

Supplementary Table S3. Proteins differently expressed in stationary phase $\Delta tonB_m$ strain compared to wild-type

gene	protein	function	localization	$\Delta tonB_m/\text{wt}$
Transport and secretion				
PP_0268*	OprQ	porin (OprD family porin)	OM	0.49
PP_0883*	OpdP	porin (OprD family porin)	OM	0.38
PP_0861	PP_0861	outer membrane ferric siderophore receptor	OM	2.38 ⁱ
PP_2817 ^a	MexC	multidrug efflux RND membrane fusion protein	IM	0.05
PP_2818 ^a	MexD	multidrug efflux RND transporter	IM	0.01 ⁱ
PP_2819 ^a	OprJ	outer membrane protein	OM	0.01 ⁱ
PP_3271	PhaK	porin (OprD family porin)	OM	0.11 ⁱ
PP_3390	PP_3390	porin	OM	0.31 ⁱ
PP_4519*	TolC	TolC family type I secretion outer membrane protein	OM	0.32
Signalling and transcription regulation				
PP_3286	PhaN	PaaX family transcriptional regulator	C	3.57 ⁱ
PP_2901	PvdQ	acyl-homoserine lactone acylase	P	0.37 ⁱ
Hypothetical and unknown function				
PP_0765*	PP_0765	hypothetical protein	OM	0.17
PP_2170	PP_2170	chorismate mutase	Unknown	0.18 ⁱ
PP_4978*	PP_4978	hypothetical protein	IM	6.25 ⁱ
PP_5298	PP_5298	glutamine amidotransferase	Unknown	2.39 ⁱ

Gene and protein name in bold indicates that the protein was differentially expressed in ZnSO_4 treated *colR*-deficient strain as well.

^a indicates that the genes are in the same operon.

* indicates that the protein is differently expressed also in exponentially growing $\Delta tonB_m$ strain compared to wild-type.

ⁱ marks that all the expression values of one strain that were used to calculate the $\Delta tonB_m/\text{wt}$ difference were imputed.

Grey values represent ON-OFF differences that are not statistically significant.

Supplementary Table S4. Proteins differently expressed in exponentially growing $\Delta tonB_m$ strain compared to wild-type

gene	protein	function	localization	$\Delta tonB_m/\text{wt}$
Transport and secretion				
PP_0101 ^U	PP_0101	sulfate transporter	IM	26.23 ⁱ
PP_0268	OprQ	porin	OM	0.44
PP_0504	OprG	OmpW family protein	OM	0.32
PP_0773 ^U	PP_0773	OmpA/MotB domain-containing protein	OM	3.91
PP_0882 ^a	DppA	peptide ABC transporter substrate-binding protein	P	0.46
PP_0883 ^a	PP_0883	porin	OM	0.41
PP_1141 ^U	BraC	ABC transporter substrate-binding protein	P	0.48
PP_1378	PcaT	metabolite/H ⁺ symporter	IM	+/- ^o
PP_1400	KgtP	metabolite/H ⁺ symporter	IM	0.21
PP_1445	OprB-II	carbohydrate-selective porin	OM	0.41 ⁱ
PP_2659	PstB1	phosphate ABC transporter ATP-binding protein	IM	4.65 ⁱ
PP_3088 ^b	TssA1	K1-T6SS core component	C	3.28
PP_3089 ^b	Hcp1	K1-T6SS core component	Extracellular	2.41
PP_3091 ^b	TssM1	K1-T6SS core component	IM	16.27
PP_3093 ^b	TssK1	K1-T6SS core component	C	7.02
PP_3092 ^b	TssL	K1-T6SS core component	C	8.89
PP_3094 ^b	TssJ1	K1-T6SS core component	IM	2.86
PP_3095 ^b	ClpV1	K1-T6SS core component, ATPase	C	5.04
PP_3097 ^{Ub}	TssF1	K1-T6SS core component	C	6.07
PP_3099 ^b	TssC1	K1-T6SS core component	C	4.89
PP_3100 ^b	TssB1	K1-T6SS core component	C	5.18
PP_3155	PP_3155	ferric siderophore receptor	OM	0.26
PP_4217	FpvA	ferripyoverdine receptor	OM	0.27
PP_4519	TolC	TolC family type I secretion outer membrane protein	OM	0.50
PP_4613	FecA	iron(III) dicitrate receptor	OM	0.34
PP_4735 ^c	LctP	L-lactate transport	IM	0.46
PP_4838	OprC	TonB-dependent copper receptor	OM	0.47
PP_4864	BraF	amino acid ABC transporter ATP-binding protein	IM	6.69 ⁱ
PP_5329	PstS	phosphate ABC transporter substrate-binding protein	IM	3.52
Stress and defence response				
PP_0089 ^U	OsmC	OsmC family protein	C	3.52 ⁱ
PP_0235	LsfA	peroxidase	C	3.30
PP_0946 ^d	SodA	superoxide dismutase	P	12.84
PP_1277 ^U	AlgA	mannose-1-phosphate guanyllyltransferase	C	6.93 ⁱ
PP_1286 ^{Ue}	Alg44	long-chain fatty acid-CoA ligase	P	2.94 ⁱ
PP_1288 ^{Ue}	AlgD	GDP-mannose 6-dehydrogenase	C	26.32 ⁱ
PP_1502 ^f	LptF	OmpA/MotB domain-containing protein	OM	7.14
PP_1749 ^{Ug}	PP_1749	acetyltransferase	C	2.89 ⁱ
PP_3822	PP_3822	cytochrome c family protein	P	0.15
PP_4812 ^U	PP_4812	3-methyladenine DNA glycosylase	C	2.46
PP_4855 ^U	OsmE	Osmotically-inducible lipoprotein	OM	4.80 ⁱ
PP_5151	CarP	SdiA-regulated domain-containing protein	C	15.78 ⁱ
PP_5288 ^U	AlgC	phosphomannomutase	C	2.86
Cell envelope homeostasis				
PP_3135	WapB	1,2-glucosyltransferase	C	6.41

PP_4032^u	PP_4032	outer membrane lipoprotein Blc	OM	3.41
PP_5037^h	PP_5037	outer membrane lipoprotein Blc	OM	6.40
Signalling and transcription regulation				
PP_0185 ^u	PprA	LytTR family two component transcriptional regulator	C	5.32
PP_0216^u	PP_0216	PAS/PAC sensor containing diguanylate cyclase	C	5.22 ⁱ
PP_0769	PP_0769	histidine kinase	C	0.47
PP_1652	PfeS-II	histidine kinase	IM	2.91 ⁱ
PP_2347	CarR	two component transcriptional regulator	C	4.38
PP_2900	PP_2900	probable Fur like regulator	C	2.65
PP_3631^u	HtrG	putative signal transduction protein	Unknown	7.10
PP_4224	PP_4224	membrane sensor signal transduction histidine kinase	IM	10.24 ⁱ
PP_4470^u	AmrZ	alginate biosynthesis regulator AlgZ	C	5.83
PP_5320	PhoB	transcriptional regulator	C	7.01
Amino acid and protein metabolism				
PP_0675	GdhA	glutamate dehydrogenase	Unknown	2.20
PP_0988 ^u	GcvP1	glycine dehydrogenase	C	0.28
PP_1750^{ug}	AsnB	asparagine synthetase	C	3.25 ⁱ
PP_3664	PssA-1	phosphatidylserine synthase	C	2.12
PP_3796	PvdA	L-ornithine 5-oxygenase PvdA	C	4.77
PP_4209	PvdR	ABC transporter permease	IM	4.84 ⁱ
PP_4213	PvdM	dipeptidase	Unknown	2.40
PP_4216	PvdE	cyclic peptide transporter	IM	6.75
PP_4218ⁱ	PP_4218	lipase/esterase	C	2.82
PP_4219ⁱ	PvdD	non-ribosomal peptide synthetase	Unknown	4.33
PP_4220ⁱ	PvdJ	non-ribosomal peptide synthetase	Unknown	3.09
PP_4221ⁱ	PvdI	non-ribosomal peptide synthetase	Unknown	6.24
PP_4222	SyrP	aspartyl hydroxylase	C	5.68
PP_4223	PvdH	diaminobutyrate-2-oxoglutarate aminotransferase	C	4.82
PP_4243	PvdL	peptide synthase PvdL	Unknown	5.50
PP_4245	PvdY	siderophore biosynthesis protein	C	4.69
PP_4621	HmgA	homogentisate 1,2-dioxygenase	Unknown	2.04
Metabolism				
PP_0100^u	CynT	carbonate dehydratase	C	5.33
PP_0236 ^k	SsuE	NAD(P)H-dependent FMN reductase	Unknown	2.64 ⁱ
PP_0238 ^k	SsuD	alkanesulfonate monooxygenase	C	12.49 ⁱ
PP_0543	EutB	ethanolamine ammonia-lyase subunit alpha	C	3.50
PP_0944^d	FumC-1	fumarate hydratase	C	18.60
PP_1755	FumC-2	fumarate hydratase	C	2.50
PP_2136^u	FadB	multifunctional fatty acid oxidation complex subunit	C	2.71
PP_2137	FadA	3-ketoacyl-CoA thiolase	C	2.56
PP_2168 ^u	Tal	transaldolase B	Unknown	2.073
PP_2437	PP_2437	acyl-CoA dehydrogenase	C	10.36 ⁱ
PP_3443	PP_3443	glyceraldehyde-3-phosphate dehydrogenase	C	2.14
PP_4043	GntZ	6-phosphogluconate dehydrogenase	C	2.16
PP_4058	GlgB	glycogen branching protein	C	2.01
PP_4060	GlgE	alpha-amylase	C	4.53
PP_4264	HemN	coproporphyrinogen III oxidase	C	0.24
PP_4736 ⁱ	LldD	L-lactate dehydrogenase	C	0.39
PP_4737 ⁱ	Dld2	D-lactate dehydrogenase	C	0.12
PP_4870	PP_4870	azurin	P	0.36

PP_5041	GlgP	glycogen/starch/alpha-glucan phosphorylase	C	2.84
PP_5210	PP_5210	zinc-containing alcohol dehydrogenase	C	6.60 ⁱ
PP_5222	CyaA	adenylate cyclase	C	2.61 ⁱ
PP_5365	PP_5365	cyclopropane-fatty-acyl-phospholipid synthase	C	2.55
Chemotaxis and motility				
PP_1087	MotY	Flagellar protein MotY precursor signature	OM	0.50
PP_4377	FlaG	flagellin	Unknown	0.44
Hypothetical and unknown function				
PP_0052	PP_0052	beta-lactamase domain-containing protein	C	3.21
PP_0085	PP_0085	hypothetical protein	Unknown	11.86 ⁱ
PP_0128	PP_0128	hypothetical protein	C	2.26 ⁱ
PP_0255	PP_0255	hypothetical protein	IM	8.40
PP_0541	PP_0541	acetyltransferase	C	2.35
PP_0765	PP_0765	hypothetical protein	OM	0.31
PP_0837^u	YcfJ	hypothetical protein	OM	5.42
PP_0886	PP_0886	hypothetical protein	P	3.38
PP_0903^u	PP_0903	type 11 methyltransferase	C	11.37 ⁱ
PP_0945 ^d	PP_0945	hypothetical protein	C	18.79
PP_1291	PP_1291	PhoH family protein	C	4.07
PP_1478 ^u	PP_1478	NADH:flavin oxidoreductase	C	2.27
PP_1487 ^u	PP_1487	hypothetical protein	IM	2.43
PP_1503^{uf}	PP_1503	hypothetical protein	Unknown	6.10
PP_1659	PP_1659	hypothetical protein	P	0.29
PP_1838	PP_1838	hypothetical protein	IM	2.26
PP_2059	PP_2059	CsbD family protein	Unknown	12.59 ⁱ
PP_2103	PP_2103	hypothetical protein	Unknown	2.22 ⁱ
PP_2132	PP_2132	universal stress protein	C	2.23
PP_2172 ^u	PP_2172	hypothetical protein	Extracellular	2.21
PP_2306 ^{Um}	PP_2306	lipoprotein	Unknown	2.66
PP_2307 ^m	PP_2307	CHAD domain-containing protein	C	2.85
PP_2356	PP_2356	phytochrome family protein	IM	2.12
PP_2891	PP_2891	acetyltransferase	IM	3.12 ⁱ
PP_3824	PP_3824	hypothetical protein	Unknown	3.25
PP_3930	PP_3930	hypothetical protein	IM	4.05
PP_4920^u	PP_4920	lipoprotein	Unknown	3.14
PP_4978	PP_4978	hypothetical protein	IM	8.93
PP_5038^{uh}	PP_5038	hypothetical protein	Unknown	53.06 ⁱ
PP_5252	PP_5252	amidohydrolase	Unknown	2.01

Gene and protein name in bold indicates that the protein was differentially expressed in ZnSO₄ treated *co/R*-deficient strain as well.

^{a-m} indicates that the genes are in the same operon.

^u shows that the gene belongs to AlgU regulon.

ⁱ marks that all the expression values of one strain that were used to calculate the $\Delta tonB_m/wt$ difference were imputed.

Grey values represent ON-OFF differences that are not statistically significant.

^o +/- indicates that protein was detected in $\Delta tonB_m$ but not in wt. $\Delta tonB_m/wt$ ratio is not presented because imputation of missing values for wild-type resulted in higher values than determined for $\Delta tonB_m$.

Supplementary Table S5. Oligonucleotides

Name	Sequence (5'-3') ^a	Use
4994Bam	aaggat <u>cc</u> ggaaaccctgacc	construction of pEMG/ $\Delta tonB_m$
4994pikk	agtactggacaacggcccaggatccagcaaccgca	construction of pEMG/ $\Delta tonB_m$
4994del	ggaccgttgc <u>c</u> act	construction of pEMG/ $\Delta tonB_m$
4994Eco	aaga <u>attc</u> agccaggcgttgtcg	construction of pEMG/ $\Delta tonB_m$
1898Xba	cgt <u>ct</u> agaggc <u>at</u> atgaccgttcc	construction of pEMG/ $\Delta pocA$ and pEMG/ $\Delta pocAB$
pocAuus	gcagcat <u>at</u> ccaaccacc	construction of pEMG/ $\Delta pocA$ and pEMG/ $\Delta pocAB$
pocA-pikk-uus	ggtgg <u>ttgg</u> atgat <u>ctgc</u> cat <u>caag</u> ctggtaagt	construction of pEMG/ $\Delta pocA$
1899Eco	cc <u>gaattc</u> gc <u>ac</u> aggat <u>cagg</u> t <u>ct</u> ag	construction of pEMG/ $\Delta pocA$
1898Xba	cgt <u>ct</u> agagg <u>c</u> at <u>at</u> gaccgttcc	construction of pEMG/ $\Delta pocB$
1899algus	Aactt <u>cacgc</u> c <u>ttgc</u> ctcc	construction of pEMG/ $\Delta pocB$
1899stop_pikk	ggagg <u>caagg</u> cg <u>tg</u> ga <u>agg</u> tt <u>agg</u> gc <u>acgc</u> ctt <u>gtgg</u> ct	construction of pEMG/ $\Delta pocB$
1899Eco	cc <u>gaattc</u> gc <u>ac</u> aggat <u>cagg</u> t <u>ct</u> ag	construction of pEMG/ $\Delta pocB$ and pEMG/ $\Delta pocAB$
pocBstop-uus	ggtgg <u>ttgg</u> atgat <u>ctgc</u> agg <u>gcacgc</u> ctt <u>gtgg</u> ct	construction of pEMG/ $\Delta pocAB$
XbaflhF	att <u>ctag</u> a <u>ag</u> ct <u>gac</u> ca <u>ca</u> g <u>ag</u> tc	construction of pEMG/ $\Delta flhF$
flhFpikk	gat <u>atgc</u> gt <u>cagg</u> cc <u>atgt</u> cg <u>ctgt</u> at <u>cttatca</u>	construction of pEMG/ $\Delta flhF$
flhFAlgus	Cat <u>ggcc</u> ct <u>gac</u> gc <u>at</u> at <u>tc</u>	construction of pEMG/ $\Delta flhF$
flhFEcoRI	at <u>gaattc</u> gg <u>ccac</u> ga <u>ag</u> gg <u>at</u>	construction of pEMG/ $\Delta flhF$
del_CDJEco	agg <u>aa</u> tt <u>cc</u> ca <u>gata</u> aa <u>acc</u> cg <u>agg</u> t	construction of pEMG/ $\Delta mexCD$ - <i>oprJ</i>
del_CDJ_pikk	ggagg <u>cttt</u> c <u>ag</u> gc <u>cc</u> tt <u>at</u> tc <u>gaac</u> ct <u>gttt</u> tg	construction of pEMG/ $\Delta mexCD$ - <i>oprJ</i>
del_CDJ	aagg <u>gg</u> ct <u>aa</u> ac <u>ag</u> gc <u>ct</u> cc	construction of pEMG/ $\Delta mexCD$ - <i>oprJ</i>
del_CDJXba	att <u>ct</u> ag <u>aa</u> ag <u>ct</u> at <u>tttt</u> gg <u>acc</u> agg	construction of pEMG/ $\Delta mexCD$ - <i>oprJ</i>
2820Eco	a <u>agaattc</u> g <u>acgg</u> tt <u>cg</u> ct <u>cg</u> ta	construction of pEMG/ $\Delta nfxB$
2820del	tg <u>caa</u> ac <u>cg</u> tt <u>gg</u> ag <u>ct</u> gt <u>ct</u> tt	construction of pEMG/ $\Delta nfxB$
2820pikk	a <u>agc</u> g <u>at</u> cc <u>c</u> ag <u>gtt</u> g <u>cat</u> ca <u>ag</u> at <u>cc</u> ac <u>ac</u>	construction of pEMG/ $\Delta nfxB$
2820Sac	a <u>agag</u> ct <u>ca</u> agg <u>gg</u> ct <u>cg</u> acc <u>aga</u> ga	construction of pEMG/ $\Delta nfxB$
oprB-1ees	cagg <u>gt</u> acc <u>at</u> tc <u>gg</u> cc <u>at</u> gt <u>cg</u> act <u>ct</u>	verification of <i>oprB1</i> deficiency
oprB-1lopp	t <u>gg</u> ct <u>tag</u> ag <u>ct</u> tt <u>gtt</u> g <u>tt</u> g <u>ag</u> at	verification of <i>oprB1</i> deficiency
prtac	a <u>atta</u> at <u>cat</u> tc <u>gg</u> ct <u>cg</u> t <u>ata</u> aa	arbitrary PCR; verification of $P_{\text{tac}}\text{-}tonB_m$
Arb6	gg <u>cc</u> ac <u>cg</u> ct <u>cg</u> act <u>gt</u> ac <u>tg</u> ac <u>nnnnnnnnnnnna</u> cg <u>cc</u>	arbitrary PCR
OEint	A <u>ctt</u> gt <u>gt</u> t <u>ata</u> ag <u>ag</u> gt <u>cg</u> ag	arbitrary PCR
Arb2	gg <u>cc</u> ac <u>cg</u> ct <u>cg</u> act <u>gt</u> ac <u>tg</u> ac <u>tt</u>	arbitrary PCR
4994alg	cc <u>aa</u> g <u>ctt</u> at <u>tc</u> g <u>act</u> g <u>ag</u> gt <u>cg</u> ct <u>gc</u> cc	construction of pBRlacI α / $tonB_m$
4994lopp	t <u>c</u> c <u>ct</u> ta <u>g</u> at <u>ac</u> t <u>cc</u> ac <u>ag</u> gt <u>tt</u> ca <u>gc</u>	construction of pBRlacI α / $tonB_m$
flhFEco	aa <u>agaatt</u> ca <u>ag</u> ca <u>gt</u> c <u>acc</u> at <u>cg</u> tt <u>g</u>	construction of pKTlacI α / $flhF$ - <i>gfp</i>
flhFXho	aa <u>act</u> cg <u>ag</u> ac <u>cc</u> ct <u>cg</u> cc <u>gt</u> gg <u>tt</u> gt	construction of pKTlacI α / $flhF$ - <i>gfp</i>

^a The sites of restriction enzymes used in cloning are underlined.