Isolation and Analysis of Salt Response of *Lactobacillus plantarum* FS5-5 from *Dajiang*

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Abstract From 15 samples of *dajiang* collected in northeast of China, three salt resistant lactic acid bacteria were isolated and identified as *Lactobacillus plantarum* through physiological studies and 16S rDNA sequence alignment. *L. plantarum* FS5-5 showed better growth in an environment with 12 % (w/v) NaCl than the other two strains. The expression of proteins extracted from *L. plantarum* FS5-5 cultured in de Man, Rogosa, and Sharp (MRS) containing 0, 3, 6 and 9 % (w/v) NaCl was analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS). The results showed that 42 kinds of proteins were identified, which could be divided into three groups: 27 kinds of proteins related to protein synthesis and degradation, six kinds of proteins related to carbohydrate metabolism and energy metabolism, nine proteins related to nucleic acid metabolism. Overexpression of these proteins imply that a series of changes have occurred in the process of protein synthesis and degradation, carbohydrate metabolism, energy metabolism and nucleic acid metabolism after *L. plantarum*

FS5-5 exposed to salt stress. All these proteins may have effects on the salt-tolerant characteristics of the *L. plantarum* FS5-5.

Keywords *Dajiang* · *Lactobacillus plantarum* FS5-5 · NaCl · Protein

Introduction

Soybean paste, also called *dajiang* in northeast of China, is one of traditionally fermented products. For centuries, *dajiang* plays an important role in people's daily diet in northeast of China. After microbial fermentation, the nutrients originally found in soybean can be easily digested and absorbed by the body. Lactic acid bacteria are proved as preponderant bacterial flora in *dajiang*, and play an important role in *dajiang* because the lactic acid and other organic acids produced by these bacteria act as natural preservatives as well as flavour enhancers.

Adding salt during fermentation of *dajiang* can prevent spoilage and enhance the flavor of *dajiang*, but also implies that lactic acid bacteria are challenged by high salt concentration. A sudden increase in the osmolarity of the environment (hyperosmotic stress) results in the movement of water from the cell to the outside, which causes a detrimental loss of cell turgor pressure, changes the intracellular solute concentration and changes the cell volume [1]. In order to maintain growth, lactic acid bacteria use several adaptation strategies to cope with unfavorable osmotic conditions. Rapid and frequent genetic transformations allow them to adjust the expression of proteins and become resilient to this detrimental factor [2]. Le Marrec et al. [3] found that Xaa-Pro dipeptidase (pepQ) showed increased activity and proline- and glutamate-containing

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peptides were protective under salt-induced stress to contribute to the adaptation of *Oenococcus oeni* to high salt. Belfiore et al. [4] found that the overexpression of proteins Hsp20, ClpB, ClpL ATPase, GrpE and DnaK occurred during growth of *Lactobacillus sakei* CRL1756 under salt stress. Zhao et al. [5] analyzed the response of *Lactobacillus plantarum* ST-III to salt stress by using RNA-seq technology to find that in the presence of Glycine-betaine (GB), the expression level of genes involved in carbohydrate transport and metabolism and ribosomal associated proteins was significantly up-regulated, which might be closely associated with salt stress resistance of *L. plantarum* ST-III. We aimed to gain an understanding of the response of *L. plantarum* FS5-5 to salt stress at the level of protein expression.

In this study, three salt resistant lactic acid bacteria strains were isolated from *dajiang* in northeast of China and identified as *L. plantarum* FS5-5, *L. plantarum* DL3-1 and *L. plantarum* DL4-5 through physiological studies and 16S rDNA sequence alignment. *L. plantarum* FS5-5 exhibited the highest salt-tolerant ability and showed better growth than the other two isolated strains. The expression of proteins extracted from *L. plantarum* FS5-5 cultured in MRS medium containing 0, 3, 6 and 9 % (w/v) NaCl were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS). This study should lay a foundation for revealing the salt-tolerant mechanism of *L. plantarum* FS5-5, and provide an important reference for directed modification of the strain in the future.

Materials and Methods

Collection of *Dajiang* Samples

In this study, a total of 15 samples of *dajiang* were collected from Shenyang, Dalian, Fushun, and Benxi City in Liaoning province of China, then immediately put the collection among ice and maintain a lower temperature back to the laboratory for the subsequent experiment in time.

Isolation and Selection of Salt Tolerance Lactic Acid Bacteria

Aliquots of 1 g from each *dajiang* sample were vortexed with physiological saline solution [NaCl 0.85 % (w/v)], serial dilution (10^{-1} – 10^{-8}) was prepared from the above mixture and was plated on MRS-agar to select lactic acid bacteria. These selected strains were tested for Gram stain and catalase reaction. Distinctly Gram-positive, catalase

negative isolates were purified by re-streaking on MRS-agar pH 6.4 and kept in MRS-agar stabs at 4 °C. Lyophilization of isolates was performed for longer storage.

The isolated strains were reactivated by growing in MRS medium at 37 °C for 24 h. Then these strains were cultured in MRS medium added NaCl at 37 °C for 24 h, the concentrations of NaCl were 4, 6, 8, 10, 12, 14, 16 and 18 % (w/v), respectively. Then the cultures were vortexed, absorbance at 600 nm ($A_{600\text{ nm}}$) was measured and compared to a control culture (without a strain).

Identification of Salt Tolerance Lactic Acid Bacteria

The morphological and physiological properties of the isolates were investigated according to Bergey's manual of systematic bacteriology [6]. Then 16S rDNA gene sequence analysis was constructed. Genomic DNA of the tested strains was extracted by the optimized cetyltrimethylammonium bromide (CTAB) method. The genomic DNA obtained was used as the template of PCR amplification, the volume of each PCR reaction system was 20 μ L. Forward primer 5'-AGA GTT TGA TCC TGG CTC AG-3' and reverse primer 5'-CTA CGG CTA CCT TGT TAC GA-3' were used to amplify the region corresponding to 1500 bp which was targeted to the whole length of *Escherichia coli* 16S rDNA gene [7]. The successfully amplified PCR products were sequenced (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China). The homology comparison of sequences obtained was performed via NCBI BLAST server (<http://www.ncbi.nlm.nih.gov/BLAST/>). The sequences were then deposited in Genbank database for obtaining the standard sequence data, and the phylogenetic tree was constructed using MAGE 5.0 software package in a method of Neighbor-Joining [8].

Protein Extraction and SDS-PAGE Electrophoresis

In order to make growth curves, the selected strain were cultured into MRS containing 3-(*N*-morpholino)propane-sulfonic acid (MOPS) and 2-(*N*-morpholino)ethanesulfonic acid (MES) (MRS-M) liquid medium with 0, 3, 6 and 9 % (w/v) NaCl, and collected in the mid-logarithmic phase (6, 6, 8 and 21 h, respectively). Whole cellular proteins were extracted according to a previously described method [9]. Cell samples were ground into fine powder in the presence of liquid nitrogen. After treatment with 10 % (v/v) trichloroacetic acid/acetone solution and lyophilization, the samples were suspended in lysis buffer that contained 7 M urea, 2 M thiourea, 4 % (w/v) 3-[(3-cholanido-propyl)dimethylammonio]-1-propanesulfonate (CHAPS), 2 % Pharmalyte pH 3–10, 1 mM phenylmethanesulfonyl

fluoride (PMSF), 2 mM EDTA, and 65 mM dithiothreitol (DTT), to give a protein concentration of 1 µg/µL.

In order to compare the protein expression difference, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate the protein using the Laemmli's buffers [10]. Protein concentration was initially determined according to the Bradford method [11], with bovine serum albumin (BSA) as the protein standard. The SDS-PAGE consisted of 5 % stacking gel and 12 % separation gel. Gradient gel was 4–20 % pre-fabricated gradient gel. Sample volume was controlled to 15 µg proteins with 5 µL of 6× bromophenol blue in each lane. Electrophoresis was conducted at a constant current of 80 V through the stacking gel and 120 V through the resolving gel using the miniprotean II apparatus (Bio-Rad). Proteins were stained with Coomassie Blue G250 (Sigma). Image Lab 3.0 software (Bio-Rad Laboratories, Inc., CA) was used to analyze the SDS-PAGE electrophoretogram to find out the distinct bands.

Bioinformatics Analysis

The six distinct protein bands were analyzed by LC–MS/MS (Beijing Protein Innovation Co., Ltd., Beijing, China). Software Mascot 2.3.01 (Matrix Science Ltd., UK) was used to retrieval protein sequence from NCBI database with the following parameter setting: Taxonomy set as lactobacillus, 50 ppm peptide mass accuracy, trypsin cleavage, one missed cleavage allowed, carbamidomethylation set as fixed modification, oxidation of methionine was allowed as variable modification, MS/MS fragment tolerance was set to 0.25 Da, The search results reached significant level ($p < 0.05$), when protein fraction was more than 19.

Results

Isolation and Selection of Salt Tolerance Lactic Acid Bacteria

Twenty-six isolates were identified as lactic acid bacteria by Gram reaction, absence of catalase and cell morphology. $A_{600\text{ nm}}$ after 24 h growth of 26 strains in MRS medium containing NaCl concentration from 4 to 18 % (w/v) was measured and showed in Table 1. With the increase of NaCl concentration, the $A_{600\text{ nm}}$ values decreased gradually. When the NaCl concentration reached 12 % (w/v), only the values of strain DL3-1, DL4-5 and FS5-5 were above 0.100. When the concentrations of NaCl were up to 14–18 % (w/v), the values of 26 strains maintained between 0.030 and 0.060, which can preliminarily concluded that they were hardly growing. These findings

suggested that DL3-1, DL4-5 and FS5-5 were better tolerant to salt than other strains, which could reach 12 % (w/v). And the salt-tolerant ability of strain FS5-5 was best.

Identification of Salt Tolerance Lactic Acid Bacteria

Physiological and biochemical reactions of the three strains of lactic acid bacteria were shown in Table 2, with their morphology shown in Fig. 1. The results showed that strain DL3-1, DL4-5 and FS5-5 all belong to the *Lactobacillus* sp.

To further confirm the species, the nucleotide sequences of the 16S rDNA gene for the tested strains of the three major isolates were determined. The homology comparison of sequences obtained was performed via NCBI BLAST server, and the phylogenetic tree was constructed (Fig. 2). Strain DL3-1, DL4-5, FS5-5 and *Lactobacillus plantarum* YML007, *Lactobacillus plantarum* IMAU32388 in the same branch, have high homology. Strain DL3-1, DL4-5 and FS5-5 were related to the specie of *L. plantarum*. The physiological results agreed with genetic identification studies.

Protein Extraction and SDS-PAGE Electrophoresis

As *L. plantarum* FS5-5 exhibited the highest salt tolerance ability and showed better growth in an environment with 12 % (w/v) NaCl than other strains. Growth curve of *L. plantarum* FS5-5 incubated in MRS with 0, 3, 6 and 9 % (w/v) NaCl were constructed (Fig. 3). When they reached the exponential phase (6, 8, 10 and 21 h, respectively), bacterial cultures were sampled for protein extraction and SDS-PAGE electrophoresis.

Results from the study using Bio-rad 18.5 × 20 cm SDS-polyacrylamide gel (12 % separation gel, 5 % concentrated gum) (Fig. 4a) and Bio-rad 4–20 % prefabricated gradient gel (Fig. 4b) both gave relative higher resolution to separate bands of protein separated from *L. plantarum* FS5-5 at different salt concentrations. However, the gradient gel was shorter than 18.5 × 20 cm SDS-polyacrylamide gel, resulting in many proteins were closely linked and not so easy to distinguish. The separation effect of Bio-rad 18.5 × 20 cm SDS-polyacrylamide gel was relatively better, so we used Image Lab 3.0 software to analyze the lanes (Fig. 5). Based on this analysis, the expression of total protein of *L. plantarum* FS5-5 in lane 2 (3 % (w/v) NaCl) was basically no difference with lane 1 (0 % (w/v) NaCl). Lane 3 (6 % (w/v) NaCl) and lane 4 (9 % (w/v) NaCl) differed from lane 1 by the absence of band 4 and 7 and the presence of one band of 14.3 kDa, and protein expression quantity increased significantly at the band of about 6.6 kDa in lane 3. So we defined band 4 in lane 1 as sample 1, band 7 as sample 2, defined band 24 in lane 3 as

Table 1 Growth situation of 26 lactic acid bacteria strains under different concentration of NaCl

Strains	$A_{600\text{ nm}}^a$															
	4 % (w/v)	6 % (w/v)	8 % (w/v)	10 % (w/v)	12 % (w/v)	14 % (w/v)	16 % (w/v)	18 % (w/v)								
SY1-1	1.945 ± 0.102	1.087 ± 0.137	0.499 ± 0.063	0.109 ± 0.015	0.047 ± 0.009	0.051 ± 0.001	0.043 ± 0.009	0.049 ± 0.003								
SY2-2	1.658 ± 0.140	0.600 ± 0.014	0.112 ± 0.017	0.043 ± 0.002	0.041 ± 0.001	0.042 ± 0.003	0.039 ± 0.006	0.051 ± 0.009								
SY2-4	1.325 ± 0.054	1.153 ± 0.083	0.103 ± 0.015	0.052 ± 0.004	0.055 ± 0.001	0.048 ± 0.002	0.051 ± 0.009	0.041 ± 0.011								
SY3-5	1.414 ± 0.127	1.021 ± 0.085	0.251 ± 0.018	0.050 ± 0.007	0.049 ± 0.010	0.051 ± 0.004	0.045 ± 0.012	0.050 ± 0.005								
SY4-2	1.856 ± 0.107	1.252 ± 0.081	0.743 ± 0.012	0.195 ± 0.006	0.098 ± 0.003	0.063 ± 0.003	0.042 ± 0.003	0.039 ± 0.001								
SY4-4	1.872 ± 0.201	1.013 ± 0.101	0.106 ± 0.033	0.071 ± 0.013	0.047 ± 0.008	0.046 ± 0.009	0.036 ± 0.005	0.041 ± 0.006								
DL1-6	1.521 ± 0.178	0.689 ± 0.060	0.105 ± 0.032	0.030 ± 0.007	0.042 ± 0.012	0.039 ± 0.005	0.052 ± 0.010	0.038 ± 0.007								
DL2-2	1.121 ± 0.133	0.543 ± 0.031	0.063 ± 0.007	0.041 ± 0.004	0.046 ± 0.005	0.056 ± 0.004	0.045 ± 0.005	0.051 ± 0.004								
DL3-1	1.981 ± 0.171	1.465 ± 0.156	0.476 ± 0.062	0.276 ± 0.022	0.103 ± 0.006	0.033 ± 0.002	0.038 ± 0.004	0.044 ± 0.005								
DL3-3	1.692 ± 0.113	1.123 ± 0.109	0.196 ± 0.031	0.071 ± 0.013	0.041 ± 0.009	0.045 ± 0.004	0.031 ± 0.004	0.034 ± 0.005								
DL3-6	1.257 ± 0.099	0.721 ± 0.023	0.343 ± 0.040	0.110 ± 0.021	0.065 ± 0.012	0.051 ± 0.007	0.045 ± 0.003	0.054 ± 0.004								
DL4-5	1.784 ± 0.165	1.103 ± 0.087	0.365 ± 0.025	0.203 ± 0.027	0.097 ± 0.013	0.030 ± 0.007	0.039 ± 0.009	0.046 ± 0.006								
DL4-6	1.636 ± 0.217	0.985 ± 0.094	0.106 ± 0.0021	0.058 ± 0.011	0.046 ± 0.008	0.037 ± 0.003	0.047 ± 0.006	0.038 ± 0.010								
FS1-8	1.432 ± 0.188	0.821 ± 0.085	0.101 ± 0.012	0.045 ± 0.007	0.023 ± 0.001	0.048 ± 0.005	0.052 ± 0.009	0.037 ± 0.007								
FS1-11	1.981 ± 0.224	1.584 ± 0.198	0.369 ± 0.059	0.212 ± 0.027	0.092 ± 0.016	0.037 ± 0.004	0.041 ± 0.013	0.034 ± 0.005								
FS2-3	1.660 ± 0.214	0.312 ± 0.017	0.245 ± 0.021	0.131 ± 0.027	0.061 ± 0.010	0.042 ± 0.008	0.058 ± 0.012	0.042 ± 0.005								
FS2-10	1.732 ± 0.124	0.583 ± 0.054	0.149 ± 0.017	0.045 ± 0.006	0.045 ± 0.005	0.056 ± 0.007	0.049 ± 0.006	0.033 ± 0.003								
FS5-5	2.176 ± 0.238	1.665 ± 0.210	0.725 ± 0.079	0.387 ± 0.035	0.137 ± 0.021	0.038 ± 0.004	0.046 ± 0.005	0.052 ± 0.005								
FS3-5	2.010 ± 0.217	0.545 ± 0.062	0.076 ± 0.014	0.052 ± 0.009	0.061 ± 0.007	0.047 ± 0.004	0.054 ± 0.006	0.058 ± 0.007								
FS3-12	1.462 ± 0.152	0.634 ± 0.067	0.143 ± 0.025	0.054 ± 0.005	0.045 ± 0.008	0.046 ± 0.003	0.037 ± 0.004	0.057 ± 0.005								
BX1-2	0.921 ± 0.085	0.325 ± 0.019	0.068 ± 0.015	0.051 ± 0.014	0.049 ± 0.009	0.043 ± 0.008	0.057 ± 0.011	0.046 ± 0.007								
BX1-8	1.313 ± 0.119	0.543 ± 0.084	0.103 ± 0.020	0.043 ± 0.005	0.049 ± 0.010	0.042 ± 0.006	0.052 ± 0.007	0.037 ± 0.004								
BX2-11	1.962 ± 0.236	0.848 ± 0.097	0.201 ± 0.039	0.052 ± 0.013	0.043 ± 0.007	0.044 ± 0.006	0.036 ± 0.005	0.038 ± 0.003								
BX3-10	1.832 ± 0.230	0.365 ± 0.021	0.094 ± 0.014	0.045 ± 0.009	0.052 ± 0.012	0.030 ± 0.003	0.037 ± 0.004	0.042 ± 0.005								
BX4-3	1.675 ± 0.247	1.150 ± 0.132	0.201 ± 0.031	0.033 ± 0.004	0.060 ± 0.009	0.049 ± 0.006	0.036 ± 0.003	0.051 ± 0.007								
BX4-12	1.467 ± 0.206	0.763 ± 0.085	0.096 ± 0.019	0.042 ± 0.005	0.047 ± 0.005	0.044 ± 0.006	0.047 ± 0.004	0.049 ± 0.006								

± indicates standard deviation from the mean

^a Presented values are means of triplicate determinations

Table 2 Physiological and biochemical characteristics of 3 salt-tolerance lactic acid bacteria

Characteristics	Groups ^a		
	1	2	3
Strains	DL3-1	DL4-5	FS5-5
Glucose acid production test	+	+	+
Glucose gas test	+	+	+
Lactose acid production test	+	+	+
Sugar acids test	+	+	+
Galactose produce acid	+	+	+
Maltose produce acid	+	+	+
Mannose produce acid	–	–	–
Sorbic acid fermentation	–	–	–
Arabinose produce acid	+	+	+
Mannitol produce acid	–	–	–
Litmus peptone	–	–	–
Litmus produce acid	–	–	–
Litmus solidification	–	–	–
MR test	+	+	+
V-P test	–	–	–
Starch hydrolysis test	–	–	–
Gelatin liquefaction test	–	–	–
Anaerobic growth test	+	+	+
Citric acid test	–	–	–
Anaerobic nitrate gas	–	–	–
Casein hydrolysis experiments	–	–	–
Indole test	–	–	–
Oxidase test	–	–	–

(+) and (–) symbols indicate the positive and negative reaction respectively

^a Groups 1–3 were all identified as *L. plantarum*

sample 3, band 31 in land 3 as sample 4. In addition, we can clearly see that the strain also significantly expressed two bands with a relative size of 16 kDa, which were defined as the sample 5 and sample 6 at 6 % (w/v) NaCl in Bio-rad 4–20 % prefabricated gradient gel electrophoresis. All the samples were shown in Fig. 4a, b.

Bioinformatics Analysis

Sample 1, 2, 3, 4, 5 and 6 were analyzed by LC–MS/MS. The six bands were all protein mixture. There were six kinds of proteins in sample 1, eleven kinds of proteins in sample 2, nine kinds of proteins in sample 3, four kinds of proteins in sample 4, fifteen kinds of proteins in sample 5, fifteen kinds of proteins in sample 6. A total of 42 kinds of proteins were identified after removing the same proteins. These proteins can be divided into three groups: 27 kinds of proteins related to protein synthesis and degradation, six kinds of proteins related to carbohydrate metabolism and energy metabolism, nine proteins related to nucleic acid metabolism (Table 3).

Discussion

The NaCl concentration of all *dajiang* collected in our study was about 8 % (w/v). High salt concentrations can inhibit the growth and reproduction of certain pathogenic and spoilage microorganisms, but it can also have an impact on the growth and the metabolic activity of lactic acid bacteria, which has a great influence on the final quality of *dajiang* [12]. The aim of this study was not only to isolate and characterize salt-tolerant lactic acid bacteria from traditionally fermented *dajiang* in northeast of China, but also to discuss effect of different salt concentrations on protein expression. Among the lactic acid bacteria collected in *dajiang*, *L. plantarum* FS5-5 showed the highest tolerance to salt. The results in our study showed that the proteins significantly expressed could be divided into three categories: 27 kinds of proteins related to protein synthesis and degradation, six kinds of proteins related to carbohydrate metabolism and energy metabolism, nine proteins related to nucleic acid metabolism.

The protein synthesis is a complex physiological process, which can be divided into initiation, elongation and termination phase, each has its own associated factors that play a role. The initiation of protein synthesis requires the

Fig. 1 The morphology of three salt-resistant lactic acid bacteria. **a** strain DL3-1; **b** strain DL4-5; **c** strain FS5-5

Fig. 2 Phylogenetic tree of three salt-tolerance strains and three standard strains

Fig. 3 Growth curve of *Lactobacillus plantarum* FS5-5 cultured in MRS medium with 0, 3, 6 and 9 % (w/v) NaCl

correct positioning of the initiator tRNA, (f)Met-tRNA_i^{Met}, in the P-site of the ribosome. In bacteria, this process is accomplished by the combined action of three initiation factors: IF1, IF-2 and IF3. IF3 promotes the dissociation of ribosomal subunits, providing a pool of free 30S subunits for initiation. IF-2 then stimulates the binding of fMet-tRNA_i^{Met} to the P-site of the ribosome with the aid of IF1. Peptide deformylase is a family of metalloenzymes that catalyzes the removal of the N-terminal formyl group in a growing polypeptide chain following translation initiation during protein synthesis. Elongation factor Tu (EF-Tu), Elongation factor G (EF-G) and Elongation factor 4 (EF4) are key proteins involved in the translocation of bacterial ribosomes along messenger RNA during protein biosynthesis. In addition to translation elongation, EF-Tu also has function in protein folding and/or protection from stress. Overexpression of EF-Tu has also been reported in *Propionibacterium freudenreichii* and *Streptococcus mutans* during acid adaptation [13]. Thus, overexpression of EF-Tu in the present study could be important in protein biosynthesis, protein folding and renaturation of *L. plantarum* FS5-5 under conditions of salt stress. Biochemical work shows that EF4 back-translocates posttranslational ribosomes for efficient protein synthesis, especially during mild stress created by high ionic strength, low pH, or low temperature [14]. Glutamyl-tRNA synthetases (GluRS) provide Glu-tRNA for protein synthesis. 30S ribosomal protein S2 (rpsB), S3 (rpsC), S5 (rpsE), S7 (rpsG) and 50S ribosomal protein L3 (rplC), L4 (rplD), L5 (rplE), L6

(rplF), L9 (rplI), L10 (rplJ), L13 (rplM), L15 (rplO), L16 (rplP), L21 (rplU) all have important functions in protein synthesis. Ribosomal proteins are thought to act as sensors of heat and cold shock. The results of the present study imply a relationship between ribosomal protein and salt response in *L. plantarum* FS5-5. RimM functions not only to promote the assembly of a few 30-domain proteins but also to stabilize the rRNA tertiary structure [15]. PurQ encoded phosphoribosylformylglycinamide (FGAM) synthetase which catalyses the reaction of 5'-phosphoribosylformylglycinamide (FGAR), glutamine and ATP to FGAM, glutamate, ADP and Pi as the fourth step in biosynthesis of purines that make up amino acids [16]. The premature termination of translation results in the release of peptidyl-tRNA molecules which are toxic to the cell. This affects the protein synthesis adversely. However, such a condition can be corrected by peptidyl-tRNA hydrolase (Pth) [17]. FtsH is a membrane-bound ATP-dependent zinc-metalloprotease which is involved in the proteolytic degradation of specific cytosolic proteins as well as integral membrane proteins. Protease HslV is a part of ATP-dependent protease HslVU. X-prolyl dipeptidyl aminopeptidase (PepX) is thought to be an important component of the proteolytic system. Kimura et al. [18] reported that PepX from *Lactobacillus helveticus* IF03809 is expressed with nearly full activity under high salt conditions. Thus, overexpression of these proteins in the present study imply that a series of changes have occurred in the process of protein synthesis and degradation after *L. plantarum* FS5-5 exposed to salt stress, leading to the difference of protein expression.

The results of proteomics analysis indicated that multiple metabolic pathways might have been involved in the adaptation of *L. plantarum* FS5-5 to salt stress, including carbohydrate metabolism, translation system, nucleotide metabolism, and amino acid metabolism. In particular, six upregulated proteins that are involved in carbohydrate metabolism (EnoA1, Probable phosphoketolase 1, gapB and mtlD) and energy metabolism (atpH, atpC) might play the most important role by supplying energy for *L. plantarum* FS5-5 to resist salt stress. Carbohydrate metabolism mainly is glycolytic pathway by which *L. plantarum* generates lactic acid. EnoA1 is a kind of homodimeric enzymes that catalyse the reversible dehydration of 2-phospho-D-glycerate to phosphoenolpyruvate as part of the glycolytic and gluconeogenesis pathways. Probable phosphoketolase 1 catalyzes the conversion of D-xylulose 5-phosphate and phosphate to acetyl phosphate, D-glyceraldehyde-3-phosphate and H₂O. GapB has been characterized as a functional glyceraldehyde-3-phosphate dehydrogenase protein. mtlD is essential for mannitol biosynthesis and catalyses the first step in mannitol biosynthesis, the reduction of fructose-6-phosphate (F-6-P)

Fig. 4 Protein profiles of *Lactobacillus plantarum* FS5-5 observed in Bio-rad 18.5 × 20 cm SDS–polyacrylamide gel electrophoresis (a) and Bio-rad 4–20 % prefabricated gradient gel electrophoresis (b) under different salt concentrations. 1 0 % (w/v) NaCl, 2 3 % (w/v) NaCl, 3 6 % (w/v) NaCl, 4 9 % (w/v) NaCl, M Marker

Fig. 5 The protein profiles analysis of *Lactobacillus plantarum* FS5-5 observed in Bio-rad 18.5 × 20 cm SDS–polyacrylamide gel electrophoresis in different lanes. a lane 1 0 % (w/v) NaCl; b lane 2 3 % (w/v) NaCl; c lane 3 6 % (w/v) NaCl; d lane 4 9 % (w/v) NaCl

to the intermediate mannitol-1-phosphate (Mtl-1-P). In lactic acid bacteria, reduction of fructose to mannitol is used to reoxidize NADH produced during glycolysis [19]. Upregulation of these proteins implies that glycolysis in *L.*

plantarum FS5-5 is enhanced after salt treatment. This agreed with previous observations by Hahne et al. [20] who reported that glycolytic enzymes were an overrepresented group during salt stress adaptation through a functional

Table 3 Analysis of proteins of *Lactobacillus plantarum* FS 5-5 induced by salt

Protein no.	Sample no.	gi in NCBI	Protein	Gene	Theoretical Mr(u)/pI	Score	Function
1	2	gil380032809	Translation initiation factor IF-2	<i>infB</i>	93605/8.92	58	A
2	5	gil380032364	Translation initiation factor IF-3	<i>infC</i>	19636/9.96	56	A
3	3	gil380032906	Peptide deformylase	<i>defI</i>	20956/4.86	140	A
4	1	gil380032877	Elongation factor Tu	<i>tuf</i>	43251/4.72	31	A ^a
5	2	gil380031164	Elongation factor G	<i>fusA1</i>	76965/4.81	103	A
6	2	gil119371360	Elongation factor 4 (EF-4)		68741/4.94	24	A
7	2	gil67461606	Glutamate-tRNA ligase GluRS		57501/5.68	21	A
8	5	gil380032822	30S ribosomal protein S2	<i>rpsB</i>	29528/5.30	22	A
9	3	gil380031973	30S ribosomal protein S3	<i>rpsC</i>	24193/9.95	768	A
10	5	gil380031984	30S ribosomal protein S5	<i>rpsE</i>	17250/9.64	29	A
11	6	gil380031964	30S ribosomal protein S7	<i>rpsG</i>	17830/9.81	53	A
12	3	gil380031967	50S ribosomal protein L3	<i>rplC</i>	22683/9.92	282	A
13	3	gil380031968	50S ribosomal protein L4	<i>rplD</i>	22623/10.11	44	A
14	4	gil380031979	50S ribosomal protein L5	<i>rplE</i>	20226/7.88	1366	A
15	5	gil380031982	50S ribosomal protein L6	<i>rplF</i>	19338/9.54	37	A
16	5	gil380031113	50S ribosomal protein L9	<i>rplI</i>	16459/9.64	185	A
17	5	gil380031624	50S ribosomal protein L10	<i>rplJ</i>	17916/5.08	1013	A
18	4	gil380032006	50S ribosomal protein L13	<i>rplM</i>	16159/9.60	141	A
19	6	gil380031986	50S ribosomal protein L15	<i>rplO</i>	15335/10.64	173	A
20	5	gil380031974	50S ribosomal protein L16	<i>rplP</i>	16065/10.46	24	A
21	5	gil380032429	50S ribosomal protein L21	<i>rplU</i>	11042/9.55	21	A
22	6	gil448821155	Ribosome maturation factor RimM	<i>rimM</i>	19311/5.00	42	A
23	6	gil380033363	Phosphoribosylformylglycinamide synthase subunit PurQ	<i>purQ</i>	24777/4.71	23	A
24	5	gil380031550	Peptidyl-tRNA hydrolase	<i>pth</i>	20280/7.01	20	A
25	2	gil448820215	ATP-dependent zinc metalloprotease FtsH	<i>ftsH</i>	80753/5.61	33	A
26	5	gil380032642	ATP-dependent protease subunit HslV	<i>hslV</i>	18591/4.99	58	A
27	2	gil380031831	Xaa-Pro dipeptidyl-peptidase	<i>pepX</i>	91548/5.25	114	A
28	1	gil380031775	Enolase 1	<i>enoA1</i>	48057/4.61	24	B
29	2	GI:38257835	Probable phosphoketolase 1		88675/5.11	54	B
30	2	gil308179802	Glyceraldehyde-3-phosphate dehydrogenase	<i>gapB</i>	36713/5.51	26	B
31	6	gil380031289	Mannitol-1-phosphate 5-dehydrogenase	<i>mtlD</i>	42808/4.99	18	B
32	6	gil448821856	H(+)-transporting two-sector ATPase, epsilon subunit	<i>atpC</i>	15688/5.05	24	B
33	5	gil308181149	ATP synthase subunit delta	<i>atpH</i>	20009/5.45	62	B
34	1	gil448822114	ATP-dependent helicase/nuclease subunit B	<i>rexB</i>	136275/5.63	20	C
35	3	gil380033016	Holliday junction ATP-dependent DNA helicase RuvA	<i>ruvA</i>	21651/5.19	25	C
36	1	gil380033344	Carbamoyl-phosphate synthase pyrimidine-specific large chain	<i>pyrAB</i>	116141/4.95	1371	C
37	1	gil380031961	DNA-directed RNA polymerase subunit beta'	<i>rpoC</i>	135746/6.63	1013	C
38	1	gil380033002	DNA mismatch repair protein MutS	<i>mutS</i>	100088/5.35	440	C
39	3	gil380032054	Xanthine phosphoribosyltransferase	<i>xpt</i>	21497/5.42	295	C
40	3	gil380033083	Uracil phosphoribosyltransferase	<i>pyrR2</i>	23071/6.97	104	C
41	5	gil380032847	Adenine phosphoribosyltransferase	<i>apt</i>	18938/5.08	27	C
42	3	gil448821774	Non-canonical purine NTP pyrophosphatase		21777/6.43	283	C

^a A proteins related to protein synthesis and degradation; B proteins related to carbohydrate metabolism and energy metabolism; C proteins related to nucleic acid metabolism

annotation analysis of proteins using the DAVID Gene Functional Classification tool. AtpH and atpC are subunits of ATP synthase and are essential for the function of ATP synthase. This enzyme is found in cell membranes of bacteria and inner mitochondrial membranes. ATP synthase is the key enzyme in the energy transduction processes which couples the transmembrane ion gradient generated by respiration (electron transport) to the synthesis of ATP from ADP and Pi. Therefore, the upregulation of atpH and atpC that was found in the present study might imply energy metabolism was also enhanced in *L. plantarum* FS5-5 when facing salt stress.

Nine proteins involved in nucleic acid metabolism were upregulated in the present study. ATP-dependent helicase/nuclease subunit B (rexB) makes up ATP-dependent helicase/nuclease which catalyzes the unwinding and degradation of double-stranded DNA (dsDNA) upon translocation [21]. The RuvA and RuvB proteins promote ATP-dependent branch migration of Holliday junctions during homologous genetic recombination and DNA repair. DNA homologous recombination is a crucial process not only for generating the genomic diversity but also for repairing damaged chromosomes [22]. Gene *pyrAB* (*carB*) encodes the large subunits of carbamoylphosphate synthetase, which catalyzes the biosynthesis of carbamoylphosphate used in the biosynthesis of both pyrimidine and arginine. They are essential units for nucleic acid structure and function. Transcription in all cellular organisms is driven by a multisubunit DNA-dependent RNA polymerase with a conserved crab claw-like shape that embraces the template DNA. Gene *rpoC* encode the beta' subunit of DNA-directed RNA polymerase. Muts belongs to the DNA mismatch repair (MMR) system which serves a vital function to correct DNA biosynthetic errors that arise during chromosomal replication and to discourage recombination between substantially diverged DNA sequences [23]. Thus, an active mismatch repair system ensures the precision of chromosomal replication maintains genomic stability and decreases mutation rate. Xanthine phosphoribosyltransferase (xpt), uracil phosphoribosyltransferase (pyrR2) and adenine phosphoribosyltransferase (apt) all are phosphoribosyltransferases (PRTases), which are responsible for the formation of all N-glycosidic bonds in nucleotides, both by salvage and de novo biosynthetic pathways, and are thus essential for all synthesis of RNA and DNA in living organisms [24]. Non-canonical purine NTP pyrophosphatase belongs to a family of nucleoside-triphosphatases that hydrolyse non-standard nucleotides such as deoxyinosine triphosphate (dITP) and xanthosine triphosphate (XTP), which may function as part of a pre-emptive DNA repair system by excluding non-standard purines from DNA precursor pool, thus preventing their incorporation into DNA and avoiding chromosomal lesions

[25]. Therefore, production of some related proteins to repair and stabilize DNA and RNA is one of the essential strategies for *L. plantarum* FS5-5 to acquire tolerance or adapt to salt stress.

In conclusion, some proteins expressed significantly under salt stress contributed to the survival and growth of *L. plantarum* FS5-5, which lays the groundwork for research the salt resistant strains. However, studies are still needed to further elucidate the salt-tolerance mechanisms of *L. plantarum* FS5-5.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

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