 mg zinc ions/L (left)



which showed that concentrations of released Zn trended toward equilibrium at a specific concentration (Song et al. 2010). Here, free zinc from nano-ZnO followed a similar pattern and seemed to trend to equilibrium while the concentration of nano-ZnO went higher than 16,700 mg/L. Most interestingly, it was found that the relationship between the released Zn and concentration of nano-ZnO was fitted with either Freundlich (adjusted $R^2=0.9797$) or Langmuir (adjusted $R^2=0.9557$) equations very well. Technically, Freundlich equation is related with the concentration of a solute on the surface of an adsorbent, to the concentration of the solute in the liquid (Yang 1998), and the Langmuir isotherm relates the coverage or adsorption of molecules on a solid surface to gas pressure or concentration of a medium above the solid surface at a fixed temperature (Goto et al. 2008). The high correlation coefficient between the free Zn and its parent nano-ZnO suggests a potential surface interaction between them, but more research is needed.

The fitted curve for $ZnCl_2$ is significantly sharper than that from nano-ZnO, which indicates that these two forms of Zn might cause toxicity to bacteria in different modes of action.

Uptake of nano-ZnO by *E. coli* cells was reported in some previous studies using TEM (Brayner et al. 2006; Huang et al. 2008; Tama et al. 2008). Kumar et al. also demonstrated the uptake of nano-ZnO in *S. typhimurium* using flow cytometry (Kumar et al. 2011). Results of our study with nano-ZnO and $ZnCl_2$ further supports the uptake and internalization of nanoparticles into cells. Here, cytotoxicity of Zn in $ZnCl_2$ was comparable to that from previous publications (Reyes et al. 2012; Zhu et al. 2011); however, Zn in nano-ZnO (321 ± 22.7 mg Zn/L) was less toxic than that (68.4 ± 6.6 mg Zn/L) in other nano-ZnO particles (Reyes et al. 2012), which is likely due in part to the particle size and stabilizer.

Because of the widespread use of nano-ZnO in various commercial products, great concern was raised on their potential adverse effects. Although toxicity of nano-ZnO had been observed in a wide array of organisms including bacteria, algae, yeast, protozoa, and zebrafish (Franklin et al. 2007; Kasemets et al. 2009; Mortimer et al. 2010; Zhu et al. 2008), the mechanism of toxicity is not completely understood. To our knowledge, this is the first report to study nano-ZnO's toxicity by use of a genome-wide transcriptional investigation

approach. Based on results of hypergeometric testing, these 387 altered genes were enriched into ten GO BP and two CC terms, five of which (structural molecule activity, structural constituent of ribosome, gene expression, RNA modification) were considered to be enriched most significantly with a *p* value less than 0.005. Actually, disturbance of translation in *Arabidopsis thaliana* roots had been reported previously exposed to nano-ZnO (Landa et al. 2012). Translation refers to a process that messenger RNA is translated into proteins in *E. coli* (Ross and Orłowski 1982), which indicated that nano-ZnO, just like some antibiotics, might exert their action by targeting the translation process in bacteria. Structural constituent of ribosome refers the actions of a molecule that contributes to the structural integrity of the ribosome. This GO term usually co-occurs with the “translation” process and is also known as one child term of structural molecule activity. These three terms might suggest that ZnO particle damage on ribosomes since the ribosome is also the place where the proteins are translated from RNA. “Gene expression” term involved in production of an RNA transcript as well as any processing to produce mature RNA or mRNA (for protein-coding genes) and translation of that mRNA into protein, which indicated that it would partly co-occur with the “translation” process. The “RNA modification” was defined as covalent alteration of specific nucleotides within an RNA molecule to produce a molecule of RNA with a sequence that differs from that coded genetically, which also posed another potential toxicity pathway of nano-ZnO.

The time- and concentration-dependent manner for expression of genes in *E. coli* has also been observed (Gou et al. 2010; Su et al. 2012). This can contribute to the calculation of transcriptional endpoints, such as no observed transcriptional effect concentration (NOTEC) and median transcriptional effect concentration (TEC₅₀). These factors had been proved to be more sensitive endpoints to assess chemical toxicity (Gou et al. 2010; Su et al. 2012) and reflect sublethal, molecular responses to a toxicant, especially for an industrial chemical/agent (such as nanoparticles) which is generally not expected to generate any beneficial biological effects through “accidental” exposure other than its industrial purpose. However, some limitations should be considered seriously before this approach is used in a regulatory context, such as the genes’ self-repair mechanisms, which were observed in bacteria exposed to nano-ZnO. As can see from the gene expression profile, some genes were altered by nano-ZnO in the first several hours, but returned to normal in the late stage. Similar results were also found in previous reports (Tuomela et al. 2013). Two of 37 altered genes, *slyA* and *alls*, were known to be transcriptional regulators, which normally bound to specific DNA sequences to control the transcription of genetic information (Latchman 1997). *slyA*, known as a MarR

family transcriptional factor, controls an assortment of biological functions in several animal-pathogenic bacteria (Haque et al. 2009). *alls* belongs to the putative transcriptional regulator LYSR-type family, which represents the most abundant type of transcriptional regulator in the prokaryotic organisms (Maddocks and Oyston 2008).

The differential patterns of gene expression in *E. coli* following exposure to nano-ZnO and free zinc ion suggested that different pathways were affected by these two chemicals and their modes of toxic action might be different. In fact, contradictions exist in terms of toxicity caused by nano-ZnO. Some researchers state that its toxicity has been attributed to the potential dissolvability of nano-ZnO into free zinc ions (Zn²⁺) (Brunner et al. 2006; Deng et al. 2009; Nel et al. 2006), while others suggested that dissolution of nano-ZnO into Zn²⁺ is not a major mechanism of cytotoxicity (Franklin et al. 2007; Lin et al. 2009; Moos et al. 2010; Yang et al. 2009). This ambiguity on the role of zinc ions as a contributory factor for all the induced toxicity by nano-ZnO need to be resolved. A recent study exhibited differential toxicity of nano-ZnO in various aqueous media and suggested that the toxicity of nano-ZnO is mainly due to free zinc ions and labile zinc complexes (Zhu et al. 2011). Results present here suggest these two chemicals might cause toxicity to bacterial but in two different manners.

Conclusions

Overall, we demonstrated that differences in cytotoxicity and profiles of expression of genes in bacteria exposed to two types of Zn from nano-ZnO (specifically Nano-ZnO) and ZnCl₂, indicating that nano-ZnO potentially causes toxicity to *E. coli* via different pathways compared to that of ZnCl₂. Then, a genome-wide approach, the live cell reporter assay system with library of 1820 modified GFP-expressing promoter reporter vectors constructed from *E. coli* K12 strains, was employed to assess the nano-ZnO’s toxicity mechanisms. Results suggested that ZnO is likely to cause toxicity to *E. coli* through several specific functional gene products synthesis processing, such as translation, gene expression, RNA modification, and structural constituent of ribosome. Further efforts might be made on the toxicity assessment of nano-ZnO exposed to eukaryotic organisms to validate whether the observed adverse effects were conserved.

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Conflict of interest The authors declare no competing financial interest.

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