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Characterisation and Structural Dynamics of Differentially Expressed Proteins of Probiotic *Escherichia coli* Nissle 1917 in Response to *Cocos nucifera* Sap

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Abstract

The aim of the present study was to evaluate and analyse the expressed protein structures in response to cocoti sap treated probiotic *E. coli* Nissle 1917 by MALDI-TOF-MS. The expressed protein spots were separated by 2-D gel electrophoresis and spots were digested with trypsin enzyme for MALDI analysis. Based on their mass-to-charge ratio, the expressed proteins sequences were collected from Mascot search data. The sequence are analysed by phyre-2 software for homology modelling. The predicted structures were also validated by Rampage software. Of the expressed proteins, only five proteins showed good structural validation in sap treated probiotics. It was concluded that the expressed protein profiles were analysed by 2D-PAGE and MALDI to understand the stress mechanisms of cocoti sap in probiotic *E. coli* Nissle 1917. Stress mechanism induces many proteins they are involved in metabolic process, regulate gene expression, SOS regulation, transferase activity, purine metabolism and other related activities (Graphical representation).

Keywords: *E. coli* nissle 1917; Stress response; *Cocos nucifera* sap; MALDI-TOF; Protein functions

Introduction

Stress response or heat-shock protein response study was the advanced research in recent years. The study of expressed proteins in response to physico-chemical conditions such as stress, heat shock and chemicals was the advanced research to understand the structure and function of the responded proteins [1]. In stress response, temporarily suppresses various biological processes such as sexual responses and digestive mechanisms. This is in an effort to focus on the stress or situation. Stress response in bacteria involves a complex network of elements that acts against the external stimulus [2]. Stress response systems can play an important role in the virulence of pathogenic organisms.

Identification of protein can be attained by analysing the Mass spectrum of protein sample through different methods. Mass spectrometry (MS/MS), Peptide mass finger printing (PMF), Protein Source Decay (PSD), MALDI-TOF (Matrix Assisted Laser Desorption/Ionization- Time of Flight) is used to analyse the stress responded proteins isolated from 2D-electrophoresis gel. MS analysis produces list of molecular weights of the fragments which is often called a peak list. MOWSE (Molecular Weight Search) method is used for identification of proteins from the molecular weight of peptides digested with Proteolytic enzymes and measured with MS. The basic concept of TOF/MS is that the ions are separated based on the time it takes for the ion to drift the flight to the detector. The method is accurate. Quick and easy to use, minimum quantity of sample requires determining the molecular weight [3]. The peptide masses are compared to protein database such as Mascot which contains protein sequence information [4].

Classical proteomics has increasingly majority of the current protein based research work [5]. In clinical proteomics MALDI-TOF MS is the platform to advanced diagnostic issues related to emerging infections, increasing of fastidious bacteria, and generation of patient-tailored phylotypes [6]. Development of systems biology/Bio-informatics is related to many biological fields including genomics, transcriptomics, proteomics, and metabolomics, with the goal of ultimately modeling cellular process *in silico* [7].

Mascot is a software search engine that uses mass spectrometry data to identify proteins from peptide sequence databases [8]. It is freely available software, most of the proteins are too big, and peptides usually fall within the limited mass range that a typical mass spectrometer can measure. MS measure the molecular weights of peptides in a sample. Mascot then compares these molecular weights against a database of known peptides. The program cleaves every protein in the specified search database in-silico according to specific rules depending on cleavage enzyme used for digestion and calculates the theoretical mass for each peptide. Mascot then computes a score based on the probability that the peptides from a sample match those in the selected protein database.

Protein structure plays a major role in determining its function. Proteins have the same structure and active sites, but they differ in functions, at the same time functions are similar but differ in their structure [9,10]. Structure and sequences of proteins are available in databases (Swissprot, RCSB PDB). 3D structure determination of protein sequence is the major challenging problem. Basically Nuclear Magnetic resonance (NMR) and X-ray crystallography techniques

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are used, which are expensive, time consuming and complex process. Therefore, advanced computational methods were developed like homology modelling, threading, and ab initio. Homology modelling is the most accurate. It is based on alignment to a known protein structure which is derived experimentally (template). However, similarity of sequence is greater than 33% and then it can act as a template. Homology modelling is also known as comparative modeling of protein. The aim of protein modelling is to predict the structure of a protein from its sequence with an accuracy that is comparable to the best results achieved experimentally. RCSB PDB is used for comparative modelling of 3-D structure [11,12].

Hence the present study was focused on the analysis and structure prediction of expressed proteins isolated from probiotic *E. coli* Nissle 1917 in response to cocoti sap treatment. The expressed proteins were analysed by 2-D gel electrophoresis and MALDI-TOF. Mascot, Phyre-2, and Rampage server software's were used for structure prediction.

Material and Methods

Extraction of protein sample

Protein sample isolated from sap treated probiotic *E. coli* Nissle 1917. Centrifuged the tube 12,000 rpm for 10 min, maintaining the temperature at 4°C, transfer the pellet to a micro centrifuge tube and washed this pellet thoroughly with phosphate buffer to remove the unwanted debris. Re-suspended pellet was sonicated on ice is enable the bacterial cells to break at 30 sec with the pulse of 1 sec at 40% amplitude, once it was completed, centrifuged the contents and collected supernatant. The supernatant was treated as crude protein sample. Proteins were extracted by the method of Trizol protein extraction method [13]. Trizol reagent consisting chloroform, isopropyl alcohol, ethanol, acetone, guanidine thiocyanide and SDS.

Added chloroform to the crude protein sample, mixed the content and place the tube on ice for few minutes, centrifuged the tube at 2000 rpm for 5 minutes. Three distinct layers were obtained at this stage the top most is the aqueous layer containing RNA, inter phase is the protein and the bottom layer contains DNA. Discarded the transparent top layer having the RNA, then added isopropyl alcohol to the remaining layers and mixed the solution as well centrifuged the contents at 2000 rpm for 5 minutes. Discarded the bottom layer, then the remaining solution treated with ethanol centrifuged the contents at 2000 rpm for 5 minutes discard the bottom layer, while the protein remains clear in the supernatant was collected in a fresh tube, and then added chilled acetone to this tube and mixed well by vortex mixture. The solution was stored at -20°C at least one hour before centrifugation. Protein pellet was collected, discarded the supernatant and dried the protein pellet at room temperature. Reconstitutes the dried pellet with rehydration buffer and stored overnight at -20°C before carry out protein quantification [13]. 2-D gel electrophoresis is very sensitive to salts and detergents. Collected protein samples were purified because sample contains some amount of salts and detergents.

Protein quantification

BCA (Bicinchoninic acid) (BCA1 SIGMA) method is a simple method to quantify the protein concentration. Standard series of protein samples and sap treated protein sample were prepared, mixed the BCA reagents A and B (50:1 (v/v)). Then 25 µl of each standard and unknown sample were pipetted and transferred to microtiter plate. 200 µl of BCA working reagent was added to each well and allowed for incubation at 37°C for 30 min, BCA protein assay Kit method utilizes a copper (Cu^{2+}) salt which can be reduced to the cuprous state by proteins. The generated Cu^{2+} ion forms an intensely coloured complex with the bicinchoninic acid reagent with a very strong absorbance band at 562 nm by using Elisa reader (Bio Rad-.Germany).

2-D gel electrophoresis

The isolated samples were separated by 2-D gel electrophoresis. In this process protein samples were, separated by two independent properties i.e. isoelectric point and based on molecular weight. 4-7 pH gradient strips were used the strip length is 24 cms. 2-DE protein sample was loaded into the strip holder, placed the strip and covered the strip by using covering gel, allowed for IEF (Isoelectric Focusing) with the help of electrodesoptimal conditions. Removed the strip from the strip holder after the IEF step was completed. Then the sample treated for rehydration with the help of equilibrium buffer Iodoacetamide (IAA). Put the strip into the casting plate, set the instrument and loaded with running buffer allowed for second dimension. After completion of the second dimension, the gel was separated from the plate and allowed for staining with colloidal coomassie brilliant blue stain was used (www.gelifesciences.com).

Isolation of differentially expressed proteins

Image analysis is the most important step in proteomics is to identify the differentially expressed proteins between control and stress samples run on a series of 2-D gels i.e., the protein spots that have been down-regulated or up-regulated the spot size. Once these gel features have been found, the proteins of interest can be identified using MS (Model voyager De-STR, applied Bio-systems, Foster, CA, USA). This goal is usually accomplished with the help of computerized image analysis systems. The colloidal coomassie blue stained gels were scanned and photographed by gel scanner (Typhon variable mode imager) with a 488 nm laser. The 2-D gel image analysis was performed with image master 2-D platinum 6.0 software and each gel was analyzed for spot detection. The gel image showing the higher number of spots and the best protein spot sets were created, pattern was chosen as a reference template, and spots in a standard gel were then matched across treated gel. Spot quantity values were normalized in each gel dividing the raw quantity of each spot by the total quantity of all the spots included in the standard gel. In order to analyse gel similarities or experimental variations such as disparities in stain intensity or sample loading, one can produce Scatter plots for groups. Scatter plots give an idea of the relationship between the spot values from two gels by searching for the linear dependence between the spot values of one gel in comparison to another gel. Spot sizes, Mean, Standard deviation, Coefficient of Variation in each group were determined. The spots in these sets were excised from gels using Spot Cutter for further analysis of spots and explain the steps involved in using Image master 2D platinum 6.0.

Data analysis

Database searching was performed using the Mascot 2.2 search engine program (http://www.matrixscience.com). The initial searching was against the NCBInr database among *E. coli* species. The search parameters were allowed for oxidation (M) of methionine, carbaminomethylation (c) of cysteine and one miss-cleavage of trypsin, 100-1200 ppm peptide tolerance and MS/MS tolerance 0.2-2 Da. Proteins were identified with confidence consider the following standards i.e. molecular weight, ion score, maximum number of hits in subjection for the significant matches ($p<0.05$), and predicted mass/pI values. Phyrol 2 software used for homology modelling, SPDBV_4.01_PC for pdb file analysis, Rampage Ramachandran's server used to visualize dihedral angles φ against ψ of amino acid residues in protein

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structure. It shows the possible conformations of φ and ψ angles for a polypeptide [14].

Results

Differentially expressed protein spots of *E. coli* Nissle 1917 with the influence of *Cocos nucifera* sap stress were characterized by using MALDI-TOF/MS/MS and the results were represented in Table 1. These differentially expressed proteins under sap stress were explained with differentially functional class in Table 2. Validation score of Ramachandran's plot scores represented in Table 3. Spectra images of differentially expressed protein spots were represented in Figure 1. Computed 3D structure of homology models of proteins under sap stress were visualized by phyre-2 database and presented in Figure 2, Validation studies by using Rampage Ramachandran server showed in Figure 3.

Discussion

In the present study, indicated that the noticed differentially expressed proteins of probiotic *E. coli* Nissle 1917 isolated from 2-D electrophoresis gel in response to cocoti sap. Two protein spots showed down- regulation (protein spot numbers- 457,595) five protein spots showed up-regulation (protein spot numbers- 348,472,468,488,427) based on the 3D view of expressed spots and statistical analysis of X,Y,Z bond angles [15]. Down-regulation proteins are Protein pmba OS=*Escherichia coli* PMBA-Eco57, UPF0401 protein ECP Y3010_ECOL5. Up regulation proteins are t-RNA-Specific adenosine deaminase monomer, Transcriptional regulatory protein BACR_ EcoL6, N-acetylgalactosamine-specific phosphotransferase enzyme IIB component 1PTPB1_ECOLI, DinI-like protein Z3305/ECs2939 in prophage CP-933V DINI1_ECO57, Formatehydrogenlyase subunit HYCE_ECOLI.

Homology modeling explains the protein structure and number of strands, helix, and sheets of the expressed proteins. Ramachandran's plot explains the dihedral angles of φ and ψ angles of amino acid residues in protein structures. It gives degree of rotation of bonds in relation to each other.

The expressed proteins involved in different metabolic activities. t-RNA-Specific adenosine deaminase monomer: Taxonomic identifier of the protein sample is 868141 and Uniprot code H4L196, length of the sequence 167AA, molecular weight of this protein sample is 26 kDa. Adenosine deaminase (ADA) is considered as one of the key enzymes of purine metabolism [16]. The high degree of amino acid sequence conservation suggests the crucial nature of ADA in the purine salvage pathway [17].

Catalysis of the reaction: adenosine+H_2O=inosine+NH_3, in a tRNA molecule

Transcriptional regulatory protein BAER_ECOL6 involved in transcription regulation, DNA binding, phosphorelay response regulator activity. Taxonomic identification number of this protein is 1181761 and the Uniprot id L4RK56, total sequence length of this protein 240 AA, molecular weight of this protein 140.6 kda. The protein responds to a phosphorelay sensor to initiate a change in cell state or

Spot	Expression protein name	Molecular weight	Calculated pI value	Protein Score	Number of Amino acids	Sequence coverage (%)
595	UPF0401 protein ECP Y3010_ECOL5	8744.9	6.54	50	77	76
472	Transcriptional regulatory protein BACR_EcoL6	27655.9	5.52	32	240	15
457	protein pmbaosE.coli PMBA-Eco57	48369.6	5.4	29	450	9
427	Formatehydrogenlyase subunit HYCE_ECOLI	64980.3	6.15	47	569	8
488	DinI-like protein Z3305/ECs2939 in prophage CP-933V DINI1_ECO57	9378.6	5.46	26	82	26
468	N-acetylgalactosamine-specific phosphotransferaseenzyme IIB component 1 PTPB1_ECOLI	17622.2	6.28	58	158	19
348	t-RNA-Specific adenosine deaminase monomer	26109.1	8.5	43	167	21

Table 1: Protein Characterization by using MALDI-TOF/MS/MS.

Protein Spot ID	Protein name	Function
348	t-RNA-Specific adenosine deaminase monomer	Breakdown of adenosine from food for the turnover of nucleic acids in
472	Transcriptional regulatory protein BACR_EcoL6	tissues Regulate gene expression.
468	N-acetylgalactosamine-specific phosphotransferase enzyme IIB component PTPB1_ECOLI	Involved in metabolic pathways
488	DinI-like protein Z3305/ECs2939 in prophage CP-933V DINI1_ECO57	Involved in SOS regulation
595	UPF0401 protein ECP Y3010_ECOL5	Participate in purine metabolism

Table 2: Differentially expressed proteins under sap stress with differentially functional class.

Protein spot No-	Spot.no-348	Spot.no-472	Spot.no-468	Spot.no-488	Spot.no-595
Number of residues in favoured region	146 (94.8%)	203 (96.2%)	148 (98.0%)	75 (94.9%)	56 (90.3%)
Number of residues in allowed region	8 (5.2%)	7 (3.3%)	3 (2.0%)	2 (2.5%)	3 (9.7%)
Number of residues in outlier region	0 (0.0%)	1 (0.5%)	0 (0%)	2 (2.5%)	3 (0.0%)

Table 3: Validation scores of Ramachandran'splot by using Rampage Ramachandran server.

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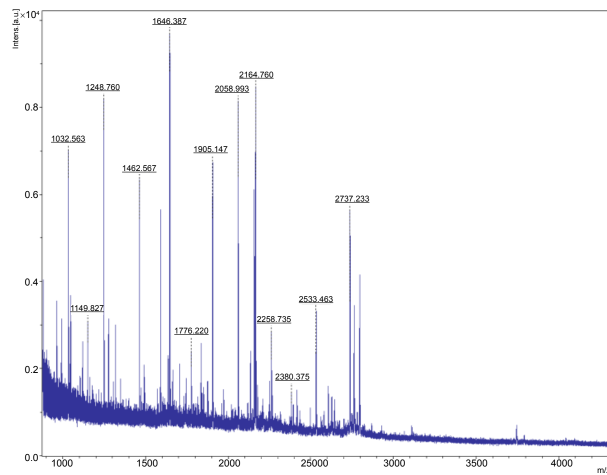
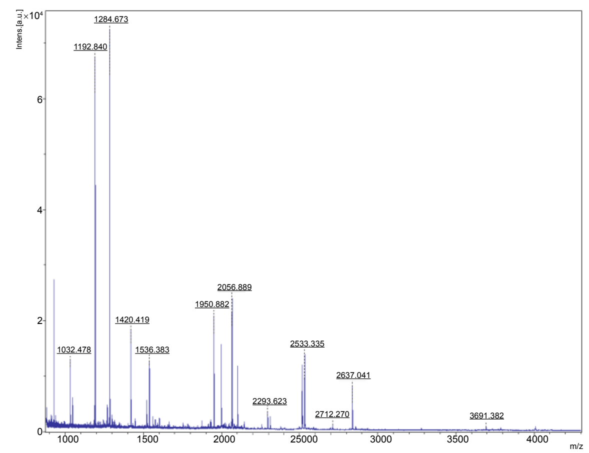
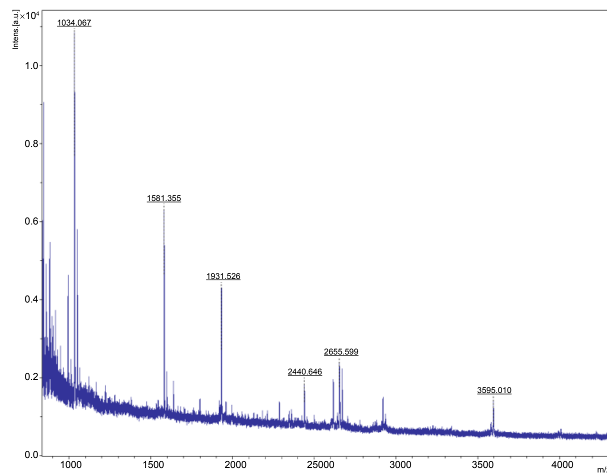


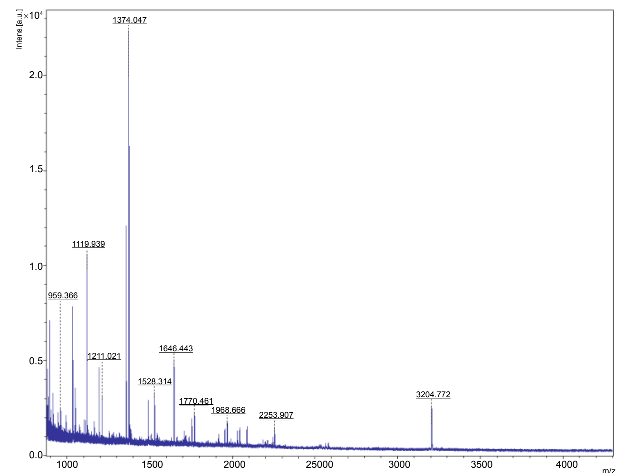
Spot.no- 348



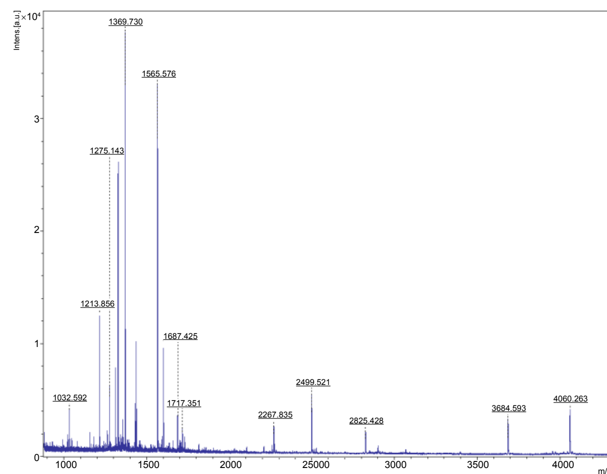
Spot.no- 472



Spot.no- 468



Spot.no- 488



Spot.no- 595

Figure 1: Spectra images of differentially expressed protein spots.

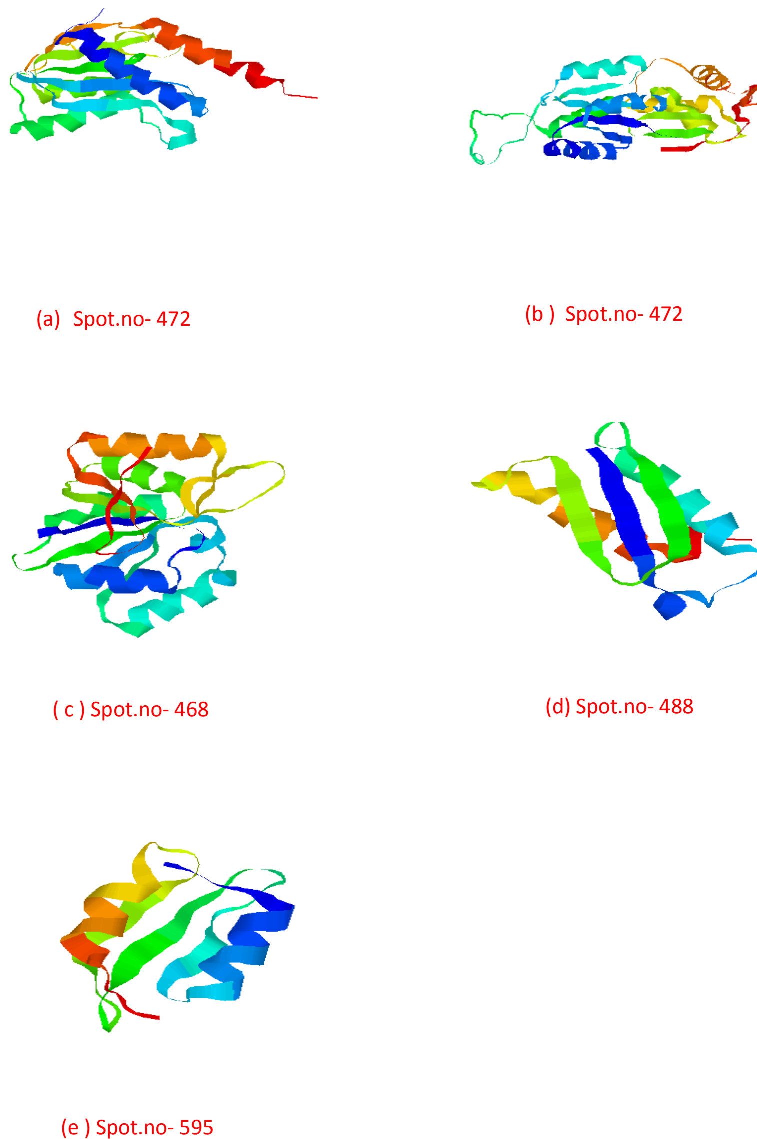
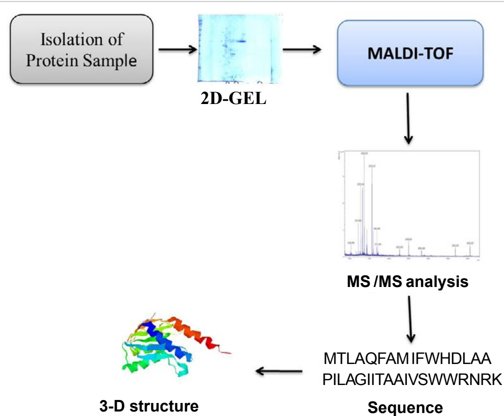


Figure 2: Computed 3D structure of homology models of sap stress protein visualized by Phyre-2 database. (a) 348- t-RNA-Specific adenosine deaminase monomer, (b) 472- Transcriptional regulatory protein BACR_EcoL6, (c) 468- N-acetylgalactosamine-specific phosphotransferase enzyme IIB component -1 PTPB1_ECOLI , (d) 488- DinI-like protein Z3305/ECs2939 in Prophage CP-933V DINI1_ECO57, (e) 595- UPF0401 protein ECP Y3010_ECOL5



Graphical representation.

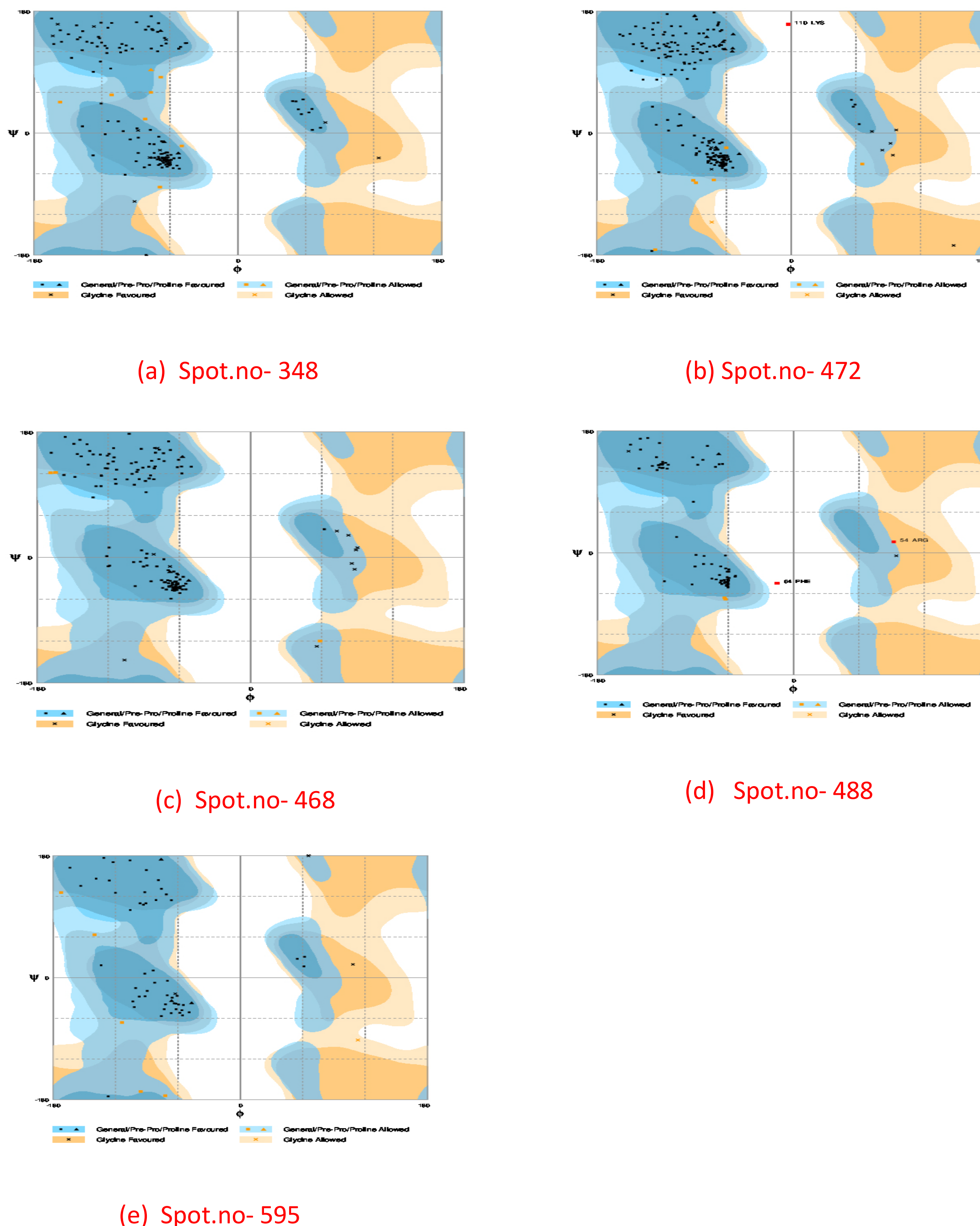


Figure 3: Validation studies by using Rampage Ramachandran server. (a) 348-Ramachandran's plot by Rampage analysis of t-RNA-Specific adenosine deaminase monomer, (b) 472–Ramachandran's plot by Rampage analysis of Transcriptional regulatory protein BACR_EcoL6, (c) 468-Ramachandran's plot by Rampage analysis of N-acetylgalactosamine-specific phosphotransferase enzyme IIB component-1 PTPB1_ECOL, (d) 488-Ramachandran's plot by Rampage analysis of Dinl-like protein Z3305/ECs2939 in prophage CP- 933V DINI1_ECO57, (e) 595- Ramachandran's plot by Rampage analysis UPF0401 protein ECP Y3010_ECOL5.

activity. The activity of the response regulator is regulated by transfer of a phosphate from a histidine residue in the sensor, to an aspirate residue in the response regulator [18].

The protein N-acetylgalactosamine specific phosphotransferase enzyme IIB component 1 involved in protein-N (PI)-phosphohistidine-sugar phosphotransferase activity. The phosphoenolpyruvate-dependent sugar phosphotransferase system (Sugar PTS), a major carbohydrate active- transport system, catalyzes the phosphorylation of incoming sugar substrates concomitantly with their translocation across the cell membrane. This system is involved in N-acetylgalactosamine transport [19]. Taxonomic identifier number of the protein is 1116033 and the Uniprot id of the protein is M8THA9.

Dinl (DNA–damage inducible protein l) like protein family is a family of short proteins (Ramirez et al.). *Escherichia coli* Dinl, a LexA-regulated SOS gene product, shut off the initiation of the SOS response when over expressed *in-vivo*. Genetic studies indicates that Dinl

physically interact with RecA to inhibit its co-protease activity [20]. Taxonomic identifier number of the protein is 868147 and the Uniprot id of the protein is H4NQH2.

UPF0401 protein ECP Y3010_ECOL5 is a phase protein, Uniprot id of the protein S1GQX9 and the taxonomic identifier number is -1182698 mostly the protein involves molecular functions like hydrolase activity acting on ester bonds and metabolic process. UPF0401 protein ECP Y3010_ECOL5 is a down regulated protein with response of cocoti sap treatment. We noticed the protein spot in pH range around 7 and the molecular mass close to 8744.9 Da.

Under mercury and cadmium stress conditions, the growth rate of *E. coli* Nissle 1917 arrest and loss of culturability was observed, 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, antibiotic and metals resistant proteins. These proteins could allow the bacteria to contend with mercury stress [21]. The Expressed proteins of probiotic *E. coli* Nissle 1917 under sap stress conditions involved regulatory and metabolic activities.

Conclusion

Expressed protein functions were studied. Protein pmba (PMBA-Eco57) involved in regulation of post-transcriptional activity, UPF0401 protein ECPY3010_ECOL5 participated in purine metabolism, t-RNA-Specific adenosine deaminase monomer breakdown the adenosine from food for the turnover of nucleic acids in tissues, Transcriptional regulatory protein BACR_EcoL6 involved in regulation of transcription, N-acetylgalactosamine-specific phosphotransferase enzyme IIB component 1PTPB1_ECOLI involved in carbohydrate metabolism, DinI-like protein Z3305/ECs2939 in prophage CP-933V DINI1_ECO57 involved in biological process DNA damage and repair, and Formaldehydrogenlyase subunit HYCE_ECOLI which act as a Co-factor in biological blue-light photo receptors.

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References

1. Requena JM (2012) Stress Response in Microbiology. Caister Academic Press.
2. Filloux AAM (2012) Bacterial Regulatory Networks. Caister Academic Press.
3. Lay JO Jr (2001) MALDI-TOF mass spectrometry of bacteria. Mass Spectrom Rev 20: 172-194.
4. Wang W, Sun J, Nimtz M, Deckwer WD, Zeng AP (2003) Protein identification from two-dimensional gel electrophoresis analysis of Klebsiellapneumoniae by combined use of mass spectrometry data and raw genome sequences. Proteome Sci 1: 6.
5. Rogowska-Wrzesinska A, Le Bihan MC, Thaysen-Andersen M, Roepstorff P (2013) 2D gels still have a niche in proteomics. J Proteomics 88: 4-13.
6. Del Chierico F, Petrucca A, Vernocchi P, Bracaglia G,Fiscarelli E, et al. (2014) Proteomics boosts translational and clinical microbiology. J Proteomics 97: 69-87.
7. Schmidt AS, Jacob SS, Ahn SB, Rupasinghe T, Kromer JO, et al. (2013) Two strings to the systems biology bow: co-extracting the metabolome and proteome of yeast. Metabolomics 9: 173-188.
8. Koenig T, Menze BH, Kirchner M, Monigatti F, Parker KC, et al. (2008) Robust predictions of the MASCOT score for an improved quality assessment in mass spectrometric proteomics. J Proteome Res 7: 3708-3717.
9. Wise E, Yew WS, Babbitt PC, Gerlt JA, Rayment I (2002) Homologous (beta/alpha)8-barrel enzymes that catalyze unrelated reactions: orotidine 5'-monophosphate decarboxylase and 3-keto-L-gulonate 6-phosphate decarboxylase. Biochemistry 41: 3861-3869.
10. Whisstock JC, LeskAM (2003) Prediction of protein function from protein sequence and structure. Q Rev Biophys 36: 307-340.
11. Martí-Renom MA, Stuart AC, Fiser A, Sánchez R, Melo F, et al. (2000) Comparative protein structure modeling of genes and genomes. Annu Rev BiophysBiomolStruct 29: 291-325.
12. Eswar N, Marti-Renom MA, Webb B, Madhusudhan MS, Eramian D, et al. (2006) Comparative Protein Structure Modeling With MODELLER, Current Protocols in Bioinformatics 15: 5.6.1-5.6.30.
13. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidiniumthiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156-159.
14. Lovell SC, Davis IW, Arendall WB 3rd, de Bakker PI, Word JM, et al. (2003) Structure validation by Calpha geometry: phi,psi and Cbeta deviation. Proteins 50: 437-450.
15. Chandrasekhar K, Sreevani S, PramodaKumari J (2014) Identification of E. coli Nissle 1917 proteins by using 2-d gel electrophoresis under the influence of Cocosnucifera sap and wine, International Journal of Pharmaceutical Science and Research 5(7): 1-8.
16. Glader BE, Backer K, Diamond LK (1983) Elevated erythrocyte adenosine deaminase activity in congenital hypoplastic anemia. N Engl J Med 309: 1486-1490.
17. Cristalli G, Costanzi S, Lambertucci C, Lupidi G, Vittori S, et al. (2001) Adenosine deaminase: functional implications and different classes of inhibitors. Med Res Rev 21: 105-128.
18. Mascher T, Helmann JD, Unden G (2006) Stimulus perception in bacterial signal-transducing histidine kinases. Microbiol Mol Biol Rev 70: 910-938.
19. Ray WK, Larson TJ (2004) Application of AgaR repressor and dominant repressor variants for verification of a gene cluster involved in N-acetylgalactosamine metabolism in Escherichia coli K-12. Mol Microbiol 51: 813-826.
20. Yoshimasu M,Aihara H, Ito Y, Rajesh S, Ishibe S, et al. (2003) An NMR study on the interaction of Escherichia coli DinI with RecA-ssDNA complexes. Nucleic Acids Res 31: 1735-1743.
21. Seshapani P, Rayalu DJ, Kumar VK, Sekhar KC, Kumari JP (2013) Insights from the molecular characterization of mercury stress proteins identified by proteomics in E. coli nissle 1917. Bioinformation 9: 485-490.