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Biosorption behavior and proteomic analysis of *Escherichia coli* P4 under cadmium stress

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HIGHLIGHTS

- Repression of succinyl-CoA ligase indicated down regulation of carbohydrate metabolism.
- Over-expression of Mn-SOD provided evidence that Cd^{+2} exposure induces superoxide free radicals.
- Expressed proteins are involved in cellular redox homeostasis, and carbohydrate catabolism.
- EnvZ/OmpR -a regulatory system observed operating to homeostat the cell's internal environment.

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ABSTRACT

Bacteria develop a variety of adaptations at transcriptomic, metabolomic and proteomic levels in order to survive potentially damaging environmental perturbations. Present study is exploring the fluctuations in proteome of *E. coli* P4 to knob Cd^{+2}-induced cytotoxicity. An attempt was also made to integrate all these approaches to gain comprehensive insight of Cd^{+2} stress response in *E. coli* P4. This study is exposing the altered behavior of various proteins and their underlying metabolic pathways which have previously not been reported with reference to Cd^{+2} stress such as sulfoquinovose biosynthesis and degradation pathway. Some of the responses studied on all integrated levels followed same dynamics and strategies to conserve energy by down regulating carbohydrate metabolism (depicted by the repression of succinyl-CoA ligase) and growth stasis (down regulation of *ftsZ*). Moreover, proteomic analysis clearly revealed the affection of Cd^{+2} stress on various proteins expression including Rrf, MdaB, DapA, GpmA,Cdd, FabI, DsbA, ZnuA and YihW found modulating key cellular metabolic pathways enabling *E. coli* P4 to withstand Cd^{+2}-induced toxic effects. Furthermore, over-expression of Mn-SOD provided evidence that Cd^{+2}exposure induces superoxide free radicals mediated oxidative stress rather than hydrogen peroxide (H_2O_2). EnvZ/OmpR -a two component cell envelope regulatory system was observed operating to homeostat the cell's internal environment. Cd^{+2} bioremediation potential of *E. coli* P4 and its kinetic and thermodynamic basis were studied by applying different isotherm models which nominated *E. coli* P4 a good bioresource for green chemistry to eradicate environmental Cd^{+2}.

1. Introduction

Survival of bacteria in a stressful environment is coupled with physiological responses at transcriptomic and proteomic levels. Transcriptomic stress responses are transitory and take the cell to a new homeostatic state similar to normal cells even under stress conditions (López-Maury et al., 2008). *Escherichia coli* has been extensively investigated for stress responses due to its various evolutionary adaptations to sense and respond to a variety of environmental perturbations to which it comes in contact during its life span (Phadtare and Inouye, 2004; Wick and Egli, 2004; Gadgil et al., 2005; Durfee et al., 2008). Some stress responses including up/down regulation of genes, growth stasis and ribosome biogenesis are same irrespective of stress nature and categorized as

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general responses, whereas some responses are regarded specific as these are unique and coupled with certain type of stress factors (Storz and Hengge-Aronis, 2000). Stress responses in bacteria including *E. coli* against heavy metals, cadmium (Cd^{+2}) in particular, are combinations of general and specific stress responses and play a key role in the regulation of various vital cellular processes. Cd^{+2} is the main culprit detected tempering the expression of a number of genes either by silencing or inducing them. Generally the genes related to energy conservation, ROS detoxification, protein folding, stringent response and DNA repair are induced whereas genes involve in energy consumption, growth, nucleotide and ribosome biosynthesis are repressed (Wang and Crowley, 2005; Khan et al., 2015a). Activation of *smt* (metallothionein) and efflux genes has been reported constituting a specific stress response against certain heavy metals including Cd^{+2} in *E. coli* (Wang and Crowley, 2005).

Transcriptional regulation is crucial as it is the first step in a series of reactions which ultimately lead to protein synthesis. This regulation is primarily mediated through σ^s (RpoS- RNA polymerase subunit) and transcription factors (TFs). σ^s is RNA polymerase subunit and has been reported to modulate the expression of more than 140 genes involved in various homeostatic functions (Weber et al., 2005). There exist huge numbers of transcription factors that govern the differential expression of genes or gene cluster under different contexts.

Presently, there is very little information about the molecular basis of *E. coli* response to environmental perturbations as the majority of global analyses are limited to transcriptional profiling. Other mature and effective approaches such as proteomics and metabolomics are largely missing (Brauer et al., 2006; Jozefczuk et al., 2010). This is also a fact that integration and parallel investigation of different molecular approaches such as transcriptome, metabolome and proteome have not been the leading emphasis of researchers (Bradley et al., 2009). Now it has grown into one of the focal challenges of cell biology and functional genomics to integrate these different molecular approaches to get a better insight of cellular response.

In the current investigation, proteomic analysis approach is employed to explore dynamic variations in *E. coli* global proteome response against Cd^{+2} stress. This task is accomplished by two-dimensional gel electrophoresis (2DE) and mass spectrometry. Moreover, bioremediation potential of *E. coli* P4 to exterminate environmental Cd^{+2} was also evaluated by applying thermodynamics, kinetics and adsorption isotherm models (Langmuir and Freundlich models).

2. Materials and methods

2.1. Strain and culturing medium

Luria-Bertani (LB) agar (1% NaCl, 1% tryptone, 0.5% yeast extract and 1.5% agar) medium was used for culturing and maintenance of *E. coli* P4 at 37 °C and pH of 7 (Khan et al., 2015b) (Fig. S1). Bacterial strain growth was obtained after incubation of 24 h.

2.2. Proteomic analysis

2.2.1. Sample preparation

Bacterial isolate was grown in minimal salt medium (MSM) in the absence (control) and presence of 10 mM Cd^{+2} (treated) overnight at optimum growth conditions. Cell pellets were harvested by centrifugation at 14,000 rpm for 10 min. The pellets were washed with 1X PBS and re-suspended in appropriate amount of lysis buffer (10% SDS, 0.5% CHAPS, 2.3% DTT, phosphatase and protease inhibitors), sonicated 4–6 times on ice for 15 s with 30 s intervals at 50 W and spun at 14,000 rpm at 4 °C for 15 min and stored at −20 °C until further usage.

2.2.2. Protein estimation

The protein quantity in the samples was measured by protein assay based on Bradford method (Bradford, 1976) using bovine serum albumin (BSA) as a standard. BSA stock solution (0.5 mg/ml) was used to prepare different BSA dilutions in 1X phosphate buffer saline pH 7.4 (0.024% KH_2PO_4, 0.02% KCl, 0.8% NaCl and 0.14% Na_2HPO_4). Each dilution was mixed with Bradford reagent (Bio-Rad), mixed well and incubated for 10 min at room temperature. The absorbance was taken at 595 nm using blank solution (800 μl buffer and 200 μl dye) as a control to prepare BSA standard curve which was used to estimate protein concentration in the samples.

2.2.3. First dimension electrophoresis

Proteins were resolved depending on their isoelectric points in first dimension electrophoresis by using (7 cm) immobilized pH gradient (IPG) strips (Bio-Rad). IPG strip, ranged 3–10 in pH, was loaded with 150 μl sample volume as 200 μg protein for Coomassie staining and 40 μg for silver staining found suitable for 7 cm strip. Each strip, carrying 150 μl total sample volume, was rehydrated in Bio-Rad re-swelling cassette with rehydration solution (2.5 M thiourea, 8 M urea, 0.5% ampholytes, 4% CHAPS and 66 mM DTT), removed protective film, placed the strip onto the cassette (gel side down) avoiding air bubbles and incubated at room temperature for 1 h. Then mineral oil was applied on each strip to prevent dehydration. For pre-focusing, ISO setup was run for 1 h at 200 V, ramping for 2 h at 500 V and final for 5 h at 4000 V to achieve 20,000 Vh.

Prior to undergo second dimension electrophoresis, each IPG strip was incubated in equilibration buffer I (0.375 M Tris pH 8.8, 30% glycerine, 6 M urea, 2% DTT and 2% SDS) and equilibration buffer II (0.375 M Tris pH 8.8, 30% glycerine, 6 M urea, 2.5% IAA, BPB in traces and 2% SDS) for 25 min each to lessen disulfide bonds and to inactivate thiol groups of cysteine residues.

2.2.4. Second dimension electrophoresis

Each strip was placed on the top of 12% polyacrylamide gel (vertical) and overlaid with agarose (1%) prepared in SDS tank buffer. The gel was also loaded with protein marker and run at 100 V for about 2 h at 4 °C. Then gel was removed and stained.

2.2.5. Gel staining and imaging

For silver staining, gel was immediately placed in formaldehyde fixer solution containing 1% acetic acid. Further steps were same as described by Blum et al. (1987). Silver stained gels were scanned with a Gel CanoScan 8400F (Canon, Tokyo, Japan). Delta 2D software version 3.6 (Decodon GmbH, Greifswald, Germany) was used for densitometric studies. The experiment was carried out in triplicate and three gels were prepared for three protein extractions. Differences in protein spots were detected by densitometric software and were statistically evaluated using unpaired Student's *t*-test. Means and standard deviations were calculated from three independent sets of experiments. The differences in protein expression with p-value < 0.05 were considered significant. Selected spots were excised and processed by in-gel protein digestion followed by mass spectrometric identification of the extracted peptides.

2.2.6. In-gel protein digestion

For in-gel digestion, selected bands were excised from silver stained gel, destained with 50 mM sodium thiosulfate/15 mM potassium ferricyanide, equilibrated with 50% acetonitrile/50 mM ammonium bicarbonate, dried under vaccume concentrator (SpeedVac SVC100 Farmingdale, NY) and rehydrated with 100 mM