

**Table S1: Proteins separated by 2D gels analysis and identified by MALDI-TOF analysis**

Spot	Protein description	AC <sup>a</sup>	Mascot search results			Localization
			Score	N. of peptides	Seq. coverage (%)	
2	Putative lipoprotein AcfD homolog	ACFD_ECOLI	76	9/13	6	Inner membrane
10	Cellulose synthase operon protein C	BCSC_ECOLI	112	10/11	8	Outer membrane
15	Protease 3	PTRA_ECO57	112	11/15	14	Periplasm
25	LPS-assembly protein LptD	LPTD_ECO57	113	11/18	17	Outer membrane
26	LPS-assembly protein LptD	LPTD_ECO57	216	24/49	42	Outer membrane
	Outer membrane protein assembly factor BamA	BAMA_ECO24	112	16/49	27	Outer membrane
27	Outer membrane protein assembly factor BamA	BAMA_ECO24	371	34/44	53	Outer membrane
28	Maltoporin	LAMB_ECOBW	162	16/32	52	Outer membrane
32	Ferrienterobactin receptor	FEPA_ECOLI	108	8/9	15	Outer membrane
33	Ferrienterobactin receptor	FEPA_ECOLI	247	24/35	40	Outer membrane
34	Ferrienterobactin receptor	FEPA_ECOLI	278	30/52	52	Outer membrane
37	Ferrichrome-iron receptor	FHUA_ECOLI	116	8/8	13	Outer membrane
38	Ferrichrome-iron receptor	FHUA_ECOLI	184	20/34	33	Outer membrane
39	Probable TonB-dependent receptor YncD	YLCD_ECOLI	162	28/96	52	Outer membrane,
	Ferrichrome-iron receptor	FHUA_ECOLI	106	23/96	35	Outer membrane,
	Ferrienterobactin receptor	FEPA_ECOLI	68	15/96	32	Outer membrane
40	Ferrichrome-iron receptor	FHUA_ECOLI	136	10/11	18	Outer membrane
42	Fe(3+) dicitrate transport protein FecA	FECA_ECOLI	148	11/12	18	Outer membrane
47	Periplasmic beta-glucosidase	BGLX_ECOLI	100	8/10	13	Periplasm
48	Periplasmic beta-glucosidase	BGLX_ECOLI	198	18/26	30	Periplasm
53	Tail-specific protease	PRC_ECOLI	154	14/19	24	Inner membrane
55	FhuE receptor	FHUE_ECOLI	104	7/7	13	Outer membrane
57	Penicillin-binding protein activator LpoA	LPOA_ECOLI	127	9/10	19	Outer membrane
58	Penicillin-binding protein activator LpoA	LPOA_ECOLI	225	18/24	34	Outer membrane
59	Penicillin-binding protein activator LpoA	LPOA_ECOLI	330	31/46	52	Outer membrane
60	Penicillin-binding protein activator LpoA	LPOA_ECOLI	147	12/16	22	Outer membrane
64	Penicillin-binding protein activator LpoA	LPOA_ECOLI	121	8/8	16	Outer membrane
65	Colicin I receptor	CIRA_ECOLI	123	8/8	18	Outer membrane
68	Outer membrane protein assembly factor BamA	BAMA_ECO24	104	9/12	12	Outer membrane
78	Alpha-amylase	AMY1_ECOLI	140	11/14	15	Outer membrane
79	2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase	CPDB_ECOLI	127	9/10	16	Periplasm
80	2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase	CPDB_ECOLI	171	13/16	24	Periplasm
88	Soluble lytic mureintransglycosylase	SLT_ECO57	208	17/19	24	Periplasm
100	Protein UshA	USHA_ECOLI	138	9/9	17	Periplasm
102	Putative periplasmictrehalase	TREA_ECO57	125	8/8	16	Periplasm
103	Uncharacterized sulfataseYdeN	YDEN_ECOLI	79	6/9	18	Outer membrane
105	Uncharacterized sulfataseYdeN	YDEN_ECOLI	146	9/9	23	Outer membrane
109	Glucans biosynthesis protein D	OPGD_ECO24	215	15/17	26	Periplasm
110	Periplasmicoligopeptide-binding protein	OPPA_ECOLI	135	10/13	20	Periplasm
111	Periplasmicoligopeptide-binding protein	OPPA_ECOLI	264	22/35	42	Periplasm
115	Polysialic acid transport protein KpsD	OPGG_ECO24	110	7/7	20	Periplasm
114	Glucans biosynthesis protein G	KPSD1_ECOLX	139	11/16	24	Periplasm
116	Glucans biosynthesis protein G	OPGG_ECO24	232	19/24	41	Periplasm

120	Glycerophosphoryldiesterphosphodiesterase	GLPQ_ECOLI	103	8/13	27	Periplasm
137	Periplasmic serine endoproteaseDegP	DEGP_ECO57	134	8/8	25	Periplasm
138	Periplasmic serine endoproteaseDegP	DEGP_ECO57	176	15/21	40	Periplasm
146	Outer membrane protein TolC	TOLC_ECOLI	206	16/24	43	Outer membrane
147	Outer membrane protein TolC	TOLC_ECOLI	99	6/6	17	Outer membrane
150	Outer membrane protein TolC	TOLC_ECOLI	155	12/18	31	Outer membrane
156	Protein YhjJ	YHJJ_ECOLI	185	13/16	34	Periplasm
157	Periplasmic pH-dependent serine endoproteaseDegQ	DEGQ_ECOLI	218	16/20	46	Periplasm
158	Cell division proteinFtsP	FTSP_ECOLI	180	15/22	33	Periplasm
164	Maltoporin	LAMB_ECO24	119	11/17	32	Outer membrane
165	Maltoporin	LAMB_ECOBW	144	14/26	44	Outer membrane
166	Maltoporin	LAMB_ECOBW	168	19/37	46	Outer membrane
167	Maltoporin	LAMB_ECOBW	181	20/40	55	Outer membrane
168	Maltoporin	LAMB_ECOBW	203	20/37	52	Outer membrane
169	Maltoporin	LAMB_ECO24	82	6/10	23	Outer membrane
170	Rare lipoprotein A	RLPA_ECOLI	166	13/24	33	Outer membrane
171	Rare lipoprotein A	RLPA_ECOLI	147	11/18	22	Outer membrane
183	Chaperone SurA	SURA_ECO57	234	24/40	55	Periplasm
192	Putative acyl-CoA thioester hydrolase YbhC	YBHC_ECOLI	116	11/17	27	Outer membrane
193	Elongation factor Tu 1	EFTU1_ECO24	107	10/26	37	Cytoplasm
	Putative acyl-CoA thioester hydrolase YbhC	YBHC_ECOLI	89	9/26	27	Outer membrane
194	Glucose-1-phosphatase	AGP_ECOLI	102	9/20	22	Periplasm
195	Glucose-1-phosphatase	AGP_ECOLI	135	13/27	30	Periplasm
196	Protein TolB	TOLB_ECO24	151	9/9	23	Periplasm
199	Protein TolB	TOLB_ECO24	207	17/27	46	Periplasm
202	Protein TolB	TOLB_ECO24	215	17/25	49	Periplasm
205	Protein TolB	TOLB_ECO24	117	7/7	18	Periplasm
210	Periplasmic serine endoproteaseDegP	DEGP_ECO57	125	8/9	25	Periplasm
211	Periplasmic serine endoproteaseDegP	DEGP_ECO57	134	8/8	21	Periplasm
225	Maltose-bindingperiplasmicprotein	MALE_ECO57	144	15/49	49	Periplasm,
	Glycerophosphoryl diester phosphodiesterase	GLPQ_ECOLI	78	11/49	38	Periplasm,
	Erythronate-4-phosphate dehydrogenase	PDXB_ECOBW	68	9/49	32	Cytoplasm
226	Maltose-bindingperiplasmicprotein	MALE_ECO57	136	17/66	52	Periplasm,
	Glycerophosphoryl diester phosphodiesterase	GLPQ_ECOLI	71	12/66	38	Periplasm,
227	Maltose-bindingperiplasmicprotein	MALE_ECO57	112	7/8	23	Periplasm
242	UncharacterizedproteinYncE	YNCE_ECOLI	126	8/10	31	Outer membrane
244	Outer membrane protein assembly factor BamB	BAMB_ECOLI	222	19/30	66	Outer membrane
245	Outer membrane protein F	OMPF_ECOLI	189	13/20	56	Outer membrane
246	Outer membrane protein assembly factor BamC	BAMC_ECOLI	110	7/9	27	Outer membrane
247	Outer membrane protein assembly factor BamC	BAMC_ECOLI	231	20/33	62	Outer membrane
248	Outer membrane protein assembly factor BamC	BAMC_ECOLI	117	7/8	27	Outer membrane
249	Iron uptake system component EfeO	EFE0_ECO57	102	6/6	16	Periplasm
263	Maltose operon periplasmic protein	MALM_ECOLI	138	10/14	25	C-frag. Periplasm
264	Glycine betaine/prolinebetaine-binding periplasmic protein	PROX_ECO57	101	6/7	24	Periplasm
266	Lipoprotein NlpI	NLPI_ECO57	188	10/10	38	Inner membrane *
267	Long-chain fatty acid transport protein	FADL_ECO57	89	7/12	19	Outer membrane
268	Phospholipase A1	PA1_ECO57	106	7/11	33	Outer membrane
273	Cell division coordinator CpoB	CPOB_ECOLI	104	8/13	23	Periplasm
272	Beta-lactamase TEM	BLAT_ECOLX	151	11/16	43	Periplasm
274	Beta-lactamase TEM	BLAT_ECOLX	280	22/36	70	Periplasm

<b>275</b>	Beta-lactamase TEM	BLAT_ECOLX	148	8/8	32	Periplasm
<b>284</b>	Probable L,D-transpeptidase YbiS	YBIS_ECO57	133	8/10	46	Periplasm
<b>286</b>	FKBP-type peptidyl-prolylcis-trans isomerase FkpA	FKBA_ECO57	154	10/15	44	Periplasm
<b>293</b>	Glutamate/aspartate import solute-binding protein	GLTI_ECOLI	100	6/7	21	Periplasm
<b>301</b>	Lipoprotein NlpE	NLPE_ECOLI	118	8/10	42	Outer membrane
<b>302</b>	Nucleoside-specific channel-forming protein tsx	TSX_ECO57	107	6/7	26	Outer membrane
<b>305</b>	Nucleoside-specific channel-forming protein tsx	TSX_ECO57	137	11/23	42	Outer membrane
<b>306</b>	MltA-interacting protein	MIPA_ECOLI	112	8/13	47	Outer membrane
<b>307</b>	MltA-interacting protein	MIPA_ECOLI	139	11/18	56	Outer membrane
<b>312</b>	Metalloprotease LoIP	LOIP_ECOLI	125	7/8	34	Outer membrane
<b>308</b>	Outer membrane protein assembly factor BamD	BAMD_ECO57	134	7/7	29	Outer membrane
<b>313</b>	Outer membrane protein assembly factor BamD	BAMD_ECO57	264	22/34	75	Outer membrane
<b>315</b>	Penicillin-binding protein activator LpoB	LPOB_ECO57	81	5/5	29	Outer membrane
<b>318</b>	ABC transporter arginine-binding protein 1	ARTJ_ECOLI	103	7/9	31	Periplasm
<b>324</b>	Probable phospholipid-binding protein MlaC	MLAC_ECOLI	111	7/10	33	Periplasm
<b>327</b>	Quorum-sensing regulator protein G	QSEG_ECO57	119	7/9	24	Outer membrane
<b>333</b>	Outer-membrane lipoprotein LolB	LOLB_ECOL6	59	3/3	18 N-frag.	Outer membrane
<b>341</b>	LPS-assembly lipoprotein LptE	LPTE_ECO57	88	5/6	24	Outer membrane
<b>364</b>	Uncharacterized lipoprotein YajG	YAJG_ECOL6	140	9/12	30	Inner membrane *
<b>365</b>	Outer membrane protein slp	SLP_ECOLI	63	3/3	26	Outer membrane
<b>369</b>	Uncharacterized protein YraP	YRAP_ECO57	193	15/24	62	Periplasm

a) Accession number in UniProtKB.

**Table S2: Bacterial strains used in this study**

Strain name	Plasmid presence	Reference
BL21(DE3) $\Delta ompA$	none	[1]
BL21(DE3) $\Delta ompA$ (pET)	pET21b	[1]
BL21(DE3) $\Delta ompA$ (pET21_Nm-fHbp)	pET21_Nm-fHbp	This study
BL21(DE3) $\Delta ompA$ (pET21_NHBA)	pET21_NHBA	This study
BL21(DE3) $\Delta ompA$ (pET21_Nm-( $\Delta$ Gly)fHbp)	pET21_Nm-( $\Delta$ Gly)fHbp	This study
BL21(DE3) $\Delta ompA$ (pET21_Nm-(Ala <sub>4</sub> )fHbp)	pET21_Nm-(Ala <sub>4</sub> )fHbp	This study
BL21(DE3) $\Delta ompA$ (pET21_Nm-fHbpDomA)	pET21_Nm-fHbpDomA	This study
BL21(DE3) $\Delta ompA$ (pET21_Nm-fHbpDomAB)	pET21_Nm-fHbpDomAB	This study
BL21(DE3) $\Delta ompA$ (pET21_Nm-fHbp_C>A)	pET21_Nm-fHbp_C>A	This study
BL21(DE3) $\Delta ompA$ (pET21_NHBA_C>A)	pET21_NHBA_C>A	This study
BL21(DE3) $\Delta ompA$ (pET21_Ag-fHbp)	pET21_Ag-fHbp	This study
BL21(DE3) $\Delta ompA$ (pET21_Nm-fHbp-vIII)	pET21_Nm-fHbp-vIII	This study
BL21(DE3) $\Delta ompA$ (pET21_NHBA-vIII)	pET21_NHBA-vIII	This study
BL21(DE3) $\Delta ompA$ (pET21_Nm-fHbpDomA-MUC1)	pET21_Nm-fHbpDomA-MUC1	This study
BL21(DE3)-C43	None	[2, 3]
BL21(DE3)-C43(pET)	pET21b	This study
BL21(DE3)-C43(pET21_Nm-fHbp)	pET21_Nm-fHbp	This study
BL21(DE3) WT	None	[4]
BL21(DE3) WT(pET)	pET21b	This study
BL21(DE3) WT(pET21_Nm-fHbp)	pET21_Nm-fHbp	This study
BL21(DE3) WT(pET21_NHBA)	pET21_NHBA	This study
BL21(DE3) $\Delta ompA$ (pACYC_slam1, pET21_Nm-fHbp)	pACYC_slam1 and pET21_Nm-fHbp	This study
BL21(DE3) WT(pACYC_slam1, pET21_Nm-fHbp)	pACYC_slam1 and pET21_Nm-fHbp	This study
BL21(DE3)-C43(pACYC_slam1, pET21_Nm-fHbp)	pACYC_slam1 and pET21_Nm-fHbp	This study

BL21(DE3) $\Delta ompA$ (pACYC pET21_Nm-fHbp)	pACYC and pET21_Nm-fHbp	This study
BL21(DE3) WT(pACYC, pET21_Nm-fHbp)	pACYC and pET21_Nm-fHbp	This study
BL21(DE3)-C43(pACYC, pET21_Nm-fHbp)	pACYC and pET21_Nm-fHbp	This study
BL21(DE3) $\Delta ompA$ (pACYC_slam1 pET21_NHBA)	pACYC_slam1 and pET21_Nm-NHBA	This study
BL21(DE3) $\Delta ompA$ (pACYC pET21_NHBA)	pACYC and pET21_Nm-NHBA	This study

**Table S3 - Oligonucleotides used in this study**

Name	Sequence
fHbp-F	GGAGATATACATATGGTGAATCGAACTGCCTTCTGCTGCC
fHbp-R	GTGATGGTGATGTTATTATTGCTTGGCGGCAAGGC
NHBA-F	GGAGATATACATATGTTTAAACGCAGCGTAATC
NHBA-R	GTGATGGTGATGTTATCAATCCTGCTCTTTTTTG
pet-R	CATATGTATATCTCCTTCTTAAAGTTAAAC
pET-ΔHIS-F	TAACATCACCATCACCATCACGATTACAAAGA
fHbpΔgly-F	AGCAGCGTCGCCGCCGACATCGGTG
fHbpΔgly-R	GGCGACGCTGCTGCAGGCCGGTCAG
fHbpGly>ALA-F	GCGGCCGCGGCCGTCGCCGCCGACATCGGTG
fHbpGly>ALA-R	GGCCGCGGCCGCGCTGCTGCAGGCCGGTCAG
fHbpΔdomB/domC-R	GTGATGGTGATGTTACACCTCTCAAGGTTTCAATATGTTTGTT
fHbpΔdomC-R	GTGATGGTGATGTTATCCGCCGGCATCGTCTG
NHBA-C>A-R	GGCAAAAATACAAGCCATTGCGATTACGCTGCG
NHBAC>A-F	GCTTGTATTTTTGCCCTTTCAGCCgcCGGGGGC
fHbpC>A-R	TATCAGGGCAGTGGTCAGAGAAAGGCAGCAGAA
fHbpC>A-F	ACCACTGCCCTGATTCTGACCGCCGCC AGCAGC
pET2-R	CATATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTC
pETHIS-F	CATCACCATCACCATCACTAAGATTACAAAGACGATGATGACAAGtga
AgfHbp-F	GAAGGAGATATACAT ATG GTT TAC CCT GTT ATA ACG
AgfHbp-R	ATGGTGATGGTGATGTTCTTT TTTACCTGCCAA ACC
Nohisflag	TAACATCACCATCACCATCACGATTACAAAGA
fHbp-R2	TTATTGCTTGGCGGCAAGGC
vIII-R	GTGATGGTGATGTTATTAGCCGGAATGGTCGGTAACCAC
vIII-F	CTTGCCGCCAAGCAAGGTTCCCTGGAAGAAAAGAAGGG
NHBA-VIII-F1	CCGGCAAAAAGAGCAGGAT ggttccctggaagaaaagaaggg

NHBA-VIII-R1	GTGATGGTGATGTTA gccggaatggtcgtaaccac
NHBA-VIII-R	ATCCTGCTCTTTTTTGCCGG
NHBA-VIII-F	TAACATCACCATCACCATCACGATTACAAAGA
R-MUCFH	GTGATGGTGATGGTGATGTTAATGCGCCGGCGGAGC
F-MUCDomA	CAAGTATACAAACAAGGGGTGACGAGCGCG
FHBP-F1	TAACATCACCATCACCATCACGATTACAAAGA
FHBPDA-R	TTGTTTGTATACTTGGA ACTCTCCACTCTC
F1-MUC	GGGGTGACGAGCGCGCCAGATACACGTCCGGCTCCT
R1-MUC	ATGCGCCGGCGGGGCCGTGAGCCAGGAGCCGGACG
F2-MUC	CCGCCGGCGCATGGAGTAACGTCAGCACCCAGACACGCGCCCG
R2-MUC	GTGGGCAGGGGAGCGGTGGATCCCGGTGCCGGGCGCGTGTC
F3-MUC	CCCCCTGCCACGGTGTACTAGTGCGCCCGATACCCGTCCA
R3-MUC	ATGCGCCGGCGGCGCGGTGGAGCCCGGTGCTGGACGGGTATC
F4-MUC	CCGCCGGCGCATGGAGTCACGTCAGCACCCGGACACTCGTCCA
R4-MUC	GTGTGCTGGAGGCGCGGTTGAACCCGGGGCTGGACGAGTGTC
F5-MUC	CCTCCAGCACACGGCGTCACCTCAGCTCCAGATACGCGCCCG
R5-MUC	ATGTGCCGGCGGAGCGGTACTGCCTGGGGCCGGGCGCGTATC
slam-F	ATAAGGAGATATACC ATGGTTATTTTTATTTTTGTGGGAAGAC
slam-R	TGATGGCTGCTGCCTTAGAACGTTTTATTA AACTCGACAAACGCC
pacyc-F	TAAGGCAGCAGCCATCACCATCATCACCAC
pacyc-R	CATGGTATATCTCCTTATTA AAGTTAAAC
pacyc-up1	GGATCTCGACGCTCTCCCT
pacyc-down1	GATTATGCGGCCGTGTACAA

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