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ORIGINAL ARTICLE

Proteomic characterization and bio-informatic analysis of differentially expressed *E. coli* Nissle 1917 proteins with response to cocoti wine stress

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Abstract The present study emphases the comparative proteomic analysis of *Escherichia coli* Nissle 1917 under cocoti palm wine stress and identified differentially expressed proteins. Protein samples were analyzed by 2-D, MALDI-TOF combined with MS access. In 2-D electrophoresis, eight differentially expressed proteins were identified: five up-regulated, two down-regulated and one newly expressed protein. Protein spots were digested with trypsin for MALDI-TOF–MS analysis; protein sequences were obtained from MASCOT search. Sequences were aligned with template using Swiss Model server. Phyre-2 was used to predict homology modeling, RasMol was used to analyze the modeling structures, PSVS server was utilized to validate the protein structure by Ramachandran's plot analysis, physical and chemical properties were analyzed using ProtParam server, Phylogenetic tree was constructed by Mega4. UniProt search helps to find protein functional information of differentially expressed proteins, involved in catalytic activities, regulation mechanisms, DNA damage stimulus, anti-termination and termination process, protein binding, electron transport mechanism, and cell signaling process functions. A detailed exploration of the proteins under cocoti palm wine stress have provided the composition, structure and functions of the expressed proteins for further investigation.

Introduction

Probiotic microorganisms provide a healthy environment in the gut region and prevent from gastrointestinal disorders (Foxx-Orenstein and Chey 2012; Ringel et al. 2012). Generally, most of the probiotic bacteria are gram-positive, but in the current study, we have used gram-negative probiotic bacteria, *E. coli* Nissle 1917 (Nissle 1918). The gastrointestinal strain *E. coli* Nissle 1917 plays a vital role in remission of Bowel syndrome disease, ulcerative colitis and diarrhea (Henker et al. 2007). *E. coli* Nissle 1917 is used as a probiotic to treat gastrointestinal disorders, because of its semi rough lipopolysaccharides, the absence of protein toxins and the iron uptake system (Grozdanov et al. 2004). Consumption of alcohol can support small intestinal bacterial overgrowth (SIBO) which may induce structural changes of the gastrointestinal tract, and intestinal disorders (Gabbard et al. 2014). Palm wine is a local drink in rural areas, which contains some percentage of alcohol and it undergoes longer fermentation to yield a stronger one, which also acts as a natural medium for pathogenic microbial flora including acidophilic bacteria. When people consumed this palm wine, it influence on microbiota of the gastrointestinal tract (Eluwa et al. 2008).

E. coli can show different changes and make adjustments on a molecular level in growth period under stress conditions (Nystrom 2004). Many studies have reported on the bacterial proteomic changes in response to various stress conditions, but this work is a comprehensive proteomic approach for better understanding of the expressed

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proteins structure and mechanism in stress conditions of *E. coli* Nissle 1917 treated with cocoti wine (Soares et al. 2013). Proteomics are widely used for characterization and identification of proteins with MS-based techniques (MALDI-TOF/LC–MS) and bio-informatic tools (Krishnamurthy et al. 2000; Dworzanski et al. 2006). Proteomic studies can focus on looking deeply into the new modifications of proteins; it gives complete information about stress response (Agrawal et al. 2005). In last decade, MS-based technology developed into a high-sensitivity access for quantitative scrutiny of protein sample. The connectedness of 2-D gel electrophoresis, MS-based techniques and bio-informatic tools encourages microbial proteomic research widely. Identification and characterization of proteins in a cell are the most important strategies to assess molecular level mechanisms (Schmidt et al. 2014). The aim of this study was to provide a basic overview of palm wine influence on gastrointestinal probiotic microbes, and to perform MALDI-TOF MS/MS and bio-informatics for identification, characterization and understanding of their functions of expressed proteins.

Materials and methods

Materials and chemicals reagents

The Palm wine sample was collected from the Tirupati rural area, A.P, INDIA. Probiotic *Escherichia coli* Nissle 1917 was obtained from Ardeypharm GmbH, Germany. Nutrient Broth, Sodium dodecyl sulfate (SDS) and other chemicals were purchased from Brass Scientifics Tirupati. BCA Protein Assay Kit was purchased from Thermo Scientific. Inc. Protein isolated Kit and 2-D cleanup kit were purchased from Bio-Rad, 2-D gel electrophoresis and MALDI-TOF facility was provided by Hyderabad Central University (HCU), Telangana State, INDIA.

Sample collection

As stated earlier, the palm wine sample was collected from the Tirupati rural area AP, India and filtered using Millipore nitrocellulose membrane filters (0.02 μ) to separate the microorganisms present in palm wine.

Culture collection and conditions

E. coli Nissle 1917 was obtained from the culture collection center of Ardeypharm GmbH, Herdecke, Germany. The bacterial culture of *E. coli* Nissle 1917 was grown in 50 ml Luria broth supplemented with 50 mg/L Ampicillin at 37 °C, overnight with shaking until the OD reached 0.5 (McFarland standard). Palm wine was added based on MIC

Table 1 Growth values of control and palm wine-treated *E. coli* Nissle 1917

Time intervals (min)	O.D Values	
	E. coli Nissle 1917 (control)	*E. coli* Nissle 1917 (Palm wine treated)
30	0.03	0.02
60	0.05	0.02
90	0.09	0.06
120	0.16	0.11
150	0.21	0.12
180	0.26	0.12
210	0.28	0.12
240	0.28	0.12

concentration, and the optical density values were recorded at 670 nm (John 2005) and the values are shown in Table 1 and Fig. 1.

Protein extraction

Cultures were grown until mid-exponential phase and proteins were isolated from control and palm wine-treated probiotic *E. coli* Nissle 1917. To achieve this, culture media were centrifuged with the culture, and the pellet was washed with phosphate buffer to remove unwanted debris. Then the pellet was re-suspended and sonicated to break at 30 s with the pulse of 1 s at 40% amplitude, and subsequently centrifuged the contents to collect the supernatant. Proteins were extracted from the supernatant (crude) by the method of the Trizol protein extraction method. Protein sample was kept at −20 °C for further analysis (Panga et al. 2013).

2-D gel electrophoresis and staining

Protein samples were purified with the 2-D cleanup kit, because the protein samples contain salts and detergent particles. 24 cm long, 4–7 pH gradient strips were used to separate proteins based on molecular weight and charge. Samples were combined with solubilizing buffer loaded into the strip holder, placed the strips and covered the strips using covering gel, allowed for Isoelectric Focusing. After completion of Isoelectric Focusing step, the strips were treated for rehydration with equilibrium buffer Iodoacetamide (IAA). Later placed the strip into the casting plate to set the instrument with loaded running buffer to allow second dimension. After 2-D gel electrophoresis, gel stained with colloidal coomassie brilliant blue stain was used (www.gelifesciences.com) (Rabilloud and Lelong 2011).

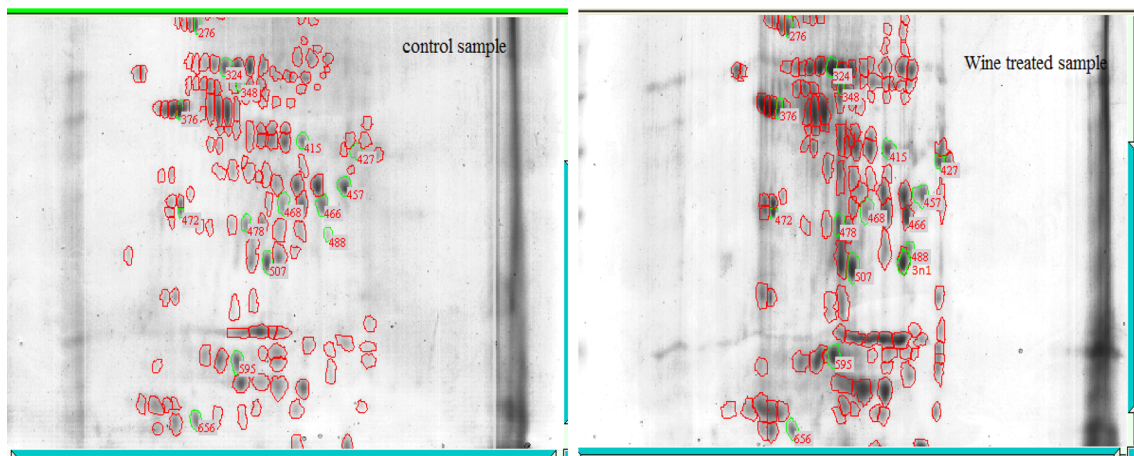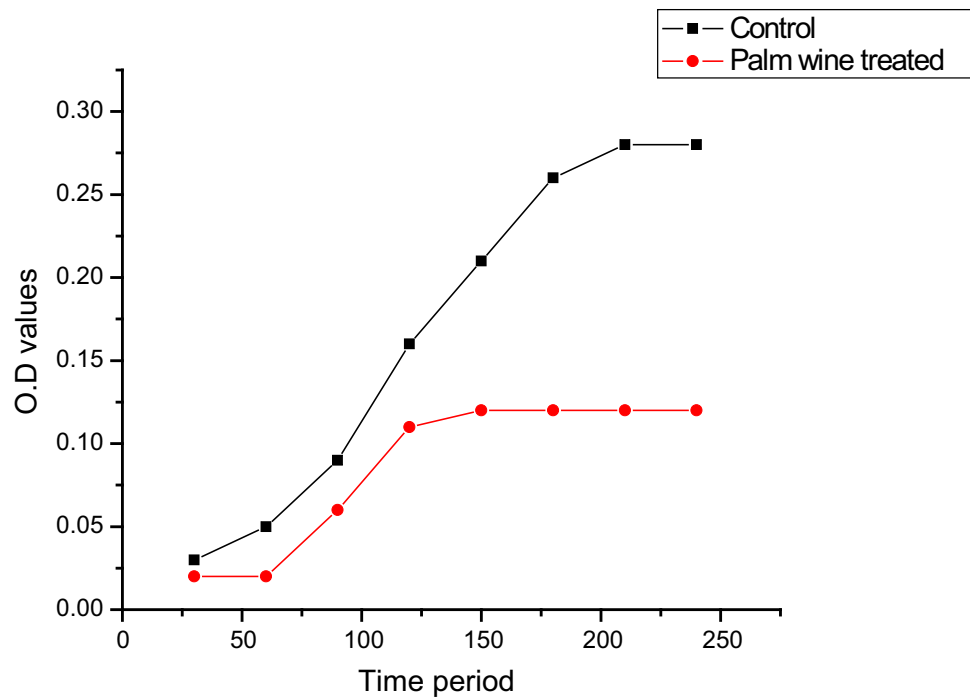**Fig. 1** Growth values of control and palm wine-treated *E. coli* Nissle 1917

Fig. 2 2-D profile of *E. coli* Nissle 1917 expressed proteins under the influence of cocoti palm wine stress

2-D gel analysis

Gels were analyzed by gel scanner (Typhon Variable Mode Imager) 2-D platinum 6.0 software program (Af-jehi-Sadat and Lubec 2007). It quantifies the protein spots, and showed the variation between the control and palm wine-treated gel samples; the spot size indicates up-regulation and down-regulation of the protein. The expressed protein spots were separated using spot cutter, and these spots can be analyzed by MS for protein identification.
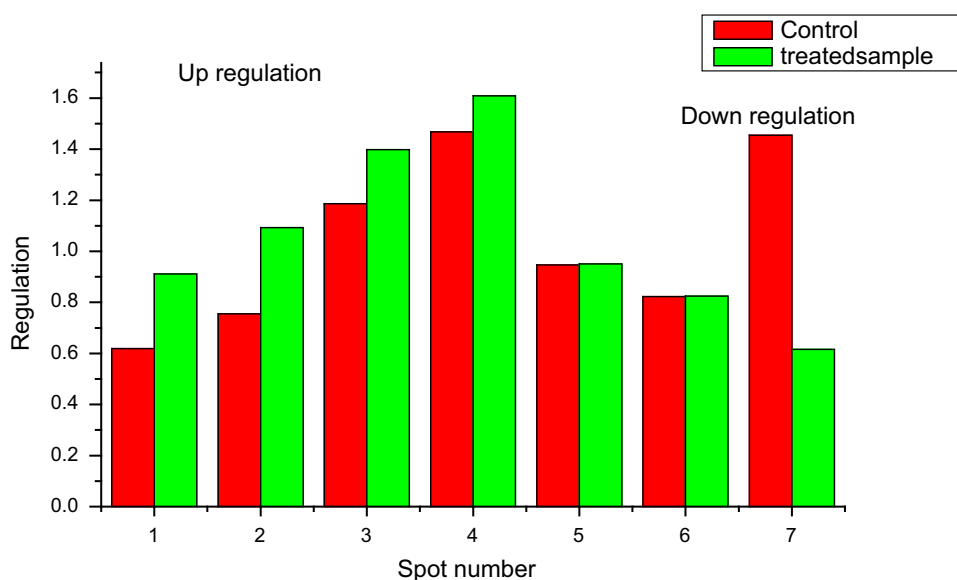
MALDI-TOF MS/MS and data analysis

Protein Spots were picked from gels, digested with trypsin, mixed with Matrix (4-hydroxy-3-methoxy cinnamic acid in acetonitrile) and allowed for MALDI-TOF MS/MS analysis (Model Voyager-DE STR, Applied Bio-systems, Foster, CA, USA). MALDI-TOF MS/MS spectra results were converted into Mascot Generic Format (MGF). Mascot is the search engine for MS spectra data (http://www.matrixscience.com). The search parameters are: peptide tolerance range is 100–1200 ppm, MS/MS tolerance range

in between 0.2 and 2 Daltons and missed cleavage of trypsin always one, search against the NCBInr database among *E. coli* species. Proteins were distinguished based on the maximum hits, molecular weight and ion score and threshold significance <0.05. Phyrol 2 software was used for homology modeling (Tang et al. 2013; Vranakis et al. 2012), RASMOL for pdb file analysis, PSVS server to visualize dihedral angles φ against ψ of amino acid residues in protein structure. It shows the possible conformations of φ and ψ angles for a polypeptide. ProtParam server was used to characterize the protein physico-chemical properties and Mega-6 for phylogenetic analysis ((Rodrigo et al. 2014; Chandrasekhar et al. 2014; Vranakis et al. 2012; Tamura et al. 2013).

Table 2 *E. coli* Nissle 1917 expressed protein up- and down-regulation values with reference to cocoti palm wine

Spot number	Control	Wine treated	Regulation
Up-regulation			
478	0.618591	0.911168	1.472973257
415	0.75465	1.09259	1.447810243
324	1.1867	1.39784	1.177921968
507	1.46744	1.60907	1.096515019
466	0.946688	0.950442	1.003965404
Down-regulation			
276	0.822424	0.824411	0.997589794
376	1.45419	0.616171	2.36004291

Results and discussion

In the present study, we have analyzed *E. coli Nissle* 1917 differentially expressed proteins with the influence of cocoti palm wine. Palm wine-treated *E. coli* showed less growth compared to controls. Trizol protein extraction method was used to isolate the protein sample for 2-D analysis. Totally, we isolated eight differentially expressed proteins and out of these eight proteins, five proteins showed up-regulation, two proteins showed down-regulation the remaining one is a newly expressed protein (Fig. 2) which are not found in control samples; protein expression levels were analyzed using an image analyzer and the regulation values are presented in Table 2 and Fig. 3 (Natale et al. 2011). The expressed proteins were analyzed by MALDI-TOF/MS and the results are represented in Fig. 4. Protein name as well as sequence coverage and calculated pI were obtained using the MASCOT search engine (http://www.matrixscience.com) (Tang et al. 2013; Sarkisova et al. 2014); the results are summarized in Table 3. Physico-chemical characters like molecular weight, instability index, aliphatic index, extinction coefficient, total number of atoms, protein molecular formula and GRAVY(grand average of hydropathy) were analyzed by ProtParam server (http://web.expasy.org/protparam/) (Vranakis et al. 2012; Tamura et al. 2013) and the results are shown in Table 4. Target sequence was aligned with templates using SWISSMODEL server (http://swissmodel.expasy.org/) and the results are represented in Fig. 5. Homology structures of expressed proteins under cocoti wine stress were visualized by phyre-2 database and the
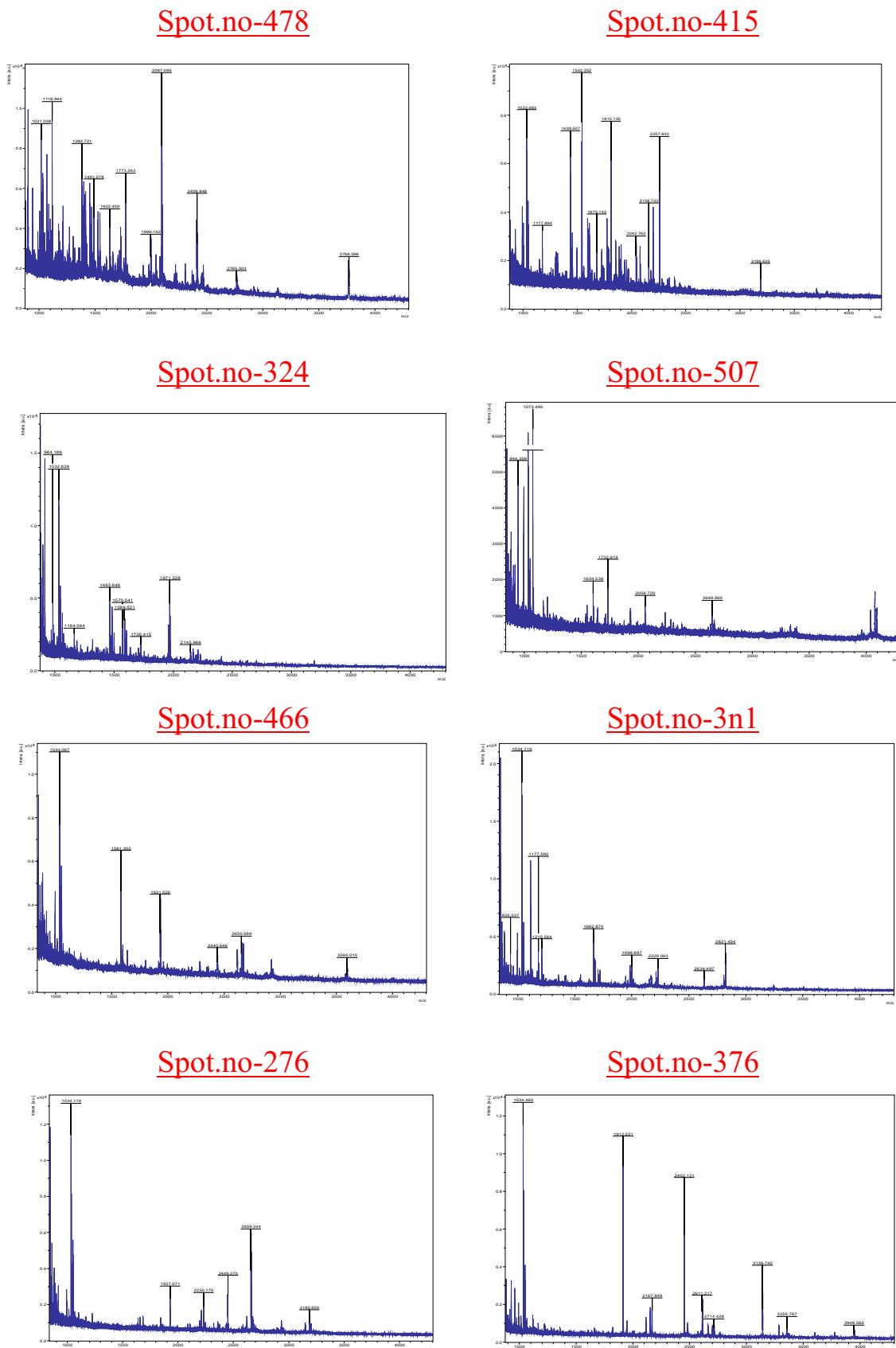
Fig. 3 *E. coli* Nissle 1917 cocoti palm wine stress expressed protein up- and down-regulation graph images

Spot.no-478

Spot.no-415

Spot.no-324

Spot.no-507

Spot.no-466

Spot.no-3n1

Spot.no-276

Spot.no-376

Fig. 4 MALDI-TOF–MS analysis of expressed proteins in response to cocoti palm wine stress

Table 3 List of newly expressed proteins of *E. coli* Nissle 1917 under cocoti wine stress identified by MASCOT search

Spot number	Protein name	No. of amino acids	Protein score	Molecular weight	Sequence coverage	Calculated PI
478	Small toxic polypeptide LDRA_ECOLI	35	25	4013.8	94	10.83
415	30S ribosomal protein RS11_ECO24	177	34	18,903.7	25	9.71
324	Transcription anti-termination protein RFAH- ECO57 NusA	162	35	18,340.2	17	8.55
507	PUR7-eco24- phosphoribosylamidazole-succinocarboxamide synthase	237	32	26,955	16	5.05
466	Xanthine dehydrogenase iron sulfur binding subunit XDHC_Eco57	159	32	16,949.7	25	6.79
3n1	P21 prophage-derived head-stabilizing proteinVG03_ECOL6	68	37	7620.8	41	10.70
276	UPF0033 protein YEDF_	77	38	8638.9	53	4.83
376	Probable adenosine monophosphate-protein transferase FIC_ECOLI	200	29	22,960	23	5.16

Table 4 ProtParam analysis of *E. coli* Nissle 1917 expressed proteins under cocoti wine stress

Spot no.	Formula	Total no. of atoms	Extinction coefficient	Molecular weight (Daltons)	Estimated half-life (h)	Instability index	Aliphatic index	GRAVY
478	$C_{190}H_{291}N_{49}O_{43}S$	575	16,500	4013.8	>10	23.18	117.43	0.760
415	$C_{837}H_{1374}N_{244}O_{247}S_3$	2705	12,950	18,903.7	>10	19.48	91.41	−0.227
324	$C_{833}H_{1313}N_{223}O_{233}S$	2607	13,075	18,340.2	>10	35.21	93.27	−0.141
507	$C_{1200}H_{1908}N_{320}O_{362}S_1$	3802	21,555	26,995	>10	37.62	88.02	−0.370
466	$C_{730}H_{1202}N_{204}O_{225}S_{16}$	2377	10,595	16,949.7	>10	42.33	88.99	0.072
3n1	$C_{324}H_{564}N_{108}O_{99}S$	1097	1490	7620.8	>10	56.26	108.97	−0.291
276	$C_{382}H_{615}N_{101}O_{116}S$	1219	6085	8638.9	>10	47.99	98.70	−0.27
376	$C_{1028}H_{1578}N_{282}O_{301}S$	3197	31,525	22,960	>10	43.13	86.90	−0.360

results are summarized in Fig. 6. The results of the RasMol server, to analyze the protein homology, total number of H-bonds, helices, strands, turns, atoms, groups and number of bonds were presented in Table 5; validation of the expressed protein was analyzed by protein structure validation software suite (PSVS) (http://psvs-1_5-dev.-nesg.org) and the results are presented in Fig. 7. Phylogenetic analysis explains the relationship of the protein presented in Fig. 8; UniProt search (http://www.uniprot.org/) helped to find the protein functional information (Alpi et al. 2015).

In the present study, we have analyzed *E. coli* Nissle 1917 differentially expressed proteins with the influence of cocoti palm wine. The probiotic *E. coli* Nissle 1917 treated with cocoti palm wine sample (as per MIC) showed a total of eight differentially expressed proteins which were separated from 2-D gels based on protein expression level. Overall, five proteins showed up-regulation compared to control and these protein spots were indicated by the numbers 478, 415, 324, 507, 466. Two proteins showed down-regulation and these protein spots were indicated by the numbers of 276,376. Only one newly expressed protein identified in wine-treated gel in the spot was indicated as 3n1. These spots were digested with trypsin and then allowed for MALDI-TOF analysis. The up-regulated proteins involved in cell signaling process, protein binding, anti-termination and termination process, electron transport mechanism and catalytic activities; the down-regulated
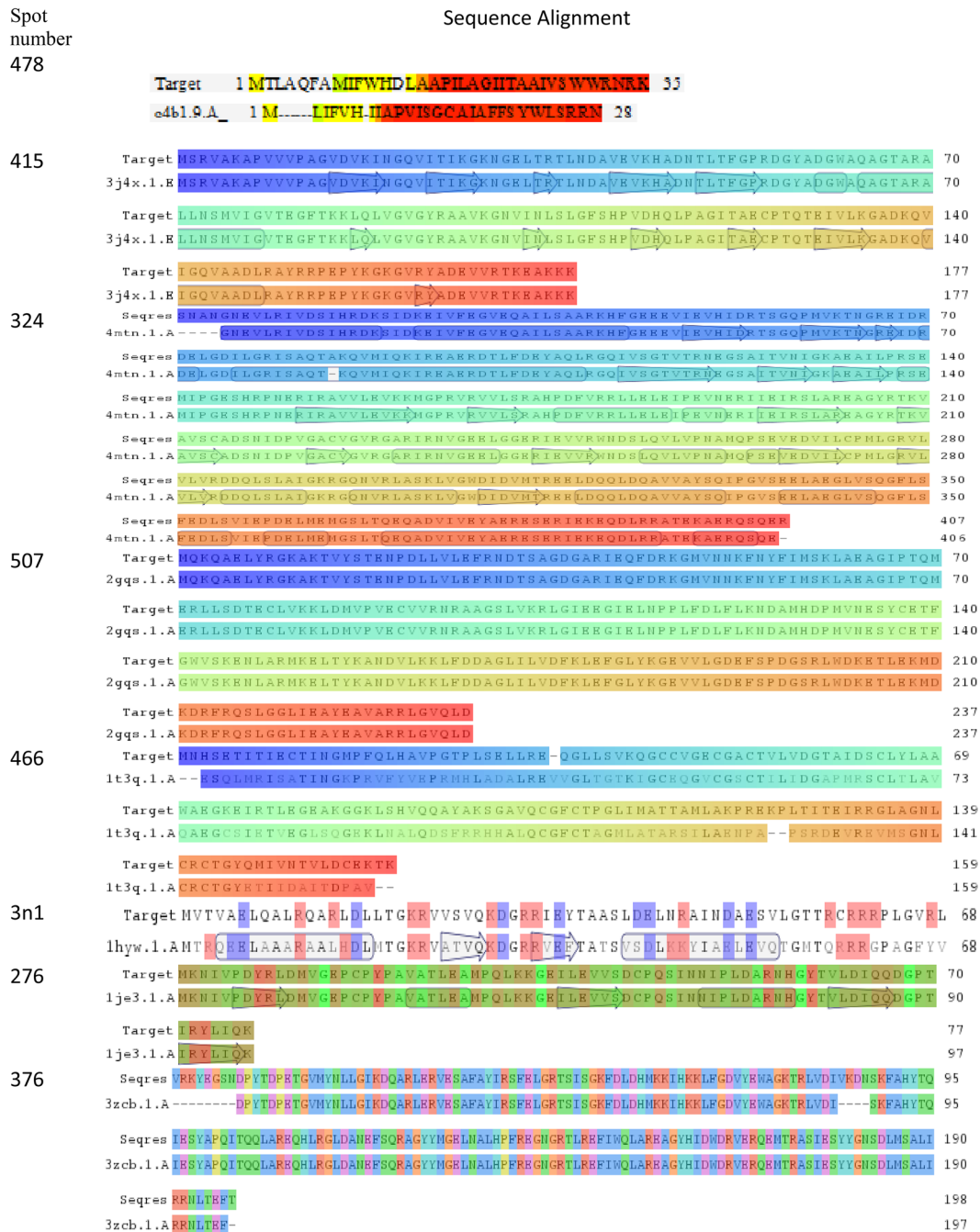
Fig. 5 Sequence alignment of *E. coli* Nissle 1917 expressed proteins with response to cocoti palm wine

proteins involved in cellular response to DNA damage stimulus and regulatory mechanisms; and the newly expressed protein involved in viral life cycle activities (Artzi et al. 2015).

Physico-chemical properties of the expressed proteins which were analyzed by ProtParam server revealed that the coefficient value range of the expressed proteins is 1490–31,525 Daltons. The half-life time indicates that half of the amount of protein disappears in a cell after its synthesis and all the expressed proteins have showed >10 h as the half-life period. Instability index explains the nature of the proteins, based on these three proteins were stable and the remaining expressed proteins were unstable. Aliphatic index explains coverage of aliphatic amino acids in the present proteins; it supports the thermal stability of globular proteins. The range of an aliphatic index of the
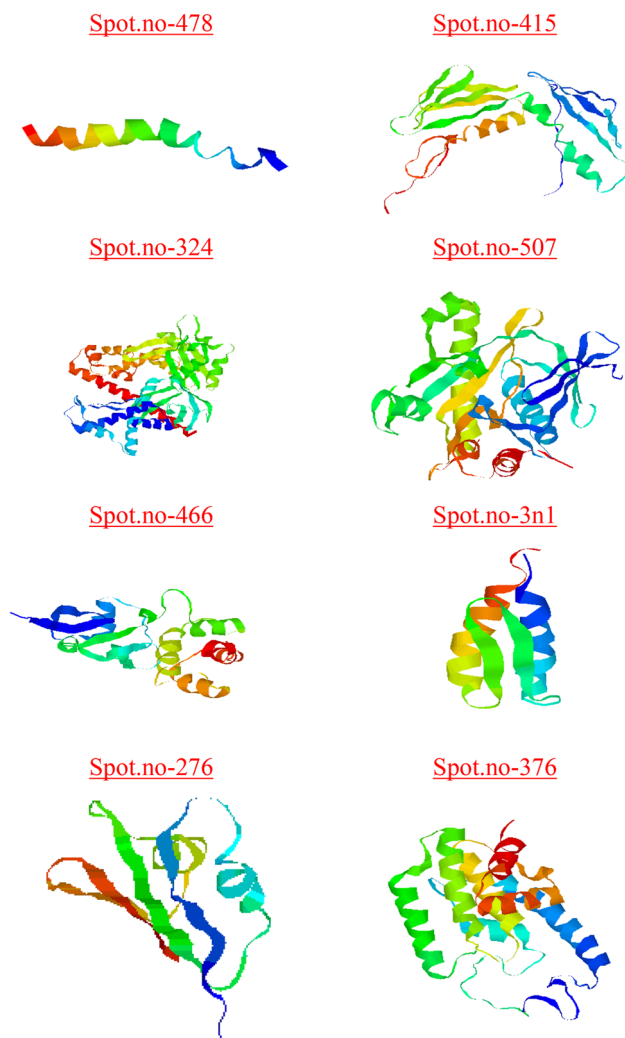
Spot.no-478   Spot.no-415

Spot.no-324   Spot.no-507

Spot.no-466   Spot.no-3n1

Spot.no-276   Spot.no-376

Fig. 6 Homology modeling of *E. coli* Nissle 1917 expressed proteins with response to cocoti wine

expressed proteins is 86.90–117.43. The grand average of hydropathicity (GRAVY) index of the proteins ranged from −0.360 to 0.760. Molecular formula of proteins is

also explained and all the results are summarized in Table 4 (Lai et al. 2003).

The target sequence was searched by Swiss model against PDB for selection of template protein. A total of eight expressed proteins was aligned in suitable templates, which were found based on the sequence superimposition up-regulated proteins aligned with C4B1, 3J5E, 4MTN, 2Z02, 1ZXI and the down-regulated proteins aligned with 1JE3, 3ZC7. These two templates and the newly expressed protein aligned with the 1HYW template as shown in Fig. 5 (Lima et al. 2009). To predict homology modeling using Phyre-2 server and to analyze these structures, we used RASMOL version 2.6 (Sheehan and Sullivan 2011) and the results are represented in Fig. 6 and Table 5. Validation of the expressed protein models was done with Protein structure validation server (PSVS). The ψ and φ distributions of the Ramachandran's plots of non-glycine, non-proline residues are summarized in Fig. 7. Phylogenetic analysis explains the evolutionary relationship between the expressed protein sequences the results are shown in Fig. 8 (Brewer et al. 2014).

Conclusion

2D Page coupled with MALDI-TOF–MS for protein identification allowed us to explore the protein complex of probiotic *E. coli* under cocoti palm wine stress. Based on comparative proteomic analysis we identified five up-regulated, two down-regulated proteins and also noticed one newly expressed protein (3N1). Complete analysis explains that the up-regulated proteins involved in cell signaling, termination and anti-termination process suppress the host pathogens, electron transport mechanism, and catalytic activities; down-regulated proteins involved in cellular responses to DNA damage stimulus and regulation mechanisms and also helps to bind the mutated gene. Newly

Table 5 Structural analysis of homology models by RasMol

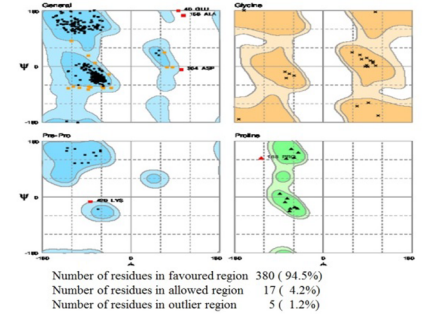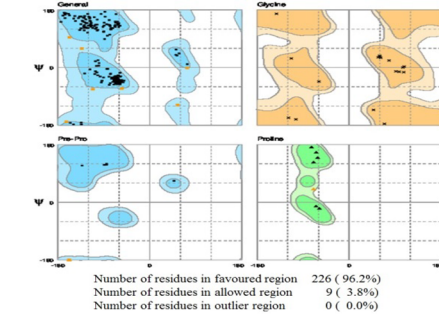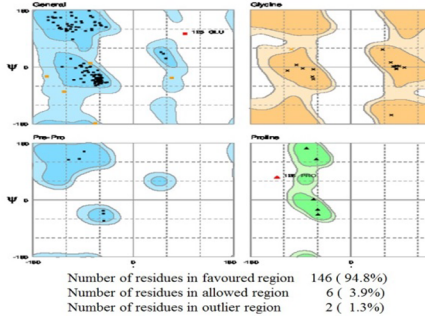| Spot number	No. of groups	No. of atoms	No. of bonds	No. of H-bonds,	No. of helices	No. of strands	No. of turns
478	28	230	241	23	3	0	1
415	175	1313	1339	89	3	14	20
324	404	3153	3211	305	19	18	29
507	237	1894	1924	172	9	22	21
466	156	1148	1175	98	10	7	21
3n1	58	448	452	41	2	2	4
276	77	765	776	48	2	6	7
376	186	1515	1557	129	9	4	17

Spot No- 478
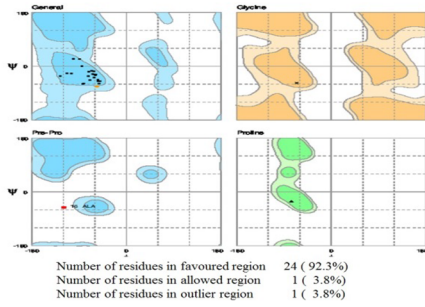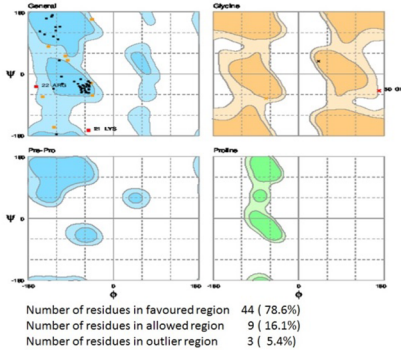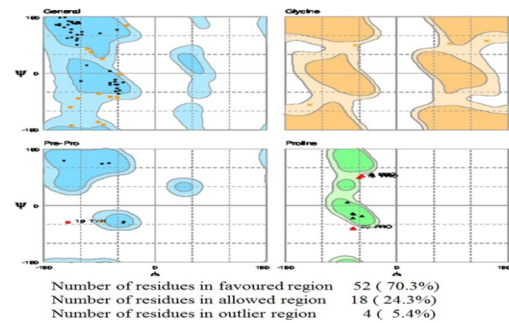
Spot No- 415
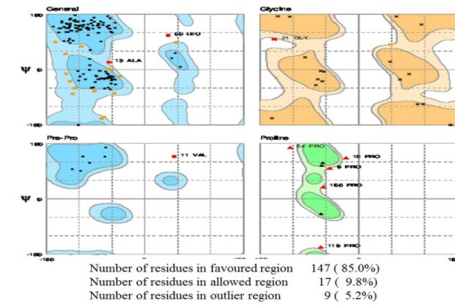
Spot No- 324

Spot No- 507

Spot No-466

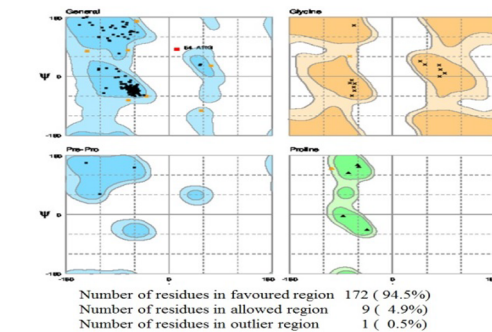Spot No- 3n1

Spot No-276

Spot No-376

Fig. 7 Ramachandran's plot analysis of expressed *E. coli* Nissle 1917 proteins under cocoti palm wine stress
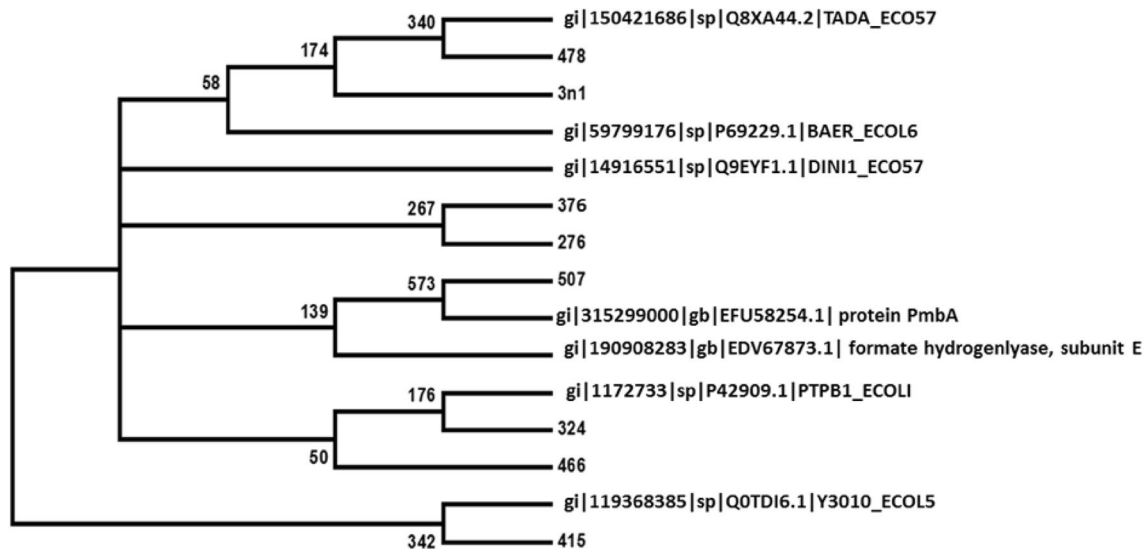
Fig. 8 Phylogenetic relationship of expressed proteins based on the Mascot search results

regulated protein (3N1) involves in viral life cycle activities. These results suggested that the cocoti palm wine stress effects on probiotic *E. coli* Nissle 1917.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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