Cryptic plasmids of the *Lactiplantibacillus plantarum* LS/07 probiotic co-determine its membrane-associated proteome and secreted proteome

Ľuboš AMBRO [1], Veronika LUKÁČOVÁ [2], Dominik HADŽEGA [3], René LINK [1], Ľuboš KĽUČÁR [3], Maksym DANCHENKO [4], Peter BARÁTH [2, 4]

1 Institute of Experimental Medicine, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Trieda SNP 1, 040 11, Košice, SK
2 MEDIREX GROUP ACADEMY, Jána Bottu 2, 917 01, Trnava, SK
3 Institute of Molecular Biology, Slovak academy of Sciences, Dúbravská cesta 21, 845 51, Bratislava, SK
4 Institute of Chemistry, Slovak academy of Sciences, Dúbravská cesta 9, 845 38, Bratislava, SK

AIM OF THE STUDY

Lactiplantibacillus plantarum LS/07 has been proven to possess various beneficial effects in preclinical testing [1, 2]. Recently, its genome and plasmids have been sequenced and fully annotated. Aim of this study was to clarify, whether these plasmids (that are thought to be cryptic) significantly contribute to the strain's secreted and membrane-associated proteome. We assesed proteome in three different cultivation conditions.

METHODS

CULTIVATION: 16 h @ 30 °C / 16 h @ 37 °C / 15.5 h @ 37 °C + 0.5 h @ 42 °C (heat-shock, HS) in defined medium w/o yeast & meat extract.

PROTEIN PRECIPITATION: bacterial culture supernatant filtered (0.2 µm) and ECV & proteins precipitated with TCA (1 h on ice).

SAMPLE PROCESSING: pellets were dissolved in 8 M urea in 100 mM TEAB, reduced by 5 mM DTT, alkylated with 40 mM iodoacetamide and quenched by additional 5 mM dithiothreitol. Resulting mixture was diluted (50 mM Tris-HCl pH 7.8), digested overnight with TPCK-treated trypsin and quenched with TFA. Concentrated peptides were dissolved in 0.1 % TFA and 2 % acetonitrile.

LC/MS ANALYSIS: trap column (PepMap100 C18, 75 µm x 20 mm, 3 µm particle size; Dionex) and nanoseparation analytical column (Acclaim PepMap C18, 75 µm x 500 mm, Dionex) attached to Ultimate 3000 RSLCnano system. Eluted peptides were sprayed directly into Orbitrap Elite mass spectrometer [3].

DATASET PROCESSING: MaxQuant v1.5.3.30 [4] with a built-in Andromeda search engine.

PEPTIDE SEARCH: performed against *L. plantarum* protein database (downloaded from https://www.uniprot.org/ on 03/06/2019 and containing 3087 entries) and *L. plantarum* LS/07 proteome (protein coding sequences extracted from both genome and plasmids).

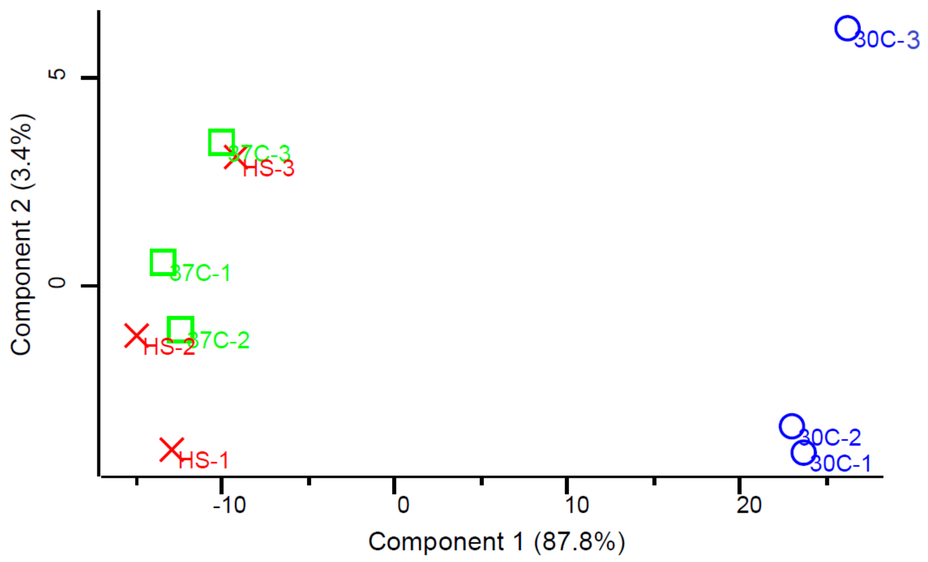
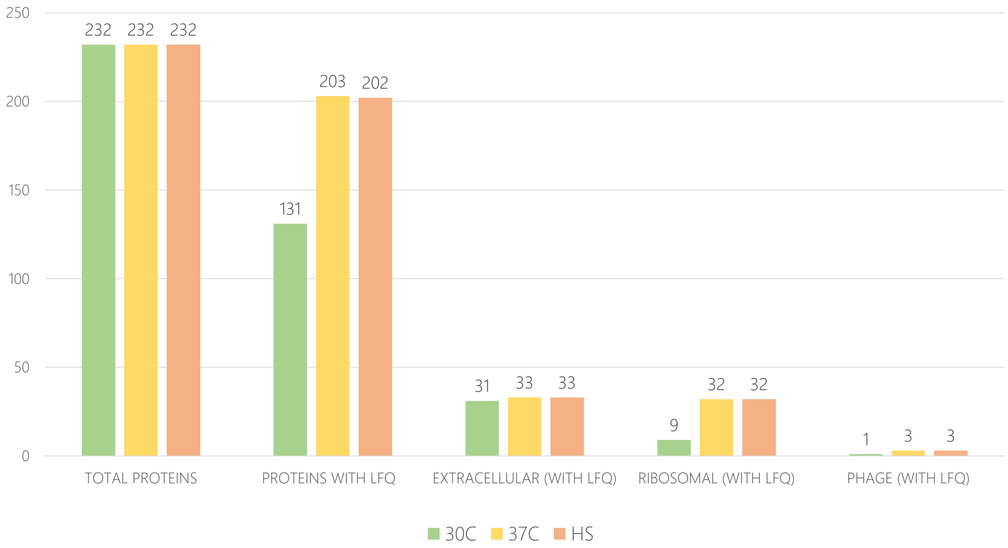


Fig. 1. PCA plot showing differences in proteomics profile of the samples resulted from cultivation of *L. plantarum* LS/07 at different temperatures (30, 37 and HS). All conditions were tested as three independent biological replicates.

Tab. B. Chosen statistics of the 30C, 37C and HS proteomics profiles: total proteins identified, proteins with assesed LFQ intensity (at least two peptides with fragmentation spectrum) and counts of extracellular / ribosomal / phage poteins with assesed LFQ intensity. The best ratio of extracellular to ribosomal proteins was observed in condition 30C.



Tab. A. Table showing top 20 proteins with highest calculated LFQ intensity. Genome-encoded extracellular proteins are highlighted with blue and cytosolic proteins with gray. Plasmid-encoded extracellular proteins are highlighted with orange. LFQ intensity values are calculated as an average of three independent biological replicates.

Protein names	MW (kDa)	Matched peptides	Sequence coverage (%)	LFQ 30C	LFQ 37C	LFQ HS
Extracellular transglycosylase | LysM peptidoglycan-binding domain	26,3	5	24,7	6,66E+08	4,58E+08	3,34E+08
Peptidoglycan endopeptidase	36,5	9	26,5	5,45E+08	3,59E+08	2,45E+08
KxYKxGKxW signal peptide domain-containing protein (plasmid)	240,6	46	77	2,47E+08	1,36E+08	1,47E+08
Extracellular transglycosylase | LysM peptidoglycan-binding domain	21,3	6	33,3	2,03E+08	2,42E+08	1,48E+08
Extracellular protein, muropeptidase	48,3	6	22,2	1,97E+08	1,60E+08	1,31E+08
Extracellular transglycosylase | LysM peptidoglycan-binding domain	21,9	6	41	1,96E+08	1,20E+08	8,07E+07
Cell wall hydrolase	82,3	35	74,5	1,68E+08	1,28E+08	1,25E+08
NADH dehydrogenase	71,9	2	7,4	1,63E+08	1,30E+08	9,85E+07
Extracellular transglycosylase | LysM peptidoglycan-binding domain	34,5	11	29,3	1,23E+08	8,79E+07	6,43E+07
Chitin-binding protein	22,2	7	42,8	9,41E+07	1,62E+08	2,64E+08
Mannose-specific adhesin | LPXTG cell wall anchor domain-containing protein	108,0	31	50,3	5,87E+07	5,64E+07	5,13E+07
Isopeptide-forming domain-containing fimbrial protein (plasmid)	107,0	63	66,3	2,93E+07	3,31E+07	3,78E+07
Metal ABC transporter substrate-binding protein	34,7	14	52,1	2,79E+07	3,57E+06	2,77E+06
Extracellular protein	23,2	5	33,5	2,50E+07	2,36E+07	1,67E+07
Extracellular protein	23,1	4	16,8	1,67E+07	9,97E+06	1,01E+07
Extracellular protein	45,8	15	50,6	1,58E+07	1,63E+07	1,72E+07
Acyltransferase	74,4	6	16,4	1,58E+07	2,12E+07	2,34E+07
Oligopeptide ABC transporter, substrate binding protein	61,1	25	45,8	1,49E+07	2,08E+06	1,80E+06
Cystine ABC transporter, substrate binding protein	29,0	5	20,2	9,29E+06	5,24E+06	5,32E+06
Extracellular protein	29,3	13	39,1	8,64E+06	3,23E+07	4,27E+07

SUMMARY

- We have shown, that our protocol is very effective for enrichment of extracellular and membrane-associated proteins. Samples 37C and HS have similar protein profiles – no significant change was observed after short heat-shock (Fig. 1, Tab. A). Contamination by cytosolic proteins was lowest in 30C samples (Tab. B).
- We confirmed production of plasmid-encoded membrane-associated KxYKxGKxW signal peptide domain-containing protein (240 kDa) and highly charged isopeptide-forming domain-containing fimbrial protein (107 kDa), both were found in samples of all conditions in relatively high concentrations.
- We also detected production of plasmid-encoded S8 family serine peptidase (162 kDa, coverage 14 %) and S41 family serine peptidase (35 kDa, coverage 26 %). Both proteases are expressed in comparable amounts in samples of all three conditions (data not shown).
- The strain contains three integrated prophages and some of the phage proteins are also found in analyzed precipitates (data not shown). Expression of several phage proteins has temperature-dependent pattern (Tab. B).
- Our findings suggest that expression of plasmid-encoded proteins can significantly affect extracellular and membrane-associated proteome of probiotic isolates.

References

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