BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Hypothesis

Volume 9(9)

Insights from the molecular characterization of mercury stress proteins identified by proteomics in *E.coli* nissle 1917

Panthangi Seshapani[1], Daddam Jayasimha Rayalu [2], Vadde Kiran Kumar[3], Kathera Chandra Sekhar[1] & Jasti Pramoda Kumari[1]*

[1]Department of Microbiology, Sri Venkateswara University, Tirupati-517 502, A.P; [2]Department of Bioinformatics, Global Institute of Biotechnology, Himaytanagar, Hyderabad-29, A.P,India; [3]Microbiology Laboratory, Global Institute of Biotechnology, Himaytanagar, Hyderabad-29, A.P, India; Jasti Pramoda Kumari - Email: pramodakumari@gmail.com; *Corresponding author

Received March 16, 2013; Revised April 03, 2013; Accepted April 05, 2013; Published May 25, 2013

Abstract:
Differently expressed proteins in probiotic *Escherichia coli* nissle 1917 under mercury stress identified by using a proteomic approach. We applied to separate proteins by using two-dimensional gel electrophoresis and proteins were identified using MALDI-TOF-MS using PMF, by mascot database search using the NCBI database. we identified six proteins after exposure to mercury stress with respect to different functional classes. It is useful to understand the molecular insights into mercury stress in probiotic *E. coli.* Next we describe a structure generated by homology modelling and functional domain identification; it is interesting to study the impact of stress on protein structures. MS characterization and computational methods together provide the opportunity to examine the impact of stress arising from mercury. The role of these proteins in metal tolerance and structure relation is discussed. To the best of our knowledge, proteomics of *E. coli* nissle 1917 overview of mercury stress has been reported for the first time.

Key words: *E. coli* nissle 1917, Mercury, 2D-PAGE, Mass spectroscopy, Homology modelling.

Background:
Next to lactic acid bacteria and the probiotic yeast *Saccharomyces boulardii*, the non-pathogenic and human faecal isolate which despite exhibiting a serotype (O6:K5:H1) *E. coli* strain nissle 1917 (EcN) is one of the best characterized probiotics and effectively colonize in the gut **[1].** The gut microorganisms form a complex and dynamic ecosystem that constantly interacts pollutants through contaminate food and water, that may have toxic effects on body results induction of a variety of stress proteins. Heavy metals are one of the major hazard pollutants, constantly added into the environment due to anthropogenic activities. This is especially true for developing countries like China and India **[2].**

When organisms or cells are exposed to low or high levels of heavy metals they adapt various types including physiological or genetic changes in response to heavy metals. So, metal-induced toxicity involves multifactor mechanisms, suggests that the toxicity of metal ions binding for metal sensitive group, especially those with high atomic numbers, e.g. Hg^{2+}, Cd^{2+} and Ag^{+2}, tend to bind SH or His moieties. Regarding health issues, Cd, Pb, Hg and as have been identified as the most toxic. Mercury (Hg), is a high potential contaminant because of its toxic nature. It exhibits neurotoxicity, nephrotoxicity and gastrointestinal toxicity **[3-6].**

A comparison of protein patterns from stress conditions versus controls allows the detection of specific changes in the protein **[7].** As result examining the protein expression that are up or down regulated could be useful for gaining insight into the molecular mechanisms of stress response **[8].** Most of the proteomic investigate that have been performed to study the toxicity of heavy metals in different organisms have shown that proteins associated with antioxidative defense mechanisms **[9-12].** MALDI-MS has been successfully applied for characterizing multi protein complexes. Two of the important mass spectrometry "bottom-up" and the less widely used "top-down" methods are the frequently applied. The bottom-up strategy involves enzymatic digestion of intact proteins to generate peptides that are analyzed by the mass spectrometer **[13-16].**

Research on the stress response has increased greatly, most of its aims at understanding the structure and function of stress proteins. Protein structures are primarily determined by using X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy. Even though these methods not possible for all proteins, the rate of newly discovered sequences grows much faster than the number of structures solved. The huge gap could possibly be resolved by computational methods. Homology modelling often provides a useful to predict 3-D model for a protein that is related to at least one known protein structure based on protein sequence and aligned to one or more proteins of known structure **[17-19].** Functional domain (FD) is the core of a protein that plays the major role in its function. As stated in Mizianty and Kurgan **[20]** having a wide range of potential applications including predicting protein subcellular location, protein (un) folding rates, DNA binding sites.

Little is known about the effect of heavy metals on the protein profile of *E. coli* strain nissle 1917. Therefore, the objective of this work is to, in combination with 2D electrophoresis, MALDI-MS is in particular applied as downstream analytical methods for protein identification. Protein structure predictions of differentially expressed proteins are formulated under mercury stress by using homology modelling.

Methodology:

Bacterial strain and minimum inhibitory concentration (MIC) of mercury

Escherichia coli strains nissle 1917, (serotype O6:K5:H1) were obtained from the culture collection of Ardeypharm, GmbH, Herdecke, Germany and was used and cultivated on LB using the streak plate method. The bacterial culture of *E. coli* nissle 1917 were grown in 5 ml Luria-Bertani (LB) broth (10 g/L tryptone, 5 g/L NaCl and 5 g/L yeast extract, pH 7.2) supplemented with 100 mg/L ampicillin at 37ºC, 150 RPM for overnight. The minimum inhibitory concentration (MIC) of mercury (Hg^{+2}) was determined by macrodilution method.

Protein extraction and two-dimensional gel electrophoresis (2D PAGE)

In preparation of protein extraction was performed and Protein content was estimated by Bradford method using Bangalore Genei Kit. For the detection of stress-induced proteins in *E.coli* nissle 1917, 2D-PAGE was carry out by using IPG strips with pH- ranges of 3-10 and followed second dimension SDS PAGE and the procedure was followed as per Bio-Rad instruction manual. In-gel digestion of the excised gel pieces was performed using sequencing grade trypsin. A basic overview of the methods is presented in **(Figure 1).**

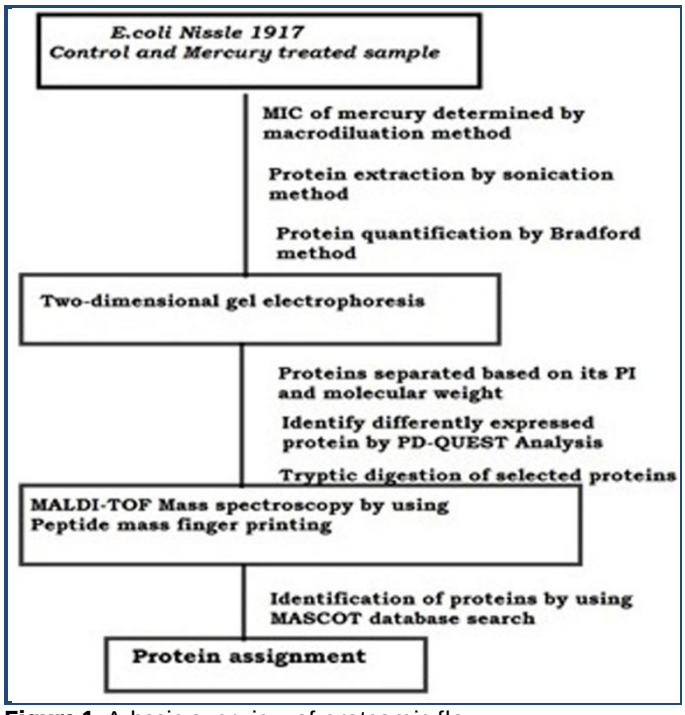

Figure 1: A basic overview of proteomic flow

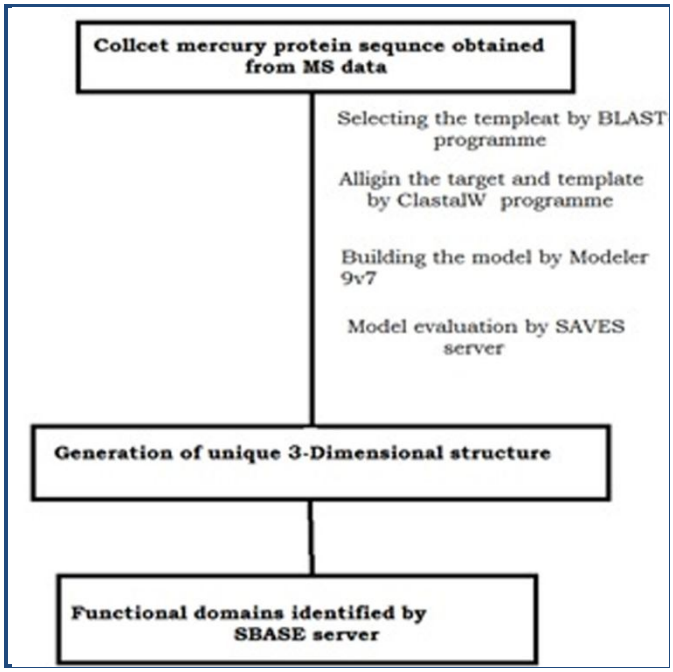

Figure 2: Classical work flow Homology modelling

Peptide mass fingerprinting MALDI-TOF- MS

Spots were excised from sypro ruby stained gels and destained for 30 minutes peptide mixture was mixed with an equal volume of matrix solution (10 mg/ml -cyano-4-hydroxycinnamic acid matrix in 50% acetonitrile containing 0.1% TFA) and the mixture was analyzed on MALDI-TOF MS (Model Voyager-DE STR, Applied Bio systems, Foster, CA, USA). Spectra were collected over the mass range of 800–3500

KDa and integrated with the MASCOT 2.2 search engine (Matrix Science, http://www.matrixscience.com) was used for spot proteins identification by querying the trypsin digested peptide fragment data using the reference database NCBI with E. *coli*. A partial modification of proteins by carbamidomethylation of cysteines and oxidization of methionines was allowed. Protein homology was assigned, if at least four peptide masses with a sequence coverage 15% or higher were matched within a maximum of 100-ppm error spread across the data set. The number of missed cleavage sites was allowed up to 1. Search result scores which are greater than 48 was considered to be of significant difference ($p<0.05$).

Homology Modelling

The sequences of mercury stress proteins were obtained from mass spectroscopy data. The modelling step can be carried out by searching the metal stress expressed protein sequences against the databases of well-defined template sequences was identified by the BLAST program **[21]** against PDB. (http://www.rcsb.org/pdb/), which shows the maximum identity with high score and less e-value designated as a template. All sequence alignments were completed using ClustalW. The 3D model was generated by using the academic version of MODELER9v7 (http//:www.salilab.org/modeller), based on the information obtained from sequence alignment **[22].** The 3D structure obtained from modeller were further refined through molecular dynamics and calibration methods using NAMD 2.5 with CHARMM 27 force field and the system contains the TIP3P water model. The energy of the structure was minimized with 10,000 steps with cutoff of 12 Å for vdW interactions. The equilibrated system was simulated with 1 picoseconds (PS); to hold back the original conformation, for that (NPT) constant pressure and 310K constant temperatures has been choosed **[23].** Finally, the structure having the least energy with low RMSD was used for further studies. The final structure obtained, was analyzed by Ramachandran's map drawn using PROCHECK v.3.0 and Verify3D. These programs accessed from the SAVES online server and the model satisfying all the parameters after the evaluation was considered for the further process. The functional domains of modelling proteins could identify by using SBASE server. A basic overview of modelling represented in **Figure 2.**

Results and Discussion:

Characterization of proteins using Peptide Mass Fingerprinting

In this study we examined differential stress responsive proteome of *E.coli* nissle 1917 before and after the exposure to mercury to identify differentially expressed proteins. Cells were treated with 0.02 mM mercury, by using 2D-PAGE and the entire differential expressed spots were subjected to peptide mass fingerprinting. After the treatment of *E.coli* nissle 1917 cells with mercury stress many proteins were up or down regulated these proteins are called stress proteins showed in **(Figure 3 & 4)**. Spot nos. 1, 2, 3, 4, 5 and 6 are differentially expressed in mercury treated cells is given in **Table 1 (see supplementary material)**. Mercury-responsive proteins were Hypothetical UPF0169 lipoprotein yfiO precursor, conserved domain protein, 50S ribosomal protein L31 type B 1, 50S ribosomal protein L31, uncharacterized HTH-type transcriptional regulator yihW and beta-lactamase SHV-24 respectively.

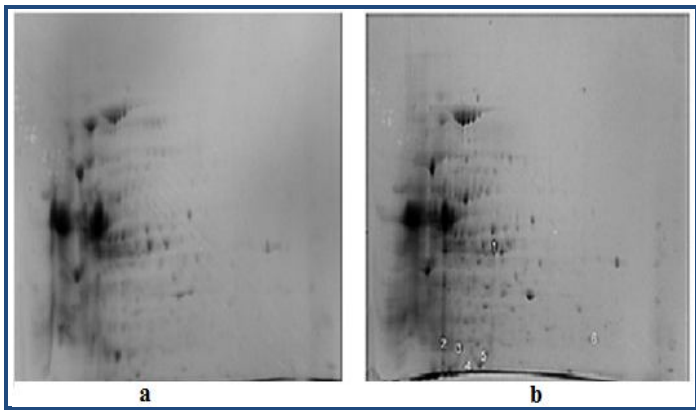

Figure 3: 2D gel images of *E.coli* nissele 1917 cells. **(a)** 2D profile of *E.coli* nissle 1917 showing the protein expression when grown in the presence without metal i.e control; **(b)** 2D profile of *E.coli* nissle 1917 showing the differential protein expression when grown in the presence of 0.02 mM.

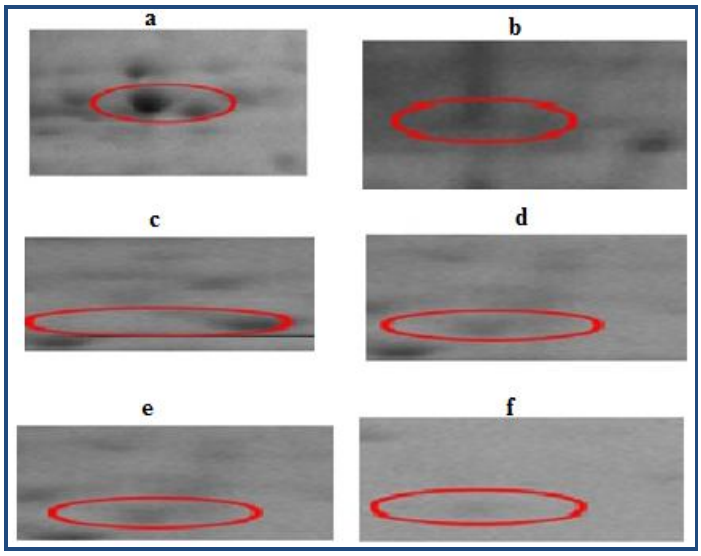

Figure 4: Zoomed 2D gels in specific locations of protein derived from cells exposure to 0.02 mM $Hgcl_2$ in *E.coli* nissle 1917. **(a)** Hypothetical UPF0169 lipo protein yfio precursor; **(b)** Conserved domain protein; **(c)** 50S ribosomal protein L31 type B 1; **(d)** 50S ribosomal protein L3 1; **(e)** Uncharacterized HTH-type transcriptional regulator yih W; **(F)** Beta-lactamase SHV-24

Homology modelling of mercury stress expressed proteins

Three homology models were obtained for the mercury stress proteins based on published X-ray structures of templates. These include (i) β-lactamase protein (ii) Ribosomal L31 protein (iii) Yfio proteins.

For β-lactamase, ribosomal L31 protein and Yfio proteins the results of BLAST search against PDB, SHV-1 betalactamase complex (PDB ID 3D4F), ribosome from *E. coli* (PDB ID 2AW4) and *E. coli* BamCD complex (PDB ID 3TGO) were selected as template proteins for homology modelling, which have high level of sequence identity with the β-lactamase domain (31%), ribosomal L31 (33%) and Yfio (34%) respectively. The alignment was done with the ClustalW program **(Figure 5 a, b, c)**. This is a good result given that, according to the literature, a sequence similarity above 25% between the protein and template is satisfactory to build a homology model **[24]**. The final stable

structure of the beta lactamase, ribosomal L31 and Yfio proteins obtained by the help of SPDBV and it is evident that β-lactamase has 10 helices, 11 sheets; Ribosomal L31; 11sheets, 2 helices; Yfio proteins 4 sheets and 11 helices as shown in the (**Figure 6: a, c, e**). The structure having the least energy with low RMSD (Root mean square deviation) which was obtained by the NAMD. The final structure was further checked by verify3D graph and validation of the model was carried out using Ramachandran plot calculations computed with the PROCHECK. Altogether 99.6% β lactamase; 96.3% ribosomal L31 and 94.1% Yfio proteins of the residues were in favoured and allowed regions. The functional domains (FD) were identified by SBASE server and visualized by SPDBV software it has been shown that beta lactamase having one FDs namely betalactamase domain; Yfio proteins having four FD including three tetratricopeptide region and Sec1- like protein domain and ribosomal L31 protein having one FD is ribosomal protein L31 domain **(Figure 6: b, d, f)**.

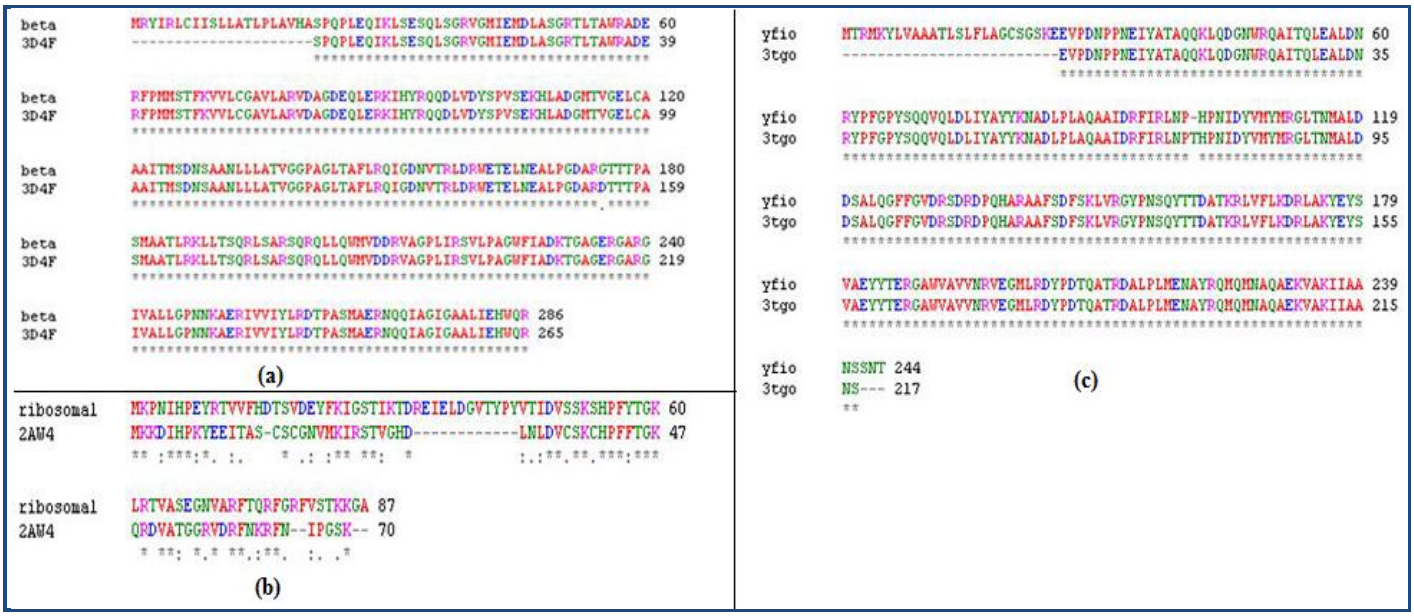

Figure 5: Pairwise alignment between the mercury stress proteins from *E. coli* nissle 1917 with respect templates. **(a)** Pairwise alignment between β-lactamase and template SHV-1 beta-lactamase complex (PDB ID-3D4F); **(b)** Pairwise alignment between Ribosomal L31 protein and template ribosome from *Escherichia coli* (PDB ID - 2AW4); (c) Pairwise alignment between Yfio protein and template ribosome from *E. coli* Bam CD complex (PDB ID: 3TGO).

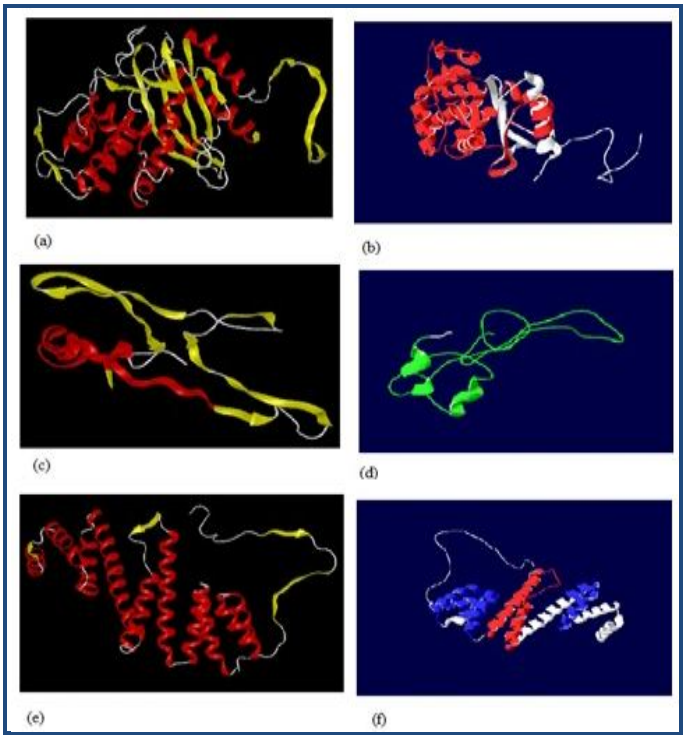

Figure 6: Computed 3D structure of homology models of mercury stress protein visualized by SPDBV software and domains identified by SBASE server. **(a)** β-lactamase has 10 helices, 11 sheets; **(b)** Beta-lactamase - like domain (Red colour); **(c)** Ribosomal L31: 11 sheets, 2 helices; **(d)** Ribosomal L31- Like domain (Green colour); **(e)** Yfio protein 4 sheets, 11 helices; **(f)** Three tetratrico peptide region = (Blue colour) and sec 1 = like protein = domain (red colour).

Conclusion:

In the present study, we described the protein profile experimentally by 2D- PAGE and mass spectroscopy to understand mercury stress. Our findings provide novel insights into the physiological role of stress proteins in probiotic *E. coli.* The growth rate of *E. coli* nissle 1917 arrest and loss of culturability was observed, and elimination of mercury ion is the most important defense mechanism of the cells against mercury stress. This is significant because, resources for a cell are limited in stress, in order to adapt that conditions probiotic *E. coli* increased synthesis of new constitutive proteins like ribosomal proteins involved in the energy metabolism protein synthesis and antibiotic and metals resistant proteins. These proteins could allow the bacteria to contend with mercury stress. Further, homology modelling performed for three unknown structures of mercury stress proteins, we got higher sequence similarity with template proteins. All the results obtained from RMSD, verify3D and PROCHECK assembled together showed the best results for homology modelling.

Acknowledgement:
My special thanks to University Grants Commission (UGC-MRP), New Delhi for their financial support and my heartfelt thanks to Dr. Ulrich Sonnenborn, Ardeypharm, GmbH, Germany, for providing *Escherichia coli* nissle 1917 (EcN Strain).

References:

[1] Grozdanov L *et al. J Bacteriol.* 2004 **186**: 5432 [PMID: 15292145]
[2] Raj S *et al*. *Atmo Envir J.* 2008 **42**: 2048
[3] Ercal N *et al. Curr Top Med Chem.* 2001 **6**: 529 [PMID: 11895129]
[4] Nies DH, *Appl Microbiol Biotechnol.* 1999 **51:** 730 [PMID: 10422221]
[5] Farrer BT & Pecoraro VL, *Curr Opin Drug Discov Devel.* 2002 **5**: 937 [PMID: 12478724]
[6] Jarup L & Akesson A, *Toxicol Appl Pharmacol.* 2009 **238**: 201 [PMID: 19409405]
[7] Blackstock WP & Weir MP, *Trends Biotechnol.* 1999 **3**: 121 [PMID: 10189717]
[8] Garcia *et al. TrAc.* 2011 **30**: 703
[9] Requejo R & Tena M, *Phytochemistry*. 2005 **13**: 1519 [PMID: 15964037]
[10] Silvestre F *et al*. *Aquat Toxicol.* 2006 **76**: 46 [PMID: 16249038]
[11] Costa VM *et al*. *Free Radic Biol Med.* 2002 **33**: 1507 [PMID: 12446208]
[12] Lee K & Pi K, *Biochemistry* (Mosc). 2010 **75:** 460 [PMID: 20618135]
[13] Geng *et al. J Chromatogr A*. 2000 **870**: 295 [PMID: 10722087]
[14] Reid GE & McLuckey SA, *J Mass Spectrom.* 2002 **37:** 663 [PMID: 12124999]
[15] Aebersold R & Mann M, *Nature.* 2003 **422**: 198 [PMID: 12634793]
[16] Delahunty CM & Yates JR, *Biotechniques.* 2007 **43**: 563 [PMID: 18072585]
[17] Marti-Renom MA *et al. Bioinformatics.* 2001 **17**: 746 [PMID: 11524379]
[18] Fiser A *et al*. *Protein Sci*. 2000 **9:** 1753 [PMID: 11045621]
[19] Petrey D & Honig B, *Mol Cell*. 2005 **20:** 811 [PMID: 16364908]
[20] Mizianty MJ & Kurgan L, *BMC Bioinformatics.* 2009 **10:** 414 [PMID: 20003388]
[21] Altschul SF *et al. J Mol Biol*. 1990 **215**: 403 [PMID: 2231712]
[22] Sundaram S *et al. Bioinformatics.* 2010 **5:** 177 [PMID: 21364783]
[23] Wang Y *et al. Comput Sci Discov.* 2011 **4**: 015002 [PMID: 21686063]
[24] Santos-Filho OA & Alencastro RB, *Quim Nova.* 2003 **26**: 253

Edited by P Kangueane
Citation: **Seshapani *et al.*** Bioinformation 9(9): 485-490 (2013)

BIOINFORMATION

open access

Supplementary material:

Table 1: List of differently expressed Proteins of *E. coli* nissle 1917 identified by Mass spectrometry by using peptide mass fingerprinting (PMF) analysis under mercury ($HgCl_2$) stress

Spot	Expression protein name	Molecular weight	Calculated pI value	Protein Score	Sequence Coverage (%)
1	Hypothetical UPF0169 lipoprotein yfiO precursor	27869	6.16	49	21
2	Conserved domain protein	14333	9.25	88	25
3	50S ribosomal protein L31 type B 1	9914	9.3	78	18
4	50S ribosomal protein L31	7866	9.46	66	46
5	Uncharacterized HTH-type transcriptional regulator yihW	28490	5.6	75	39
6	Beta-lactamase SHV-24	31317	8.49	58	19