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DATASET BRIEF

Exo- and surface proteomes of the probiotic bacterium *Lactobacillus acidophilus* NCFM

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Running title: Subproteomes of *Lactobacillus acidophilus* NCFM

Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GIT, gastrointestinal tract; LABSEM, semisynthetic lactic acid bacteria medium; MucBP, mucin-binding protein; NCFM, *Lactobacillus acidophilus* NCFM

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Abstract

Lactobacillus acidophilus NCFM is a well-known probiotic bacterium extensively studied for its beneficial health effects. Exoproteome (proteins exported into culture medium) and surface proteome (proteins attached to S-layer) of this probiotic were identified by using 2DE followed by MALDI TOF MS to find proteins potentially involved in bacteria-host interactions. The exo- and surface proteomes included 43 and 39 different proteins from 72 and 49 successfully identified spots, respectively. Twenty-two proteins were shared between the two proteomes; both contained the major surface layer protein that participates in host interaction as well as several well-known and putative moonlighting proteins. The exoproteome contained 9 classically-secreted (containing a signal sequence) and 10 non-classically secreted proteins, while the surface proteome contained four classically-secreted and 8 non-classically secreted proteins. Identification of exo- and surface proteomes contributes describing potential protein-mediated probiotic-host interactions.

Lactobacillus acidophilus NCFM (NCFM) is an extensively used, Gram-positive, lactic acid-producing, and genome-sequenced (2.0 Mb) probiotic bacterium [1,2]. The surface layer (S-layer) is a crystalline array of proteins forming the outermost part of the bacterial cell wall [3] that contributes to the shape, protection from external factors, mediation of adhesion, and host immune regulation [4]. Several *lactobacilli* including *L. acidophilus, L. crispatus,* and *L.*

brevis possess S-layers involved in host interactions, especially with extracellular matrix (ECM) components, mucin, collagen, and fibrinogen [4]. In addition, some secreted extracellular proteins are available for contact with host mucosa and epithelial cells [5,6]. Previously we have mapped the acidic and alkaline whole-cell proteomes of NCFM, comprised of 275 and 102 identified proteins, respectively [7,8]; and the present identification of exo- and surface proteomes complements these previous results.

L. acidophilus NCFM (NCFM 150B, FloraFIT® Probiotics; 1.50×10^{10} CFU/g DuPont, USA Inc., Madison, US) grown aerobically without shaking (37 °C; 50 mL batch cultures) in semisynthetic lactic acid bacteria medium (LABSEM) containing 1% glucose (Sigma-Aldrich) was harvested at late log-phase (three biological replicates) [7]. The exoproteome was prepared by centrifugation (3200x*g*, 10 min), filtration (0.22 μm syringe filters; VWR), and concentration (Amicon® Ultra-15 Centrifugal Filter Devices; Millipore) of the supernatant. The surface proteome was prepared by treating cell pellets with 5 M LiCl (RT, 30 min), followed by centrifugation and filtration of supernatants as above [9,10]. Proteins in the filtrate were precipitated by three volumes chilled 10% TCA in acetone (20°C, overnight), centrifuged (20000x*g*, 20 min), washed thrice with acetone, dissolved in rehydration buffer (8 M urea, 2 M thiourea, 2% CHAPS, 1% IPG buffer pH 3−10; GE Life Sciences) and analysed for protein concentration (2D Quant kit, GE Life Sciences).

Protein samples (50 μg) were separated by 2-DE followed by image analysis and MS identification [11]. Briefly, the first dimension was performed using IPG strips (pH 3–10, 11 cm; GE Healthcare) on Ettan™ IPGphor (GE Healthcare) to a total of 30 kVh. Strips were then treated with 65 mM DTT followed by 135 mM iodoacetamide in equilibration buffer (6 M urea, 30% glycerol, 50 mM Tris-HCl pH 8.8, 0.01% bromophenol blue, 2% SDS), and the second dimension (SDS-PAGE) was run using 12.5% Tris-HCl precast gels and Criterion™

Midi-format electrophoresis cell (Bio-Rad). Gels were stained by colloidal CBB [12], scanned (Microtek Scan maker 9800 XL; Microtek), and image-analyzed (Progenesis SameSpots, version 3.3). In-gel trypsin digestions of spots were performed as previously described [7,11]. Aliquots (1 μL) of the tryptic digests of protein spots were applied onto an Anchor Chip target (Bruker-Daltonics), covered by matrix solution (1 μL 0.5 mg/mL CHCA in 90% ACN, 0.1% TFA) and washed (2 μL 0.02% TFA). MS spectra were obtained by MALDI-TOF MS (Ultraflex II, Bruker-Daltonics) in auto-mode using Flex Control v3.0 (Bruker-Daltonics) and calibrated ~~by~~ with tryptic β-lactoglobulin peptides (5 pmol/mL). MS spectra were used to search the NCBIprot database or SwissProt for Firmicutes using the MASCOT 2.0 software (http://www.matrixscience.com) integrated with BioTools v3.1 (Bruker-Daltonics). Protein identifications by PMF were approved with a MASCOT score of at least 80 (60 for SwissProt), $p \leq 0.05$, and a minimum of six matched peptides. Signal peptides of identified proteins were predicted using SignalP (ver. 4.1) [13], and SecretomeP (ver. 2.0) was used for non-classical secretion [14].

Analysis of the exoproteome of NCFM revealed 106 spots by 2-DE. Forty-three individual proteins (Figure 1A, Table 1, Supplementary Table S1 and Figure S1A) were identified from 72 spots. Nine of these are predicted to be secreted by the canonical Sec secretion system, 10 to be non-classically secreted, 2 are membrane proteins, and 22 are cytoplasmic and include previously reported or putative moonlighting proteins, defined as proteins playing multiple functions that do not result from gene fusions, families of homologous proteins, splice variants, or promiscuous enzyme activities [15]. Mucin- (or mucus-) binding proteins are generally large (> 200 kDa), containing mucin-binding protein (MucBP) domains, and secreted *via* the Sec secretion system [16]. Mucus binding protein (spot 3, Figure 1A) has three MucBP domains and a Tar (methyl-accepting chemotaxis protein) domain that is

considered to be responsible for cell motility and signal transduction [17]. The mucus binding protein precursor Mub (spot 2, Figure 1A) has one MucBP domain [17] and is considered to be important in interactions with the host gastrointestinal tract (GIT) [18]. Additionally, fibrinogen-binding protein (spot 6, Figure 1A) in the exoproteome may be important for adhesion to the host.

Surface proteome identification was reported for *L. acidophilus* NCFM using 1D SDS-PAGE and LC-MS/MS [19]. However, 2DE proteome analysis has some advantages; i) visualization of protein spots prior to MS; ii) tracking of protein speciation and possible PTMs; and iii) simpler data analysis [20]. Thirty-nine individual proteins were identified in 49 spots from in total 58 spots (Figure 1B, Table 1, Supplementary Table S2 and Figure S1B) including the S-layer protein, a cell division protein, hypothetical protein LBA0695, and thermostable pullulanase, which are predicted to have a signal peptide, 8 non-classically secreted, one transmembrane, and 26 cytosolic proteins. The dominant S-layer protein (SlpA; spots 1 and 2, Figure 1B) plays a key role in GIT adhesion and host immune system modulation [10,21]. Recently the pullulanase-deficient mutant was found to have reduced adhesion to mucin, compared to the wild-type NCFM [11].

Several proteins, in particular in the exoproteome, occurred in more than one spot (Table 1). Thus, multiple forms of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) with the same molecular mass, but different pIs were found in 8 and 4 spots in the exo- (Figure 1A, Table 1) and surface proteomes (Figure 1B, Table 1), respectively, reflecting different PTMs and maybe corresponding to distinct physiological roles [22,23].

The exo- and surface proteomes shared 22 proteins, while each contained 21 and 17 specific proteins, respectively (Figure 2). The SlpA dominance in the surface proteome, however, perhaps suppressed observation of low abundance proteins. Furthermore, the presence of e.g.

adenylate kinase (Spot 64) and transcription elongation factor NusA (Spot 27) found in the exoproteome have not been previously identified or suggested as putative moonlighting proteins, and therefore their presence possibly indicates cell lysis releasing intracellular contents [24].

Several exo- and surface proteome (Table 1) proteins have been reported to be moonlighting proteins for other bacteria that may bind to ECM components (fibrinogen, collagen, plasminogen, mucin) and host epithelial cells [11,24,25]. GAPDH is a universal moonlighting protein that has different roles in bacteria and eukaryotic cells [25,27]. Recently, recombinant GAPDH of NCFM was shown to bind to mucin carbohydrate moieties [28]. The NCFM subproteomes (Table 1) contained putative moonlighting proteins previously found in an extracellular environment or on bacterial surfaces, including trigger factor [29,30]; phosphoketolase [30]; D-lactate dehydrogenase [30], elongation factor Ts [31]; adenylosuccinate synthetase [32]; cell division protein FtsZ [33]; S-ribosylhomocysteinase [30]; transcription elongation factor GreA [34]; glutamyl-tRNA synthetase [35]; Mn-dependent inorganic pyrophosphatase [36]; dipeptidase [37]; 30S ribosomal proteins S1 and S2 [35,38]; 50S ribosomal proteins L1, L3, L5, L6, L10 [33,38–40]; phosphate starvation inducible protein [41]; and lysine tRNA ligase [36].

In our mapping of whole-cell proteomes of NCFM [7,8], very few proteins (SlpA, LBA0169; lysin, LBA1918) contained a signal peptide, as opposed to the subproteomes where both signal peptide- and non-classically secreted proteins, moonlighting and putative moonlighting proteins were identified. Exo- and surface proteomes of NCFM have been made available at the World-2DPAGE Repository (http://world-2dpage.expasy.org/repository/; please contact the authors for accession numbers).

In conclusion, probiotics are gaining increasing attention due to their benefits on human health, and identification of bacterial host-interacting proteins is needed to investigate and disclose details of the bacteria-host interplay at the molecular level. The proteins identified in this study encompass secreted and surface-layer associated proteins that may be involved in attaching to human ECM components and intestinal cells.

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The authors have no conflict of interests.

References

[1] Sanders, M.E., Klaenhammer, T.R., Invited review: the scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. *J. Dairy Sci.* 2001, 84, 319–331.

[2] Altermann, E., Russell, W.M., Azcarate-Peril, M.A., Barrangou, R., et al., Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. *Proc. Natl. Acad. Sci. U. S. A.* 2005, 102, 3906–3912.

[3] Sara, M., Sleytr, U.B., S-layer proteins. *J. Bacteriol.* 2000, 182, 859–868.

[4] Hynönen, U., Palva, A., *Lactobacillus* surface layer proteins: structure, function and applications. *Appl. Microbiol. Biotechnol.* 2013, 97, 5225–5243.

[5] Sánchez, B., Bressollier, P., Urdaci, M.C., Exported proteins in probiotic bacteria: adhesion to intestinal surfaces, host immunomodulation and molecular cross-talking with the host. *FEMS Immunol. Med. Microbiol.* 2008, 54, 1–17.

[6] Sánchez, B., Urdaci, M.C., Margolles, A., Extracellular proteins secreted by probiotic bacteria as mediators of effects that promote mucosa-bacteria interactions. *Microbiology* 2010, 156, 3232–3242.

[7] Majumder, A., Sultan, A., Jersie-Christensen, R.R., Ejby, M., et al., Proteome reference map of *Lactobacillus acidophilus* NCFM and quantitative proteomics towards understanding the prebiotic action of lactitol. *Proteomics* 2011, 11, 3470–3481.

[8] Majumder, A., Cai, L., Ejby, M., Schmidt, B.G., et al., Two-dimensional gel-based alkaline proteome of the probiotic bacterium *Lactobacillus acidophilus* NCFM. *Proteomics* 2012, 12, 1006–1014.

[9] Lortal, S., Heijenoort, J. Van, Gruber, K., Sleytr, U.B., S-layer of *Lactobacillus helveticus* ATCC 12046: isolation, chemical characterization and re-formation after extraction with lithium chloride. *J. Gen. Microbiol.* 1992, 138, 611–618.

[10] Konstantinov, S.R., Smidt, H., de Vos, W.M., Bruijns, S.C.M., et al., S layer protein A of *Lactobacillus acidophilus* NCFM regulates immature dendritic cell and T cell functions. *Proc. Natl. Acad. Sci. U. S. A.* 2008, 105, 19474–19479.

[11] Celebioglu, H.U., Ejby, M., Majumder, A., Køber, C., et al., Differential proteome and cellular adhesion analyses of the probiotic bacterium *Lactobacillus acidophilus* NCFM grown on raffinose - an emerging prebiotic. *Proteomics* 2016, 16, 1361–1375.

[12] Candiano, G., Bruschi, M., Musante, L., Santucci, L., et al., Blue silver: A very sensitive colloidal Coomassie G-250 staining for proteome analysis. *Electrophoresis* 2004, 25, 1327–1333.

[13] Petersen, T.N., Brunak, S., von Heijne, G., Nielsen, H., SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nat. Methods* 2011, 8, 785–786.

[14] Bendtsen, J.D., Kiemer, L., Fausbøll, A., Brunak, S., Non-classical protein secretion in bacteria. *BMC Microbiol.* 2005, 5, 58.

[15] Jeffery, C.J., Moonlighting proteins. *Trends Biochem. Sci.* 1999, 24, 8–11.

[16] Juge, N., Microbial adhesins to gastrointestinal mucus. *Trends Microbiol.* 2012, 20, 30–39.

[17] Marchler-Bauer, A., Derbyshire, M.K., Gonzales, N.R., Lu, S., et al., CDD: NCBI's conserved domain database. *Nucleic Acids Res.* 2015, 43, D222–D226.

[18] Van Tassell, M.L., Miller, M.J., *Lactobacillus* adhesion to mucus. *Nutrients* 2011, 3, 613–636.

[19] Johnson, B., Selle, K., O'Flaherty, S., Goh, Y.J., Klaenhammer, T., Identification of extracellular surface-layer associated proteins in *Lactobacillus acidophilus* NCFM. *Microbiology* 2013, 159, 2269–2282.

[20] Rabilloud, T., Chevallet, M., Luche, S., Lelong, C., Two-dimensional gel electrophoresis in proteomics: Past, present and future. *J. Proteomics* 2010, 73, 2064–2077.

[21] Buck, B., Altermann, E., Svingerud, T., Klaenhammer, T.R., Functional analysis of putative adhesion factors in *Lactobacillus acidophilus* NCFM. *Appl. Environ. Microbiol.* 2005, 71, 8344–8351.

[22] Schlüter, H., Apweiler, R., Holzhütter, H.-G., Jungblut, P.R., Finding one's way in proteomics: a protein species nomenclature. *Chem. Cent. J.* 2009, 3, 11.

[23] Jungblut, P.R., Holzhütter, H.G., Apweiler, R., Schlüter, H., The speciation of the proteome. *Chem. Cent. J.* 2008, 2, 16.

[24] Jacobs, A.C., DiDone, L., Jobson, J., Sofia, M.K., et al., Adenylate kinase release as a high-throughput-screening-compatible reporter of bacterial lysis for identification of antibacterial agents. *Antimicrob. Agents Chemother.* 2013, 57, 26–36.

[25] Kainulainen, V., Korhonen, T.K., Dancing to another tune Adhesive moonlighting proteins in bacteria. *Biology (Basel).* 2014, 3, 178–204.

[26] Mani, M., Chen, C., Amblee, V., Liu, H., et al., MoonProt: A database for proteins that are known to moonlight. *Nucleic Acids Res.* 2015, 43, D277–D282.

[27] Henderson, B., Martin, A.C.R., Protein moonlighting: a new factor in biology and medicine. *Biochem. Soc. Trans.* 2014, 42, 1671–1678.

[28] Patel, D.K., Shah, K.R., Pappachan, A., Gupta, S., Singh, D.D., Cloning, expression and characterization of a mucin-binding GAPDH from *Lactobacillus acidophilus*. *Int. J. Biol. Macromol.* 2016, 91, 338–346.

[29] Aleljung, P., Shen, W., Rozalska, B., Hellman, U., et al., Purification of collagen-binding proteins of *Lactobacillus reuteri* NCIB 11951. *Curr. Microbiol.* 1994, 28, 231–236.

[30] Izquierdo, E., Horvatovich, P., Marchioni, E., Aoude-Werner, D., et al., 2-DE and MS analysis of key proteins in the adhesion of *Lactobacillus plantarum*, a first step toward early selection of probiotics based on bacterial biomarkers. *Electrophoresis* 2009, 30, 949–956.

[31] Beck, H.C., Madsen, S.M., Glenting, J., Petersen, J., et al., Proteomic analysis of cell surface-associated proteins from probiotic *Lactobacillus plantarum*. *FEMS Microbiol. Lett.* 2009, 297, 61–66.

[32] Ashida, N., Yanagihara, S., Shinoda, T., Yamamoto, N., Characterization of adhesive molecule with affinity to Caco-2 cells in *Lactobacillus acidophilus* by proteome analysis. *J. Biosci. Bioeng.* 2011, 112, 333–337.

[33] Genovese, F., Coïsson, J.D., Majumder, A., Pessione, A., et al., An exoproteome approach to monitor safety of a cheese-isolated *Lactococcus lactis*. *Food Res. Int.* 2013, 54, 1072–1079.

[34] Sibbald, M.J.J.B., Ziebandt, A.K., Engelmann, S., Hecker, M., et al., Mapping the pathways to staphylococcal pathogenesis by comparative secretomics. *Microbiol. Mol. Biol. Rev.* 2006, 70, 755–788.

[35] Katakura, Y., Sano, R., Hashimoto, T., Ninomiya, K., Shioya, S., Lactic acid bacteria display on the cell surface cytosolic proteins that recognize yeast mannan. *Appl. Microbiol. Biotechnol.* 2010, 86, 319–326.

[36] Espino, E., Koskenniemi, K., Mato-Rodriguez, L., Nyman, T.A., et al., Uncovering surface-exposed antigens of *Lactobacillus rhamnosus* by cell shaving proteomics and two-dimensional immunoblotting. *J. Proteome Res.* 2015, 14, 1010–1024.

[37] Waśko, A., Polak-Berecka, M., Paduch, R., Jóźwiak, K., The effect of moonlighting proteins on the adhesion and aggregation ability of *Lactobacillus helveticus*. *Anaerobe* 2014, 30, 161–168.

[38] Berlec, A., Zadravec, P., Jevnikar, Z., Štrukelj, B., Identification of candidate carrier proteins for surface display on *Lactococcus lactis* by theoretical and experimental analyses of the surface proteome. *Appl. Environ. Microbiol.* 2011, 77, 1292–1300.

[39] Bohle, L., Riaz, T., Egge-Jacobsen, W., Skaugen, M., et al., Identification of surface proteins in *Enterococcus faecalis* V583. *BMC Genomics* 2011, 12, 135.

[40] Dreisbach, A., Hempel, K., Buist, G., Hecker, M., et al., Profiling the surfacome of *Staphylococcus aureus*. *Proteomics* 2010, 10, 3082–3096.

[41] Dumas, E., Desvaux, M., Chambon, C., Hébraud, M., Insight into the core and variant exoproteomes of *Listeria monocytogenes* species by comparative subproteomic analysis. *Proteomics* 2009, 9, 3136–3155.

Figure legends

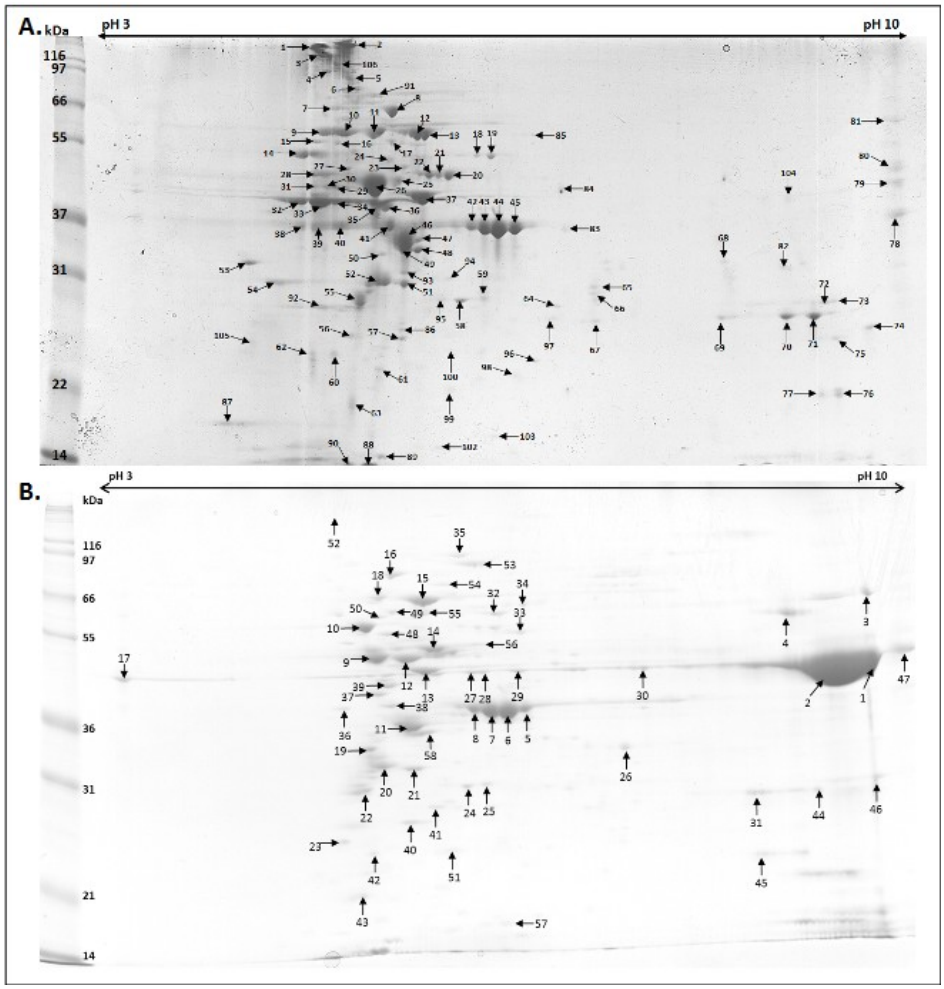

Figure 1: Representative 2-DE images of (A) exoproteome and (B) surface proteome of *Lactobacillus acidophilus* NCFM. Numbered spots were picked and used for protein identification by in-gel digestion and MALDI-TOF MS.

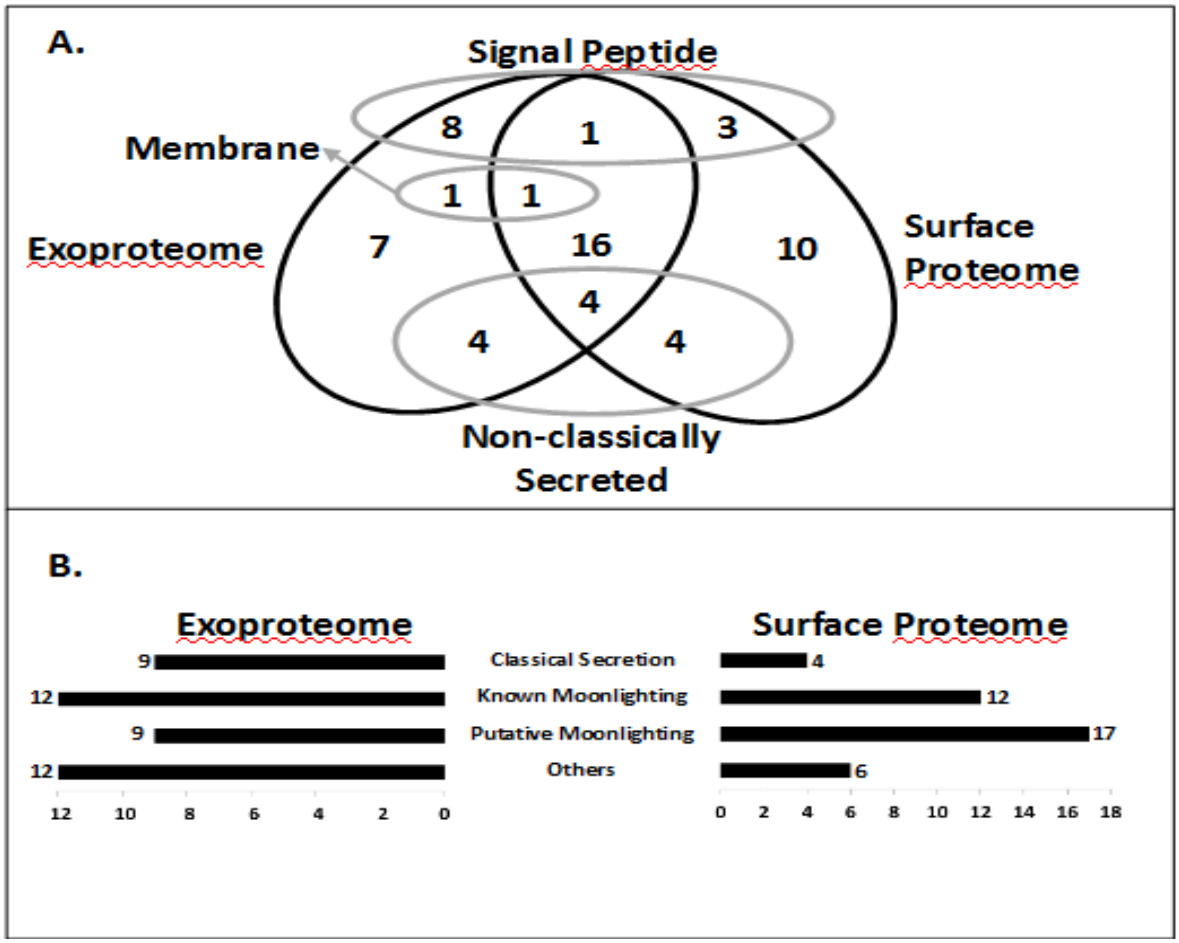

Figure 2: A. Venn diagram for exo- and surface proteome identifications of *Lactobacillus acidophilus* NCFM. Signal Peptide, proteins that contain a signal peptide; Membrane, proteins predicted to be membrane proteins; Non-classically Secreted, proteins predicted to be secreted by non-classical secretion systems [13, 14] **B.** Protein categories of exo- and surface proteomes of *Lactobacillus acidophilus* NCFM. Classical Secretion, proteins normally secreted; Known Moonlighting, proteins previously described as moonlighting for other bacteria; Putative Moonlighting, proteins previously found extracellularly for other bacteria.

Table 1: Identified proteins from 2DE of exo- and surface proteomes of *Lactobacillus acidophilus* NCFM grown on 1% glucose

Spot No	Exoproteome	Surface Proteome	Spot No
Common Proteins			
32-34, 82	S-layer Protein [A]		**1, 2, 27-30**
38-40,42-45,78	GAPDH*		**5,6,7,8**
26, 35	Enolase*		**9**
14	Trigger factor [B#]		**10**
46, 49	L-lactate dehydrogenase		**11**
25	Elongation factor Tu*		**12**
37, 79	Phosphoglycerate kinase*		**13**
22, 28	30S ribosomal protein S1[#]		**14**
9, 10, 12-13, 81	Pyruvate kinase*		**15**
7, 8, 17	Elongation factor G*		**16, 41, 50**
52	Fructose-bisphosphate aldolase*		**20**
55	Triose phosphate isomerase*		**22**
58, 95	Glycoprotein endopeptidase [B]		**24**
91	Phosphoketolase [B#]		**35**
41	Lactate dehydrogenase[#]		**38**
36	Elongation factor Ts[#]		**39**
24	ATP synthase FOF1 subunit alpha [C]		**48**
15, 17	Molecular chaperone GroEL*		**49**
51, 54	Phosphoglyceromutase*		**21**
20,21	Adenylosuccinate synthetase[#]		**56**
103	Hypothetical protein LBA1769 [B]		**57**
47, 48, 93	6-phosphofructokinase*		**58**

Spot No	Exoproteome	Surface Proteome	Spot No
Distinct proteins			
1	Surface Protein [A]	Cell separation protein cdpA [A]	**3**
2	Mucus binding protein precursor Mub [A]	Hypothetical protein LBA0695 [A]	**4**
3	Mucus-binding protein [A]	Transcription elongation factor GreA [B#]	**23**
6	Fibrinogen-binding protein [A]	30S ribosomal protein S2[#]	**25**
11	Molecular chaperone DnaK [B]*	Cysteine synthase*	**26**
16	Cell division protein FtsZ [B#]	50S ribosomal protein L1[#]	**31**
18, 19	Aspartyl/glutamyl-tRNA amidotransferase subunit A	Glutamyl-tRNA synthetase[#]	**32**
23	Hypothetical protein LBA1642	Mn-dependent inorganic pyrophosphatase[#]	**36**
27	Transcription elongation factor NusA	Dipeptidase[#]	**37**
29, 30, 104	SlpX [A]	50S ribosomal protein L10[#]	**42**
53	Hypothetical protein LBA0733 [B]	50S ribosomal protein L5[#]	**45**
61	Peptide deformylase	50S ribosomal protein L3 [B#]	**46**
64	Adenylate kinase	Phosphate starvation inducible protein stress-related[#]	**51**
72, 92	N-acetylmuramidase [A]	Thermostable pullulanase [A]	**52**
74	Lysin, partial [A]	Ribonucleoside tri-P reductase [B]	**53**
76, 77	50S ribosomal protein L6 [B#]	BipAEFTU family GTP-binding protein [B]	**54**
89	Phosphotransferase system enzyme II [C]	Lysine tRNA ligase[#]	**55**
96	Adenine phosphoribosyltransferase		
102	S-ribosylhomocysteinase[#]		
106	Mucus-binding protein, partial [A]		

[A] Signal peptide-dependent secretion [13]

[B] Non-classical secretion [14]

[C] Transmembrane protein

* Known moonlighting proteins

[#]Putative moonlighting proteins