Comprehensive Characterization of the *E. coli* Cell Envelope Using a nanoLC-LTQ Orbitrap MS

M Papanastasiou, M Frantzeskos-Sardis, G Orfanoudaki, J Giapitzaki, M Aivaliotis, S Karamanou, A Economou

Institute of Molecular Biology & Biotechnology, FoRTH, Iraklio-Crete, Greece

Introduction

• The *E. coli* envelope is a complex assembly that comprises the outer membrane, the periplasmic space and the inner membrane. It is estimated that about one third of the total *E. coli* proteome is associated with the cell envelope.

• Proteins embedded in the membranes are key components for viability and pathogenicity. Characterizing their function is crucial therefore for understanding of how a cell operates.

• The hydrophobic nature of those proteins, however, has hampered the efforts to perform mass spectrometry analysis and most proteomic studies carried out to date have dealt mainly with the soluble counterparts of the cell (cytoplasm, periplasm). The inner and outer membrane proteomes are not as well defined.

• In this study, we couple traditional biochemical techniques with mass spectrometry in an effort to identify previously undetected membrane proteins.

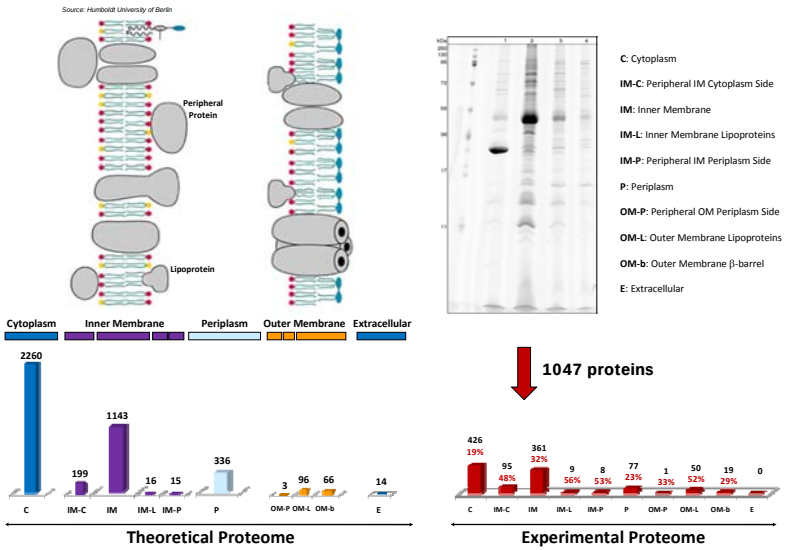
• This work will serve as a basis for the analysis of the complete cell envelope complexome of laboratory and enteropathogenic *E. coli* strains and other pathogenic bacteria.

Inner Membrane proteins reported in this study that remained undetected

Protein	Accession No	Molecular Function	Description	Signal Peptide	Predicted TMs
SBMA	P0AFY6	Catalytic Activity	Protein sbmA	32	8
POTB	P0AFK4	Catalytic Activity	Spermidine/putrescine transport system permease protein potB	0	6
YCAI	P37443	DNA Uptake	Uncharacterized protein ycaI	0	7
APPB	P26458	Electron transport	Cytochrome bd-II oxidase subunit 2	0	8
C56H	P76345	Electron transport	Cytochrome b561 homolog 1	0	4
FLK	P15286	Flagellum Biogenesi	Flagellar regulator flk	0	1
FXSA	P37147	Phage Infection	UPF0716 protein fxsA	0	3
LGT	P60955	Transferase	Prolipoprotein diacylglyceryl transferase	0	5
MRAY	P0A6W3	Transferase	Phospho-N-acetylmuramoyl-pentapeptide-transferase	0	10
UBIA	P0AGK1	Transferase	4-hydroxybenzoate octaprenyltransferase	0	7
ARNT	P76473	Transferase	Undecaprenyl phosphate-alpha-4-amino-4-deoxy-L-arabinose arabinosyl transferase	0	12
MARC	P0AEY1	Transporter	UPF0056 inner membrane protein marC	0	5
DINF	P28303	Transporter	DNA-damage-inducible protein F	0	13
CHAA	P31801	Transporter	Calcium/proton antiporter	0	11
KCH	P31069	Transporter	Putative potassium channel protein	0	7
YHAM	P42626	Transporter	UPF0597 protein yhaM	0	0
XYLH	P0AG94	Transporter	Xylose transport system permease protein xylH	0	10
GALP	P0AEP1	Transporter	Galactose-proton symporter	31	12
PANF	P16256	Transporter	Sodium/pantothenate symporter	0	13
YHO	P32136	Transporter	Uncharacterized symporter yihO	0	11
GLTP	P21345	Transporter	Proton glutamate symport protein	0	10
RHAT	P27125	Transporter	L-rhamnose-proton symporter	0	10
ALX	P42601	Transporter	Inner membrane protein alx	0	9
AROP	P15993	Transporter	Aromatic amino acid transport protein aroP	0	12
BETT	P0ABC9	Transporter	High-affinity choline transport protein	0	12
FSR	P52067	Transporter	Fosmidomycin resistance protein	0	10
NUPG	P0AFF4	Transporter	Nucleoside permease nupG	0	12
PSTC	P0AGH8	Transporter	Phosphate transport system permease protein pstC	0	6
YCHM	P0AFR2	Transporter	Putative sulfate transporter ychM	0	11
YFCJ	P77549	Transporter	UPF0226 protein yfcJ	0	12
YFEH	P39836	Transporter	Uncharacterized protein yfeH	29	8
YGAZ	P76630	Transporter	Inner membrane protein ygaZ	0	5
YHAO	P42628	Transporter	Inner membrane transport protein yhaO	0	11
YOAE	P0AEC0	Transporter	UPF0053 inner membrane protein yoaE	0	7
YQEG	P63340	Transporter	Inner membrane transport protein yqeG	0	10
YPIK	P27837	Transporter	Probable transport protein ydiK	0	12
YQIK	P77306	Transporter	Inner membrane protein yqiK	0	2
MMUP	Q47689	Transporter	Probable S-methylmethionine permease	0	12
YFBV	P0A8D9	Uncharacterized	UPF0208 membrane protein yfbV	0	2
YIDI	P31446	Uncharacterized	Inner membrane protein yidI	35	3
YJDF	P39270	Uncharacterized	Inner membrane protein yjdF	29	6

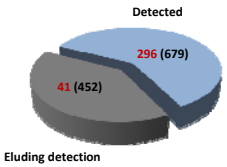
Results & Discussion

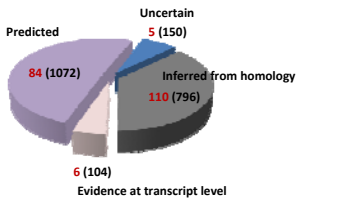
A. Analysis of the E. coli Cell Envelope



C: Cytoplasm
IM-C: Peripheral IM Cytoplasm Side
IM: Inner Membrane
IM-L: Inner Membrane Lipoproteins
IM-P: Peripheral IM Periplasm Side
P: Periplasm
OM-P: Peripheral OM Periplasm Side
OM-L: Outer Membrane Lipoproteins
OM-b: Outer Membrane β-barrel
E: Extracellular

Protein Curation EchoLOCATION was used for P, IM-L, OM-L, OM-b, E and C proteins (Horler *et al*, 2009). The IM proteome was taken from Bernsel & Daley, 2009. Proteins were cross checked with experimental and theoretical lists (Riley *et al*, 2006, Weiner & Li, 2008, Lopez-Campistrous *et al*, 2005, Zhang *et al*, 2006). Prediction tools (LipoP, SignalP, TMHMM, BOMB) and Blast were used to check cytoplasmic proteins manually.

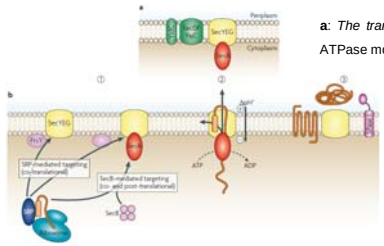




Inner Membrane Proteins Identified in Proteomic Studies (for a detailed list of references please see Bernsel & Daley, Trends in Microbiology, 2009). Proteins detected in this study are noted in red.

Types of Protein Existence for EcoliK12 (UniProtKB) Proteins that have not been identified experimentally are noted in parenthesis. Proteins identified in this study are noted in red.

B. Sec Pathway and Translocase



Source: Papanikou et al, Nature Reviews, 2007

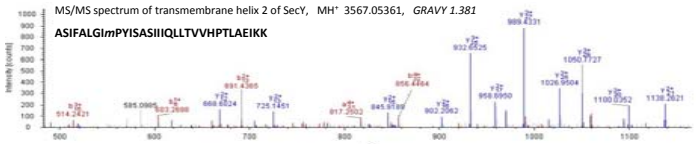
a: *The translocase core* consists of the SecYEG heterotrimer, the ATPase motor SecA and a subset of partner proteins.

b: *Translocation of proteins:* Proteins to be exported are synthesized as pre-proteins in the cytoplasm with N-terminal signal peptides and are targeted to the translocase either co-translational by the SRP or co- and post-translational by the SecB chaperone. FtsY and SecA act as receptors for SRP and SecB respectively. Translocation occurs through the SecYEG channel.

Protein	Accession No	MW kDa	% Coverage	GRAVY	Pred. TMs*	Sequence Coverage
SRP54	P0AG07	50	83.0	-0.243	-	
FtsY	P10121	55	11.1	-0.334	-	
SecB	P0AG86	17	19.4	-0.188	-	
SecA	P10408	102	71.7	-0.497	-	
SecY	P0AGA2	49	46.0	0.552	10	
SecE	P0AGA2	14	31.5	0.876	3	
SecG	P0AG99	11	76.4	0.375	2	
SecD	P0AG90	67	64.2	0.116	6	
SecF	P0AG93	35	31.6	0.400	6	
YajC	P0AD27	12	59.1	0.395	1	
YidC	P25714	62	47.3	-0.121	3	
SPaseI	P00803	36	53.1	-0.156	2	
SPaseII	P00804	18	ND	0.673	4	

* Predicted TMs are noted with lines below the sequence coverage

0 20 40 60 80 100
MW (kDa)



Acknowledgements The research leading to these results has received funding from the European Commission (EC) Sixth Framework Programme agreement n° LSHC-CT-2006-037834 «Streptomics». The proteomics facility of the IMBB was established through the European Community's Seventh Framework Programme agreement 229823 Capacities-FP7-REGPOT-2008-1/ project «ProFI».M.F.S. and G.O. are Onassis Foundation pre-doctoral fellows

Materials & Methods