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Cytosolic Proteomes of *Lactobacillus rhamnosus* ATCC27773 Cells Grown in pH 5.5 and 6.5

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Abstract

Lactobacillus rhamnosus is an important bacterium from a health and industry perspective. Cytoplasmic proteomes of *L. rhamnosus* ATCC2773 were studied to elucidate the specific growth condition variations and the changes occurring in the protein expression patterns of this bacterium when cultivated in a standard rich medium under two different pH conditions (pH 6.4 and 5.5). A total of 220 proteins were identified and compared by quantitative proteomic analysis using iTRAQ LC-MS/MS. A functional classification of the cytoplasmic proteins of *L. rhamnosus* revealed translation and carbohydrate metabolism associated proteins as the major groups. A comparison of proteome data showed that the majority of proteins (89%) have similar expression patterns in cells grown in pH 6.5 and 5.5. The key differences observed in the proteomes of *L. rhamnosus* grown in pH 6.5 and 5.5 were: 19 proteins were present at pH 5.5 but not expressed at pH 6.5 and six proteins were expressed at pH 6.5 but not at pH 5.5. Specific proteins were induced by each condition: 14 and 23 proteins in response to pH 6.5 and pH 5.5, respectively. This study illustrated the impact of culture conditions on the proteome of *L. rhamnosus*.

Abbreviations

GI: Gastrointestinal; LAB: Lactic Acid Bacteria; iTRAQ: Isobaric Tags for Relative and Absolute Quantitation; MRS: Mann Rogosa Sharpe; SCX: Strong Cation Exchange; LC-MS: Liquid Chromatography–Mass Spectrometry; NCBI: National Center for Biotechnology Information

Introduction

Lactobacilli are members of the microbial community associated with the human gastrointestinal (GI) tract. There is documented evidence that lactobacilli are beneficial for their host due to their ability to inhibit the growth of potential harmful bacteria in the GI tract [1-4]. One of the most commonly marketed probiotic lactobacilli is *Lactobacillus rhamnosus* GG. *L. rhamnosus* is frequently isolated from a large variety of ecological niches that include fermented food products, the human gastrointestinal tract and oral and vaginal cavities. It is classified as a facultatively hetero-fermentative lactic acid bacterium (LAB). Several strains of *L. rhamnosus* (GG, HN001, ATCC53103) demonstrate probiotic effects. *L. rhamnosus* GG and HN001 are the most prominent probiotic strains [5-9] and have been extensively studied. The use of bacteria in an expanding array of microbial applications creates stressful conditions for their survival, thus, bacteria like *L. rhamnosus* have developed various metabolic responses to cope with these conditions. A significant amount of research has focused on bacterial stress responses. Accordingly, *L. rhamnosus* has been shown to respond to stress factors such as heat shock [10,11], presence of lactic acid [12], bile [13,14] and low pH [15].

Proteomics is considered to be a new technology-driven approach dedicated to the identification of proteins on a genome-wide scale [16]. The latest high-throughput proteomic approaches offer new options to study probiotic bacteria from a different perspective. This technique allows not only the identification of proteins but also the extensive characterisation of their primary structures (maturation, post-translational modifications and degradation), topology (structural proteomics), interaction networks (interactomics), function (chemical proteomics), regulation (quantitative proteomics) and turnover [17]. Proteomics is an emerging field in probiotic research. The first important application of proteomics is to obtain a proteome map, or overview, of the bacterial protein content; secondly, understanding the adaptation to gut conditions, such as low pH and bile acids, is an important research theme; thirdly, proteomics is a tool to answer important questions about the molecular biology of potential probiotic bacteria. Previous reviews have discussed the proteomics of LAB in general [18-20] but only a few have focused on the proteomics of probiotic bacteria [21,22] are available.

In a recent study, Savijoki et al. compared the proteomes of two *L. rhamnosus* strains, GG and Lc705 [23]. The GG strain is a well-known for probiotic applications and strain Lc705 is used by the dairy industry. Proteomics analysis resulted in the identification of more than 1600 proteins in each strain. The identified proteins make up nearly 60% of the predicted proteomes of *L. rhamnosus*. Comparative analysis revealed that the expression of more than 90 and 150 proteins were uniquely present in GG and Lc705, respectively. Differences were in the proteins with functionalities in biofilm formation, phage-related functions, reshaping the bacterial cell wall and immunomodulation. These differences did not come as a surprise, as lactobacilli such as *L. casie*, *L. plantarum* and *L. rhamnosus* have diverse habitats and, thus, the variation in the protein expression patterns of different

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strains adapted to diverse ecological niches is natural. However, more detailed information on the dynamics of global protein expression patterns during the growth of these microorganism is needed and, more importantly, studies are required to detect the specific differences under different physiological conditions.

In this work, we investigated growth-related variations in the cytoplasmic proteome of *L. rhamnosus* strain ATCC27773, a strain with potential uses as a probiotic research and in industry applications. This strain has been applied in microbiological assays to determine folate in serum and red cells [24,25]. *L. rhamnosus* ATCC27773 also showed a positive effect as a probiotic for the treatment of functional dyspepsia [26]. In order to achieve a better understanding of strain-specific differences in the protein expression patterns of industrial strains adapted to specific applications, we report on the cytoplasmic proteome of *L. rhamnosus* and differences seen in the proteomes of cells grown in two pH conditions (6.4 and 5.5).

Material and Methods

Bacteria and growth conditions

L. rhamnosus ATCC27773 was procured from the New Zealand Reference Culture Collection (ESR Ltd New Zealand) and long-term stock was maintained in 50% MRS-glycerol at -80 °C. An overnight culture was prepared by inoculating10 mL of Mann Rogosa Sharpe (MRS) broth (Oxoid) and incubating at 37 °C under anaerobic conditions. Harvested cells were transferred into a modified MRS broth [27] prepared using 0.3 M phosphate buffer to maintain the required pH (5.5 and 6.5) during the incubation period. The starting OD_{600nm} of the cultures was adjusted to ~0.2. All cultures were incubated at 37 °C for 8 h under anaerobic conditions. The cells were then harvested by centrifugation at 10,000 x *g* for 10 min at 4 °C and washed twice with 40 mM Tris-buffer (pH 7.0) before re-suspending them in appropriate volumes of 40 mM Tris buffer to achieve a final OD_{600nm} of 20. Bacterial cell suspensions were stored at -80 °C until further use. Each growth condition was tested in duplicate and the experiments were repeated at least twice.

Cytosolic protein samples preparation

The frozen bacterial suspensions were thawed and lysed using a mini-bead beater. Half a gram of sterile zirconium beads (0.1 mm) were mixed with 0.5 ml of cell suspension before mechanically lysing the cells through four beating cycles, e.g. one x 90 s cycle was followed by three beating cycles of 60 s each (with a five minute cooling on ice between each beating cycle). The cytosolic protein rich supernatant was collected after removing the cell debris by centrifugation at 13,000 x *g* for 30 min at 4 °C. Cytosolic protein samples were stored at -80 °C until used for proteomic analysis.

iTRAQ labelling of the protein sample

Eighty micrograms of cytosolic proteins from each sample were mixed with ten µg of trypsin for digestion and then incubated at 37 °C overnight. The trypsin digests were dried and suspended each in 20 µl 0.5M TEAB and labeled using an iTRAQ reagents-8plex k it (AB SciexPte. Ltd) according to the manufacturer's instructions. Every sample was labeled twice and the aliquots of iTRAQ were combined with peptide mixtures from different samples, and incubated at room temperature for 1 h.

Identification of proteins through LC-MS/MS and data analysis

The iTRAQ labeled peptides were fractionated through a 5 µM BioX-SCX column (Dionex, Auckland, New Zealand) into 10 fractions with 0, 1, 5, 10, 20, 40, 60, 80, 100% 2 M ammonium formate in 2%. The SCX fractionation was performed using high-pressure liquid chromatography. For LC-MS/MS analysis, two microlitres of sample were loaded on a C18AQ nano trap (Bruker, 75 µm × 2 cm, C18AQ, 3 µm particles, 200 Å pore size) using nanoAdvance UPLC coupled to a maXis impact mass spectrometer equipped with a CaptiveSpray source (Bruker Daltonik, Bremen, Germany). The column oven was heated to 50 °C. Elution was with a gradient from 0% to 40% B in 90 min at a flow rate of 800 nl/min. Solvent A was LCMS-grade water with 0.1% FA and 1% ACN; solvent B was LCMS-grade ACN with 0.1% FA and 1% water. Samples were measured in the auto MS/MS mode, with a mass range of m/z 50-2200, followed by one full MS scan, the 10 most abundant ions were subjected to MS/MS analysis. The acquisition speed was maintained at 2 Hz in MS and 10 or 5 Hz in MS/MS mode depending on precursor intensity. Precursors were selected in the m/z 400-1400 range, with charge states of 2-5 (single charge ions were excluded) and active exclusion was activated after 1 spectrum for 0.3 min.

Data analysis was conducted by submitting peak list files (mgf format) to an in-house Mascot server (v2.4) (Matrix Science, UK). The search parameters included: taxonomy *Lactobacillus rhamnosus*; enzyme semi trypsin; cysteine modification carbamidomethyl; MS tolerance 0.02 Da; MS/MS tolerance 0.1 Da; 1 missed cleavage; instrument specificity ESI-QUAD-TOF. Mascot iTRAQ parameters included variable iTRAQ8plex (N-term, K, Y), with reporter ions defined as appropriate for the experiment. Peptides with a score below 20, and proteins with fewer than two peptides were discarded. Only unique peptides were used for this quantification. Normalisation was based on division by channel sum. Protein functionalities were searched in several online proteomics databases (http://www.ncbi.nlm.nih.gov/; http://www.matrixscience.com/; http://www.uniprot.org/) and appropriate literature. Proteins were classified into different groups through manual processing.

Results and Discussion

The gel free proteomics technique, iTRAQ LC-MS/MS analysis, was used to identify constitutively expressed proteins in the cytosolic extract of *L. rhamnosus grown* in MRS broth under anaerobic conditions. This study also described the dynamics of the cytosolic proteome of *L. rhamnosus* in response to two pH conditions (5.5 and 6.5). An increase of 1.5-fold or more in the relative expression of a protein was considered for differential expression.

Cytosolic proteome of *L. rhamnosus*

In this study, a total of 220 different proteins were identified in the cytosolic proteome of *L. rhamnosus* (Supplementary Table). The identified proteins were grouped according to their functionalities (Figure 1). Functional grouping revealed the distribution of the proteins: translation (n=46, 21.60%), carbohydrate metabolism (n=27, 12.68%), general and unclassified proteins (n=22,12.68%), unknown proteins (n=17, 10.33%), transcription and regulation (n=14, 6.57%), cell division and growth (n=11 5.16%), transport,

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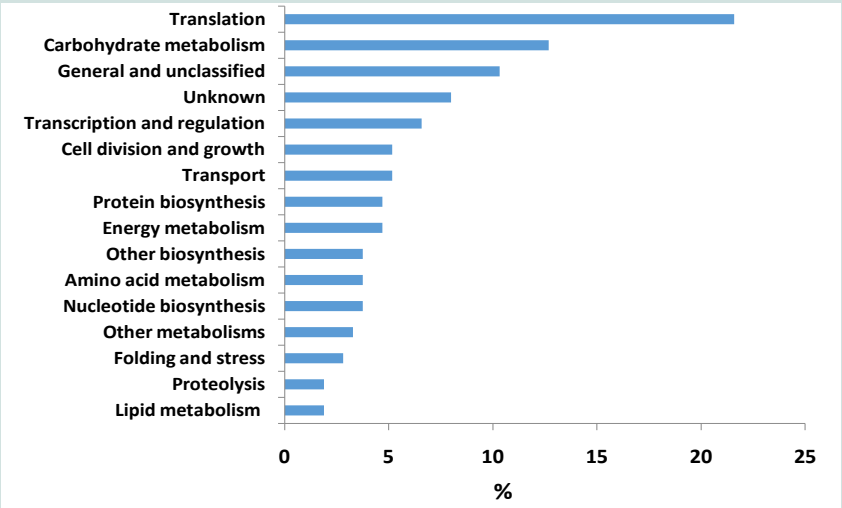

Figure 1: Functional grouping of 231 proteins identified in the cytosolic fraction of *L. rhamnosus* ATCC27773 cells using iTRAQ analysis.

(n=11,5.16%), protein biosynthesis (n=10, 4.69%), energy metabolism (n=10, 4.96%), other biosynthesis (n=8, 3.76%), amino acids (n=8, 3.76%), nucleotides (n=8, 3.76%), other metabolisms (n=7, 3.29%), folding and stress (n=6, 2.82%), proteolysis (n=4, 1.88%) and lipid metabolism (n=4, 1.88%). This study showed that proteins involved in translation and carbohydrate metabolism were the major functional groups in the cytoplasmic proteome of *L. rhamnosus* ATCC 27773. Koskenniemi et al. reported that growth of *L. rhamnosus* LGG in a laboratory medium (MRS broth) increased the expression of translation proteins [24].

Overall, the assessment of the cytosolic proteomes generated from the cells cultured in MRS broth adjusted and maintained at two different pH conditions (6.5 and 5.5) showed noticeable variations (Figure 2). There were proteins with varying degrees of expression with a 1.50- to 4.27-fold relative increase, or some proteins were absent in the cells grown at a particular pH (6.5 or 5.5). Nineteen proteins were expressed only in the cells grown at pH 6.5 and were not detected in the cells harvested from cultures at pH5.5. Similarly, six proteins were only present in the cytosolic proteome of the cells grown at pH 5.5. Around 89% proteins were commonly expressed in both cytosolic proteomes. However, fourteen proteins were expressed in greater quantities in the cells grown at pH 6.5 rather than at pH 5.5. Similarly, 23 proteins were induced in the cells in response to pH 5.5. Changes to the homeostatic environment of bacteria (including pH) causes the bacteria to adjust their metabolism, growth, and translational and transcriptional biochemistry by producing enzymes (proteins) to help them adjust to changes in their environments [28-30].

It has been shown from the literature that the growth pattern of lactobacilli and biomass yield was influenced by pH and temperature. There were noticeable differences between different species and strains in their response to pH conditions. Reports suggested that a lower pH of fermentation (pH 5 vs pH 6) produced cells with diverse and robust physiological attributes [31]. In this study, induction of higher numbers of proteins in pH 5.5 compared to pH 6.5 was observed, which clearly demonstrated a stronger physiological response of *L. rhamnosus* cells at the lower pH. Deepika et al. evaluated the effect of fermentation conditions (pH 5, 5.5, 6, 6.5 and temperature 25, 30 and 37 °C) on the surface properties of *L. rhamnosus* GG and its adhesion to Caco-2 cells [32]. The rationale to compare optimal growth conditions (pH 6.5 and temperature 37 °C) to suboptimal conditions (pH <6 and temperature <37 °C) was that important changes could take place when growing the cells in such environments.

Proteins induced at pH 6.5

Fourteen proteins were induced in response to pH 6.4 and five of them had an induction factor in the range of 2.02 - 4.27 (Table 1). Among the highly expressed proteins were: acyl carrier protein, a transport protein, was expressed 4.27-fold; cell division protein GpsB (homology with *L. rhamnosus* LRHMDP2) increased 3.14 times; a transcriptional protein of the MarR family had a 3.08-fold increase; a cysteine synthase protein and a glycine cleavage system protein H were over-expressed 2.10- and 2.02-fold, respectively. Other differentially expressed proteins (increased 1.56 to 1.92 times) were phosphotransacetylase, a lysine transporter protein, uracil phosphoribosyltransferase, lipoate-protein ligase A, HPrkinase/phosporylase, a cell division ATPase protein, glycerol-3-phosphate oxidase, L-lactate dehydrogenase and phosphocarrier protein HPr.

Nineteen proteins were detected in the cells of *L. rhamnosus* grown at pH 6.5 that were not present in cultures of *L. rhamnosus* grown at pH 5.5. These proteins included pyruvate oxidase, adenyl succinate synthase, carbamoyl-phosphate synthase, isoleucyl-tRNA synthase, glutamine synthetase repressor, thiamine biosynthesis ATP pyrophosphatase, ribonuclease P protein component, iron-binding alcohol dehydrogenase, Hsp33-like chaperonin, 3-oxoacyl (acyl carrier protein) synthase III, cell division protein sepF partial, MutT/nudix family phosphohydrolase, RNA ribosyltransferase-isomerase Que A, phage-related tail-host specificity protein, phosphohistidine-sugar phosphotransferase, ATP/GTP hydrolase, putative extracellular matrix binding protein, a conserved hypothetical protein and preprotein translocate subunit YajC.

Most of the proteins produced and/or expressed by the cells at

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pH5.5	pH6.5	Protein
0.889	1.099	Glyceraldehyde-3-phosphate dehydrogenase
1.019	0.893	Pyruvate kinase
0.819	0.842	non-specific DNA-binding protein HBsu
0.575	0.740	chaperonin GroEL
0.745	0.825	GroEL
1.037	1.061	ribosomal protein L10
1.036	1.234	elongation factor Tu
1.310	0.458	Tuf
0.836	1.301	L-lactate dehydrogenase
0.314	3.307	Acyl carrier protein
1.077	1.113	30S ribosomal protein S1
0.962	0.971	Ribosomal protein S2
1.010	0.849	Enolase
1.010	0.844	Enolase
0.933	1.068	elongation factor G (EF-G)
1.194	1.183	oligoendopeptidase F
1.161	1.645	nitroreductase family protein
1.295	0.907	F0F1-type ATP synthase subunit beta
1.077	0.903	50S ribosomal protein L22
1.685	2.009	cold shock protein
0.921	1.173	NADH peroxidase
0.668	0.869	co-chaperonin GroES
0.874	0.966	chaperone protein DnaK
1.052	1.061	ribosomal protein L7/L12
1.079	0.923	50S ribosomal protein L13
1.025	0.934	Pyridoxine 5'-phosphate oxidase
1.067	1.142	ribosomal protein L18
0.958	0.893	tagatose-bisphosphate aldolase
1.003	0.927	phosphoenolpyruvate-protein phosphotransferas
0.958	0.912	triosephosphate isomerase
1.073	0.945	ribosomal protein S5
0.986	1.011	NADH oxidase
0.941	0.919	50S ribosomal protein L4
1.428	0.892	Mannitol-1-phosphate 5-dehydrogenase
1.001	1.292	glucose-6-phosphate isomerase
1.044	0.910	dTDP-4-dehydrorhamnose 3,5-epimerase
1.086	0.739	30S ribosomal protein S19
0.872	0.971	phosphoglycerate kinase
0.934	0.946	Ribosomal protein L1
0.981	0.933	thiol peroxidase
0.630	1.085	6-phospho-beta-glucosidase
0.710	1.139	Phosphotransacetylase
1.318	1.113	Uracil phosphoribosyltransferase
0.909	0.946	GlnAm protein
1.034	1.102	50S ribosomal protein L14
	1.683	pyruvate oxidase
1.366	1.020	ATP synthase subunit D
1.072	0.933	Phosphoglycerate mutase 1
0.678	1.421	cysteine synthase
0.978	0.893	transcription termination factor NusA
1.279	0.952	Aldo/keto reductase related enzyme
1.268	1.082	Ribosomal protein L15
0.941	0.778	F0F1 ATP synthase subunit alpha
1.211	1.188	ribosomal protein S4
0.912	1.845	glycine cleavage system protein H
0.956	1.313	Mannose-6-phosphate isomerase
0.936	0.877	RmlD
1.314	0.965	hypothetical protein LRH_01568
0.791	1.322	glycerol-3-phosphate oxidase
0.956	0.960	thioredoxin reductase
1.284	0.883	adenylate kinase
1.079	0.874	glycine--tRNA ligase
0.703	0.710	30S ribosomal protein S8
0.999	1.104	tRNA binding domain-containing protein
1.009	0.921	50S ribosomal protein L27
0.674	1.108	HPr kinase/phosphorylase
	0.884	carbamoyl-phosphate synthase
1.000	1.004	50S ribosomal protein L6
0.981	0.907	6-phosphogluconate dehydrogenase
0.943	0.967	dipeptidase PepV
0.500	0.902	Cell division ATPase
0.707	1.355	phosphocarrier protein HPr
0.966	0.937	50S ribosomal protein L11
	0.804	Adenylosuccinate synthetase
0.722	0.625	prolyl-tRNA synthetase
0.909	1.189	Ribosomal protein L29
1.435	0.916	preprotein translocase subunit SecA
1.476	0.985	Cell division protein FtsH
1.083	0.973	Ribosomal protein L23
0.384	1.205	Cell division protein GpsB
1.196	1.049	50S ribosomal protein L2
1.081	1.105	Ribosomal protein S3
1.077	0.976	50S ribosomal protein L16
0.765	0.905	catabolite control protein A
0.949	1.272	M3B subfamily peptidase
0.857	1.007	phosphoglucomutase
1.039	1.067	threonine--tRNA ligase
	0.778	isoleucyl-tRNA synthetase
1.226	1.035	peptidyl-tRNA hydrolase
1.009	1.280	ribosomal subunit interface protein
	1.067	glutamine synthetase repressor
0.907	0.787	dipeptidyl aminopeptidase
	0.981	Thiamine biosynthesis ATP pyrophosphatase
0.866	0.764	30S ribosomal protein S10
1.118	1.092	ABC-type dipeptide
1.104	1.080	ribosomal protein L17
	1.145	ribonuclease P protein component
1.021	1.323	CspA family cold shock transcriptional regulator
1.033	1.002	30S ribosomal protein S13
0.673	0.910	ATP-dependent Clp protease proteolytic subunit
0.828	1.102	pyruvate dehydrogenase
	0.389	Iron-binding alcohol dehydrogenase
0.984	0.938	Signal recognition particle GTPase
1.161	1.117	Ribosomal protein L32
1.142	0.970	50S ribosomal protein L5
1.254	0.569	Predicted phosphosugar isomerase
1.067	0.778	dipeptidase A, partial
0.794	0.907	xre family transcriptional regulator
0.712	1.035	Lipoate-protein ligase A
1.165	0.800	DNA-directed RNA polymerase
1.027	0.928	ribosomal protein S16
1.033	1.063	Phosphotransferase system,
0.880	0.935	Substrate-binding lipoprotein
0.927	1.060	Periplasmic component
0.753	0.892	Short-chain alcohol dehydrogenase
1.037	1.585	Valyl-tRNA synthetase
0.584		DegV family protein
0.973	0.984	phosphoketolase
1.124	0.960	Ribosomal protein L31
1.194	1.252	ABC-type uncharacterized transport system
1.960	0.544	Ribosomal protein S15P/S13E
0.848	0.786	L-lactate dehydrogenase
	1.020	cell division protein sepF, partial
0.774	0.761	Aldo/keto reductase family enzyme
1.119	1.013	Dioxygenase
0.740		ribose-5-phosphate isomerase A
0.775	0.845	glutaminyl-tRNA synthase b subunit
1.007	1.079	ABC-type sugar transport system
1.038	0.808	RmlA [
1.135	0.826	hypothetical protein LRHMDP2_1625
1.100	0.986	NLPA lipoprotein
0.944	0.983	hypothetical protein LRH_13901
1.115	0.737	HflX subfamily GTP-binding protein
1.046	0.863	UTP--glucose-1-phosphate uridylyltransferase
0.932	0.785	Ribosomal protein S21
0.886	1.150	pilus specific protein
1.125	0.843	dihydroorotate dehydrogenase 1A
0.778	0.709	tyrosine recombinase xerD
0.982	1.014	2-dehydro-3-deoxyphosphogluconate aldolase
1.194	0.931	DNA-directed RNA polymerase, beta subunit
1.065	0.864	Asparagine synthase
0.831	1.046	50S ribosomal protein L33
1.052	1.380	Ribosomal protein S17
1.103	1.137	chromosome partitioning ATPase
1.220	1.503	oxidoreductase
1.232	1.240	thioredoxin
0.837	1.570	oligopeptide ABC transporter
1.106		GMP synthase
1.076	0.773	GMP synthase
0.794	0.585	bifunctional GMP synthase
	0.958	MutT/nudix family phosphohydrolase
1.237	0.911	Transcription elongation factor
1.082	0.693	Proline dipeptidase
	2.010	RNA ribosyltransferase-isomerase GueA
2.132	1.761	two-component response regulator
3.108		hypothetical protein LC705_01627
0.886	1.118	conserved hypothetical protein
1.011	0.622	hypothetical protein LRH_10752
1.061	0.739	ribose-phosphate diphosphokinase
0.883	0.726	hypothetical protein HMPREF0533_1302
	0.373	phage-related tail-host specificity protein
1.403	0.755	hypothetical protein LRH_00422
1.050	1.110	D-alanyl-D-alanine carboxypeptidase
1.218	1.148	ATP-dependent Clp protease A
1.184	1.371	cation transport ATPase
1.054	0.942	3-hydroxymyristoyl dehydratase
1.039	1.046	Ribosomal protein L9
0.675	0.935	conserved hypothetical protein
1.055	0.748	Fe-S cluster assembly ABC transporter
	1.478	phosphohistidine--sugar phosphotransferase
	2.433	ATP/GTP hydrolase
1.389	0.683	peptidylprolyl isomerase
0.831	0.934	D-glutamate--L-lysine ligase
1.008	0.624	diguanylate cyclase
0.982	0.870	PTS system mannose-specific transporter
	1.084	putative extracellular matrix binding protein

Figure 2: Map of protein expression changes in cytosolic proteomes of *L. rhamnosus* ATCC27773 cells grown at pH 5.5 and 6.5. Legends: ***Red colour***, up-regulated protein; ***Green colour***, down-regulated protein; ***White colour***, protein absent in a particular condition; Matching colour, similar expression pattern.

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Table 1: The identity and expression of proteins induced by pH 5.5 and 6.5 in *Lactobacillus rhamnosus* ATCC27773.

Protein name [Homology match]	Metabolic pathways or protein function	Accession no.	Mass (Da)	Fold change
pH 6.5				
Acyl carrier protein [*Lactobacillus rhamnosus* HN001]	Fatty acid metabolism	gi|199597462	8957	+4.27
Phosphotransacetylase [*Lactobacillus rhamnosus* HN001]	Pyruvate and propanoate metabolism	gi|199597298	35204	+1.72
Uracil phosphoribosyltransferase [*Lactobacillus rhamnosus* HN001]	Transport	gi|199597583	22780	+1.60
Cysteine synthase [*Lactobacillus rhamnosus* LMS2-1]	Signal transduction	gi|229553598	32586	+2.10
Glycine cleavage system protein H [*Lactobacillus rhamnosus* GG]	Amino acid metabolism	gi|258508188	10787	+2.02
Glycerol-3-phosphate oxidase [*Lactobacillus rhamnosus* LMS2-1]	Glycerolphospholipid metabolism	gi|229553666	67070	+1.67
Phosphocarrier protein HPr [*Lactobacillus casei* ATCC 334]	Carbohydrate metabolism	gi|116495240	9248	+1.92
Cell division protein GpsB [*Lactobacillus rhamnosus* LRHMDP2]	Cell growth and death	gi|421770856	15147	+3.14
HPr kinase/phosphorylase [*Lactobacillus rhamnosus* HN001]	Carbohydrate metabolism	gi|199597253	35325	+1.64
Cell division ATPase [*Lactobacillus rhamnosus* HN001]	Cell growth	gi|199597237	25493	+1.80
L-lactate dehydrogenase [*Lactobacillus casei* ATCC 334]	Carbohydrate metabolism	gi|116495980	35508	+1.56
Lipoate-protein ligase A [*Lactobacillus rhamnosus* HN001]	Protein lipoylation	gi|199598387	38434	+1.54
MarR family transcriptional regulator [*Lactobacillus rhamnosus* GG]	Transcription	gi|258509122	18863	+3.08
Lysine transporter protein [*Lactobacillus rhamnosus* GG]	Transport	gi|385827176	49813	+1.61
pH 5.5				
DNA mismatch repair protein [*Lactobacillus rhamnosus* HN001]	Replication and repair	gi|199597366	71751	+2.41
Galactose-1-phosphate uridylyltransferase [*Lactobacillus rhamnosus* HN001]	Galactose metabolism	gi|199596969	54599	+3.53
Peptidylprolyl isomerase [*Lactobacillus rhamnosus* LMS2-1]	Protein folding	gi|229552580	33583	+2.02
Diguanylate cyclase [*Lactobacillus rhamnosus* R0011]	Biofilm formation	gi|418072587	44802	+1.61
Hypothetical protein LRH_00422 [*Lactobacillus rhamnosus* HN001]	Unknown	gi|199598119	12182	+1.86
Hypothetical protein LRH_10752 [*Lactobacillus rhamnosus* HN001]	Unknown	gi|199598624	18734	+1.63
Proline dipeptidase [*Lactobacillus rhamnosus* LRHMDP2]	Cellular process	gi|421770354	40557	+1.56
Ribosomal protein S15P/S13E [*Lactobacillus rhamnosus* HN001]	Translation	gi|199598193	10307	+3.68
50S ribosomal protein L19 [*Lactobacillus rhamnosus* LMS2-1]	Translation	gi|229552441	14555	+2.57
Bacterial cell division membrane protein FtsW [*Lactobacillus rhamnosus* LMS2-1]	Cell growth	gi|229551987	46222	+1.69
DNA-directed RNA polymerase, beta' subunit [*Lactobacillus rhamnosus* ATCC 21052]	Transcription	gi|423078557	135986	+1.50
Predicted phosphosugar isomerase [*Lactobacillus rhamnosus* HN001]	Carbohydrate metabolism	gi|199599301	43041	+2.20
Preprotein translocase subunit SecA [*Lactobacillus rhamnosus* HN001]	Protein transport	gi|199597235	89455	+1.63
Cell division protein FtsH [*Lactobacillus rhamnosus* LRHMDP2]	Cell growth and death	gi|421770643	78161	+1.50
Adenylate kinase [*Lactobacillus rhamnosus* LMS2-1]	Nucleotide metabolism	gi|229552729	23339	+1.50
Fructose/tagatose bisphosphate aldolase [*Lactobacillus rhamnosus* HN001]	Carbohydrate metabolism	gi|199597065	31686	+1.50
Tuf [Lactobacillus rhamnosus]	GTP catabolic process	gi|38488993	25919	+2.86
F0F1-type ATP synthase subunit beta [*Lactobacillus rhamnosus* GG]	Energy metabolism	gi|385827851	56680	+1.50
Mannitol-1-phosphate 5-dehydrogenase [*Lactobacillus rhamnosus* LMS2-1]	Energy metabolism	gi|229551152	42605	+1.60
30S ribosomal protein S19 [*Lactobacillus rhamnosus* ATCC 8530]	Translation	gi|385836313	10552	+1.50
Pyruvate carboxylase [*Lactobacillus rhamnosus* Lc 705]	Pyruvate carboxylase	gi|258539534	125248	+1.62
HflX subfamily GTP-binding protein [*Lactobacillus rhamnosus* R0011]	GTP binding	gi|418072452	48214	+1.51
Ribose-phosphate diphosphokinase [*Lactobacillus rhamnosus* LMS2-1]	Nucleotide biosynthesis	gi|229551493	35478	+1.50

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pH 6.5 were associated with cell growth and active metabolic cellular processes. Previous studies showed that pH 6.5 and 37 °C were the best conditions for *L. rhamnosus* GG cells' adhesion ability [32]. Our observations on the basis of proteomics data supported that cells produced a range of enzymes required for vigorous growth and normal cell activities at pH 6.5 and 37 °C. Induction of protein-related stress factors or cellular damage was not seen under these conditions.

Proteins induced at pH 5.5

Twenty three proteins were up-regulated at pH 5.5 with the increase ranging from1.50- to 3.68-fold; seven of these proteins had a differential expression value of >2-fold. These highly expressed proteins included a DNA mismatch repair protein (2.41-fold), galactose-1-phosphate uridylytransferase (3.53-fold), ribosomal protein S15P/S13E (3.68-fold), 50S ribosomal protein L19 (2.57-fold), predicted phosphosugar isomerase protein (2.20-fold) and Tuf protein (2.86-fold). Preprotein translocase subunit SecA, fructose/tagatose bisphosphate aldolase, ribose-phosphate diphosphokinase, mannitol-1-phosphate 5-dehydrogenase, bacterial cell division membrane protein FtsW, diguanylate cyclase, prolinedipeptidase protein, cell division protein FtsH, 30S ribosomal protein S19, HflX subfamily GTP-binding protein, pyruvate carboxylase protein, hypothetical proteins LRH_0042 and LRH_10752 were also detected in relatively higher amounts in the cells growth at pH 5.5. Six proteins expressed at pH 5.5 but not at pH 6.5 included: DegV family protein, ribose-5-phosphate isomerase A, GMP synthase, hypothetical protein LC705_01627, adhesion exoprotein and translation initiation protein.

Cells grown at the relatively suboptimal pH (5.5) had a higher expression of proteins involved in DNA repair, translation, protein folding and transport, biofilm formation and adhesion abilities. Induction of hypothetical proteins was also seen in cells grown at lower pH. The pH conditions used in this study were harsh enough to impose acidic stress on bacterial cells as generally used by other authors [33]; thus discussion on acid stress responses will be irrelevant. This proteomic study was to identify and characterize protein expression in *L. rhamnosus* in two different growth conditions, which were important to understand its functionality and characteristics when used as a probiotic and industrial microorganism. Further proteomics work is needed to develop better understanding of protein expression differences in different *Lactobacillus* strains in relation to specific growth conditions.

Conclusions

This study was initiated to construct the cytosolic proteome of *L. rhamnosus* ATCC27773 and evaluate the impact of culture conditions (pH) on the proteomic profile of cells. This present cytosolic proteome is the first study to catalogue *L. rhamnosus* ATCC27773 proteins to date. The temperature and the pH of cultures influenced several physiological and technological properties such as growth, mass yield, surface composition, metabolic by-products and the level of adhesion of lactobacilli. Comparison of changes in the cytosolic proteomes of cells grown at pH 6.5 and 5.5 clearly showed that the strain had difference in their proteomics expression under the different culture conditions. Suboptimal growth conditions generally improved physiological responses as shown by the expression of greater numbers of proteins seen in the case of pH 5.5. This attribute was thought to increase the robustness and technological properties of the cells. In conclusion, this study provided a detailed cytosolic proteome of a lactobacilli strain and paved the way to understand its functionality and characteristics for industrial use.

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