

1 SUPPLEMENTAL FILES FOR:

2 Putative horizontally acquired genes, highly transcribed during *Yersinia pestis* flea
3 infection, are induced by hyperosmotic stress and function in aromatic amino acid
4 metabolism

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22 Running title: Putative horizontally acquired genes of *Y. pestis*

23
24 Key words: *Yersinia pestis*, flea infection, hyperosmolarity stress

25 **TABLE S1 Bacterial strains and plasmids used in this study**

26 -----

27	Strain or plasmid	Genotype or description	Reference or source
28	-----		
29	Strains		
30	<u><i>Y. pestis</i></u>		
31	KIM6+	Pgm+, pCD-, pMT1+, pPst+, parental strain	(1)
32	VV150	KIM6+ derivative, $\Delta y3550$	This study
33	VV151	KIM6+ derivative, $\Delta y3555$	This study
34	VV278	KIM6+ derivative, $\Delta y3550\Delta y3551$	This study
35	VV279	KIM6+ derivative, $\Delta y3555\Delta y3550$	This study
36	VV280	KIM6+ derivative,	
37		$\Delta y3555\Delta y3554\Delta y3553\Delta y355x\Delta y3551\Delta y3550$	
38		('All' mutant)	This study
39	VV267	KIM6+ derivative, $\Delta y3555\Delta y3554\Delta y3553$	(2)
40	VV670	KIM6+ derivative, WT <i>glmS-pstS::kan^R</i>	This study
41			
42	<u><i>E. coli</i></u>		
43	DH5 α	laboratory cloning strain	Invitrogen
44	DG44	$\Delta tyrB \Delta aspC$	CGSC Strain#: 5802
45	DM12740	BL21A1 contains pET20b with <i>S. enterica ridA</i>	(3)
46	DM16279	BL21AI contains pET28a:: <i>y3551</i>	This study
47			
48	<u><i>Salmonella enterica</i> serovar Typhimurium LT2</u>		
49	DM15847	wildtype contains pBAD24 (empty vector)	Downs lab collection
50	DM16509	DM15847 contains pTRc99A	This study

51	DM16510	DM15847 contains pTRc55	This study
52	DM16150	DM15847 <i>aspC789::Km</i>	Downs lab collection
53	DM16434	DM16150 contains pTRc99A (empty vector)	This study
54	DM16435	DM16150 contains pTRc55	This study
55	DM14828	<i>S. enterica</i> wildtype	Downs lab collection
56	DM16281	DM14828 pET28a:: <i>y3550</i>	This study
57	DM16282	DM14828 pET28a:: <i>y3551</i>	This study
58	DM14829	DM14828 derived <i>ridA1::Tn10(Tc)</i>	(4)
59	DM16283	DM14829 contains pET28a:: <i>y3550</i>	This study
60	DM16284	DM14829 contains pET28a:: <i>y3551</i>	This study
61	DM16293	DM14829 contains pET28b (empty vector)	This study
62	DM16294	DM14829 contains pET28b:: <i>ridA</i> (K12)	This study
63			
64	Plasmids		
65	pC26-pac	pSC101 derivative contains promoterless	
66		<i>luxCDABE</i> reporter, Km ^R	(5)
67	pCS:: <i>P55</i>	pCS26-pac derivative containing <i>55-lux</i>	
68		transcriptional fusion carrying nucleotides	
69		-881/+153 of <i>y3555</i> gene, Km ^R	This study
70	pCS:: <i>P54</i>	pCS26-pac derivative containing <i>54-lux</i>	
71		transcriptional fusion carrying nucleotides	
72		-428/+130 of <i>y3554</i> gene, Km ^R	This study
73	pCS:: <i>P53</i>	pCS26-pac derivative containing <i>53-lux</i>	
74		transcriptional fusion carrying nucleotides	
75		-445/+116 of <i>y3553</i> gene, Km ^R	This study
76	pCS:: <i>P55-54</i>	pCS26-pac derivative containing	

77		transcriptional fusion carrying nucleotides	
78		-881/+1355 of <i>y3555</i> gene, Km ^R	This study
79	pCS::P5x	pCS26-pac derivative containing	
80		transcriptional fusion carrying nucleotides	
81		-915/+54 of <i>y3551</i> gene, Km ^R	This study
82	pCS::P50	pCS26-pac derivative containing	
83		transcriptional fusion carrying nucleotides	
84		-403/+135 of <i>y3550</i> gene, Km ^R	This study
85	pCS::P5x-51-50	pCS26-pac derivative containing	
86		transcriptional fusion carrying nucleotides	
87		-915/+800 of <i>y3551</i> gene, Km ^R	This study
88	pKOBEG	λRed and <i>sacB</i> plasmid for mutagenesis,	
89		Cm ^R	(6)
90	pKD4	pANTsg derivative template plasmid	
91		containing the kanamycin cassette for λRed	
92		recombination, Ap ^R	(7)
93	pTRc99A	IPTG-inducible strong hybrid <i>trp/lac</i> promoter	
94		containing plasmid, Ap ^R	(8)
95	pCR4-TOPO	Cloning vector, Km ^R , Ap ^R	Invitrogen
96	pCR4::3550	pCR4-TOPO derivative expressing	
97		<i>y3550</i> , Km ^R , Ap ^R	This study
98	pCR4::3555	pCR4-TOPO derivative expressing	
99		<i>y3555</i> , Km ^R , Ap ^R	This study
100	pUC18R6KminiTn7T	pUC18 derivative plasmid enabling	
101		insertion of genes into <i>attTn7</i>	
102		transposon insertion site located between	

103		the <i>glmS</i> and <i>pstS</i> genes in the	
104		chromosome, Ap ^R	(9)
105	E5049:: <i>km</i> ^R	pUC18R6KminiTn7T plasmid with	
106		kanamycin resistance cassette cloned	
107		in between transposition mediating sites	This study
108	pTRc50	pTRc99A derivative contains <i>y3550</i> gene	This study
109	pTRc51	pTRc99A derivative contains <i>y3551</i> gene	This study
110	pTRc55	pTRc99A derivative contains <i>y3555</i> gene	This study
111	pTRc50_55	pTRc99A derivative contains <i>y3550</i> and <i>y3555</i>	This study
112	pTRc51_55	pTRc99A derivative contains <i>y3551</i> and <i>y3555</i>	This study
113			
114	pET28a	Protein Expression vector allowing 6xHis	
115		tagging of a gene	EMD Biosciences
116	pET28a:: <i>y3551</i>	pET28a derivative contains <i>y3551</i> gene	This study
117	pET28a:: <i>y3550</i>	pET28a derivative contains <i>y3550</i> gene	This study
118	pET28a:: <i>y3555</i>	pET28a derivative contains <i>y3555</i> gene	This study
119	pFLP3	Source of Flp recombinase, Tc ^R , Ap ^R	(9)

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121 The coordinates for the *lux* fusions are indicated with respect to the translational start codon for each
122 gene. Kan^R, kanamycin resistance; Ap^R, ampicillin resistance; Tc^R, tetracycline resistance.

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124 **TABLE S2 Oligonucleotides used in this study**

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126	Primer	Sequence (5'-3')	RE
127	-----		
128			
129	<u>For lux fusions</u>		
130	50R-BHI	TAAGGATCCATTGGCATTCCATGCCAC	BamHI
131	50F-XhoI	TAACTCGAGTTTATGCTGATTATTTTAAAGGAAC	XhoI
132	5xR-BHI	GAGGGATCCGGCCAATAGCGGAAGGTGC	BamHI
133	5xF-XhoI	AGGCTCGAGGAACTTTTATACAATGGAACGTG	XhoI
134	53R-BHI	AACGGATCCAGGATGATAATCTGAAAATTC	BamHI
135	53F-XhoI	TGCCTCGAGCTGTAGTAGTCGACTGGCTC	XhoI
136	54R-BHI	AGCGGATCCAATGCGGTCATGTCAAAG	BamHI
137	54F-XhoI	AATCTCGAGCGTTCTCTTTGGCTGTACCG	XhoI
138	55R-BHI	CGGGGATCCAAGGATCTGCCCAGCACAAC	BamHI
139	55F-XhoI	GAACTCGAGTGATTCATGATTAACCGAAATGC	XhoI
140			
141	<u>For RT-PCR analysis</u>		
142	5051RTR (B _{rt})	CATCAGCAGGCGTGGCCC	
143	Y3555Set2Fw (A _{rt})	AAATACCTCTTCTCAGGCGGGCAT	
144			
145	<u>Gene deletion</u>		
146	3550-H1P1	CAATAATGGAGAGTTATCATGAATAGACGTGTAATCAAT	
147		<u>CCCTGTGTAGGCTGGAGCTGCTTCG</u>	
148	3550-H2P2	CTACTCTTCATTTTATTATCAGCCCAAGCAAGCAGTCGC	
149		<u>TTCCATATGAATATCCTCCTTAGTTC</u>	
150	3555-H1P1	TTATTAAAGGGGAATACCATGAATACGATTAGAAGAAAA	

151 ATGTGTGTAGGCTGGAGCTGCTTCG

152 3555-H2P2 CGCTAACTGCATTAATGCCTGTTGGATTTCGAGTCAAAC

153 TGCCATATGAATATCCTCCTTAGTTC

154 3551-H1P1 TAAAATAGAGGATATATCATGAAGTCAGTCATTGAAACA

155 AAATGTAGGCTGGAGCTGCTTC

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157 For cloning

158 p270 ACGGATCCATGAATAGACGTGTAATCAATCCCG

159 p271 CACTCGAGTCAGCCCAAGCAAGCAGTCGC

160 p272 ACGGATCCATGAAGTCAGTCATTGAAACAAAAAATGC

161 p273 CACTCGAGTCAAACCCCAGCAATAACTTCTATTTTC¹

162 p274 ACGGATCCATGAATACGATTAGAAGAAAAATGCGTC

163 p275 CACTCGAGTTATCCATGGCAGATCCCCTTTTCC

164 p508 CGATAAGCTTGATTTAGTAAGCCTGTGATTTGC

165 p509 TGTGAAGCTTATCCATGGCAGATCCCCTTTTCC

166 p395/pKD4KanF ATTAGGATCCGAACACGTAGAAAGCCAGTCC

167 p396/pKD4KanR CAGCGAATTCTCAGAAGAACTCGTCAAGAAGGC

168 pstSup2 GCTATACGTGTTTGCTGATCAAGATGC (9)

169 pTn7R CACAGCATAACTGGACTGATTTTC (9)

170 3550-R CGC CCA TTT AAT GAT GAG TGC

171 3550-F TCC CAC TGC TGA TTA GTA TCG

172 3555-R CAA CAC CGG ACT GAG CGA TC

173 3555-F ATT TTG TGT CAA CTG ACT GAT TG

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175 For qPCR

176 y3550Set1Fw GCATGGAATGCCAATGGCGAGTTA

177	y3550Set1Rev	TAGACGTACAACATCAGCAGGCGT
178	y3551Set1Fw	TGCTTCAGGGCAGATACCGATCAA
179	y3551Set1Rev	GTTCCAGCCTGTAGAAGAACAGCA
180	y3553Set1Fw	TCATCCTGGTGCTGTTTCGTAGT
181	y3553Set1Rev	TCCATCAGACATTTCAACTCGCCG
182	y3554Set3Fw	GCATCATTGGTATTCTGCGTGCCA
183	y3554Set3Rev	CGCCAACTAATGCGGTTGCGATAA
184	y3555Set2Fw	AAATACCTCTTCTCAGGCGGGCAT
185	y3555Set2Rev	ATGAGAGGAACCCACTTTCAGGCA
186	gyrBFw	TCGCCGTGAAGGTAAAGTTC
187	gyrBRv	TTTGCTCAGTTTCACCCACC

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Protein %ID

1: Y3555	100.00	20.17	19.88	15.80	18.97
2: Y2760_AspC	20.17	100.00	85.86	41.41	42.42
3: Se_AspC	19.88	85.86	100.00	42.17	42.42
4: Y0579_TyrB	15.80	41.41	42.17	100.00	72.80
5: Se_TyrB	18.97	42.42	42.42	72.80	100.00

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194 Y3555 1 ----MNTIRRKMRPEFQGLQGGFLFSSVKKKADVGTAVLD-----LMANGVDM--L
195 Y2760_AspC 1 MEASDMF-----EKKIT-AAPADPILG-LTDIFRADDRAHK-INLIGIG
196 Y0579_TyrB 1 ----MF-----QNVDA-YAGDPILS-LMSEFKADNRAHK-VNLSIG
197 STM0998_AspC 1 ----MF-----ENIT-AAPADPILG-LADLFRADDRPGK-INLIGIG
198 STM4248_TyrB 1 ----MF-----QKVDAYAGDPILS-LMERFKDDSRHDK-VNLSIG

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201 Y3555 44 CWADPFFPDPVLP--EHTSQAVIKSMEE--NGSASHYTMPICNPPELKEKTIALKLQRYNNLT
202 Y2760_AspC 40 VYKDETGKTPVLT---SVKKAEQYLLE-N-EATKKNYLIGIDGLPVFASCTQELLFGANSAT
203 Y0579_TyrB 35 LYYNEQGEIPQMQ---AVDAEAAQLSTQP-HCTPVYLPMEGLQSYRTAIQQLLFGHDHPM
204 STM0998_AspC 35 VYKDETGKTPVLT---SVKKAEQYLLE-N-ETTKKNYLIGIDGLPEEFARCTQELLFGKGSAL
205 STM4248_TyrB 35 LYYNEDGIIPQLK---IVAEAEARLNAQP-HCASLYLPMEGLINTYRHTIAPLLFGADHPV

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208 Y3555 100 VEAQR-NILITPGSDSGLLFAMPPFIN---NDDEVLIHSPSYPSNFLNVELLGGKPIISVE
209 Y2760_AspC 95 IADKRARTAQTGGTGLLRIAA-DFTIAHQTSAKRVVWSNPSWPNHKNVFEAAGLEVVEYA
210 Y0579_TyrB 91 LVQQRVATIQTVGGSGALKVGA-DFLNHYFPDSQVWVSDPTWENHVAIFSGAGFKVNTYYP
211 STM0998_AspC 90 IADKRARTAQTGGTGLLRIAA-DFLAKNTPVKRVVWSNPSWPNHKSVEFNAAGLEVREYA
212 STM4248_TyrB 91 LVQQRVATIQTVGGSGALKVGA-DFLKRYFPDAGVWVSDPTWENHVAIFAGAGFEVSTYYP

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215 Y3555 156 LKAENNFQDIKDFENKITEK-TKMVILTN--PNNPTGTVLRRESLQAIADFIIA-----
216 Y2760_AspC 154 YYDAANHALDFDGLLNSLSEAQAGDVVLFHGCCHNPTGIDPTETQWSQLAELISVA-----
217 Y0579_TyrB 150 YFDNDKLAVKFDPMLATLQQLPARSIVLLHPCCHNPTGSDLTNAQWDRLIDVVKE-----
218 STM0998_AspC149 YYDAENHTLDFEALQASLSEAQAGDVVLFHGCCHNPTGIDPTLEQWQVLAELISVE-----
219 STM4248_TyrB150 WYDDATNGIRENDLLATLNTLPARSIVLLHPCCHNPTGADLTPSQWDVAIEIVKA-----

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222 Y3555 208 -HDLILVVVDQAFE---DAIFDEIEFISIASIPGMWERTVSVFVSFKMGISGFRVGYLVA
223 Y2760_AspC 209 -KGLVPLFDFAFAYQGFANGLBEDAQG--IRIFAATHQELIVCSSYSKNFGLYNERVGACTL
224 Y0579_TyrB 205 -RELIPFLDIAYQGFAGLNEDAYA--IRAMAAGVLPCLISNSFSKIFISLYNERVGGLSV
225 STM0998_AspC204 -KGLVPLFDFAFAYQGFANGLBEDAEG--IRAFAAHKELVASSYSKNFGLYNERVGACTL
226 STM4248_TyrB205 -RDLIPFLDIAYQGFAGMDDDAYV--IRATASAGLPALVSNFSKIFISLYGERVGGLSV

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229 Y3555 264 ---DAQIVDVLFGCTVNVVVGATNTS---SQAGMIAAI-DEPSFMCEYITQIFER-----
230 Y2760_AspC 266 VAADSNVADTAESQVKAVIRANYSNPPAHGASVVATILSNAALRAIWEQELTDMRORIQR
231 Y0579_TyrB 262 VCESDEAAGRVLGQLKATVRRNYSSPPNFGAQVVSKVINDTVLRAQWQAEVEQMRLRILD
232 STM0998_AspC261 VAADAETVDRAESQMKSAIRANYSNPPAHGASIVATILSNDALRAIWEQELTDMRORIQR
233 STM4248_TyrB262 VCEDAETIARVLGQLKATVRRNIYSSPPCFGAQVVATVVGDEALKAGWLAEVDAMRNRIIS

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236 Y3555 310 RRKVVVFEMNNAIPGVCMVMPESGFLSWIDISKLGTSTFICDYLLQHAHVMVNSGVVPGQG
237 Y2760_AspC 326 MRQLFVNTLQ-----EKGAQQDF-----SFIINQNGMFSFSGLTIKEQV
238 Y0579_TyrB 322 MRHTLVEALK-----ASIPERNE-----DYLLQQRGMFSYTGFSQAQV
239 STM0998_AspC321 MRQLFVNTLQ-----EKGANRDF-----SFIINQNGMFSFSGLTIKQV
240 STM4248_TyrB322 MRQTLVKELK-----AEMPDRNE-----DYLLQQRGMFSYTGFSSEEQV

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243 Y3555      370 GEGYIRLVHGCKDDEKIYAVLT-RIQQALMQLAKEKGI CHG-----
244 Y2760_AspC 364 ----LR-----LRDEFVAVYAVNSGRVNVAGMTPDNMAPLCEA-----
245 Y0579_TyrB 360 ----ES-----LRREFGVYLIASGRMCMAGVNHQNVQVAVA-----
246 STM0998_AspC359 ----LR-----LRREFGVYAVASGRVNVAGMTPDNMAPLCEA-----
247 STM4248_TyrB360 ----DR-----LRREFGVYLIASGRMCMVAGLNASNVHRVAKA-----

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250 Y3555      -----
251 Y2760_AspC 397 IVAVL
252 Y0579_TyrB 393 FAAVQ
253 STM0998_AspC392 IVAVL
254 STM4248_TyrB393 FAAVM

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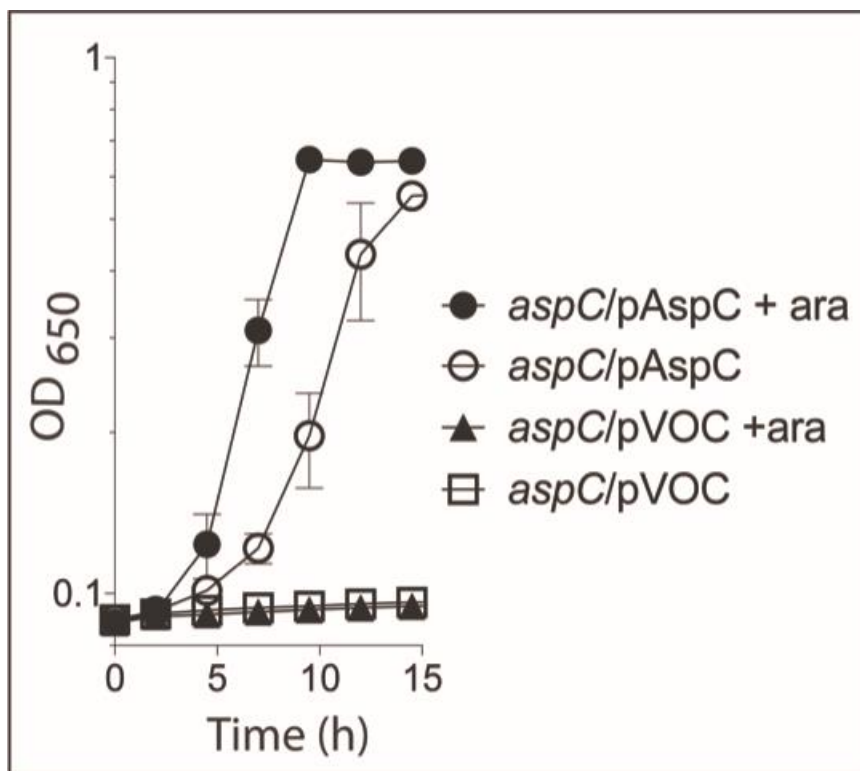
257 **Figure S1. Alignments of the Y3555 and *Y. pestis* KIM6+ and *S. enterica* AspC and**

258 **TyrB.** * denotes the glutamate instead of aspartate that is predicted to coordinate the

259 pyridine nitrogen of PLP in the active site

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Figure S2. *S. enterica aspC* expressed *in trans* complements growth defects of an *S.*

***enterica aspC* mutant** The *S. enterica aspC789::km* mutant (DM16150) with either a

pBAD24 empty vector control (*aspC/pVOC*), or a pBAD24 plasmid expressing *S. enterica*

aspC (*aspC/pAspC*) were grown in minimal glucose (11 mM) medium without, or with 0.2%

arabinose (+ara) as indicated in legend. Error bars represent mean(\pm SEM) from three

biological replicates.

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278 Protein %ID

1: Y3555	100.00	86.59	86.59	86.59	86.83	19.60	19.03	16.43	18.70
2: 07704	86.59	100.00	98.05	98.05	98.29	19.03	18.47	16.71	17.85
3: 072382	86.59	98.05	100.00	100.00	99.76	19.60	18.75	15.86	17.85
4: 093506	86.59	98.05	100.00	100.00	99.76	19.60	18.75	15.86	17.85
5: Se_AspB	86.83	98.29	99.76	99.76	100.00	19.60	18.75	15.86	17.85
6: Y2760_AspC	19.60	19.03	19.60	19.60	19.60	100.00	85.86	41.41	42.42
7: Se_AspC	19.03	18.47	18.75	18.75	18.75	85.86	100.00	42.17	42.42
8: Y0579_TyrB	16.43	16.71	15.86	15.86	15.86	41.41	42.17	100.00	72.80
9: Se_TyrB	18.70	17.85	17.85	17.85	17.85	42.42	42.42	72.80	100.00

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Y3555	1	MNTIRRKMRPEFQGLQGGLFSSVKKADVGTAVL	DLMANGVDM-----LCWADPFFPDP
Y2760_AspC	1	MEASD-----MFEKITT-AAPADPILGLT	DI FRADDRAHKINLIGICVYKDETG
Y0579_TyrB	1	M-----FQNVDA-YAGDPIISLME	SFKADNRAHKVNLISIGLYYNEQG
STM0998_AspC	1	M-----FENIT-AAPADPILGLADL	FRADDRPGKINLIGICVYKDETG
STM4248_TyrB	1	M-----FQKVD-AYAGDPIISLME	RFKD DSRHDKVNLISIGLYYNEGD
07704	1	MSTIRRKMRPVFQGLQGGLFSSVKKADVGTAVL	ELMANGVDM-----LCWADPFFPDS
072382	1	MSTIRRKMRPVFQGLQGGLFSSVKKADVGTAVL	ELMANGVDM-----LCWADPFFPDS
093506	1	MSTIRRKMRPVFQGLQGGLFSSVKKADVGTAVL	ELMANGVDM-----LCWADPFFPDS
Se_AspB	1	MSTIRRKMRPVFQGLQGGLFSSVKKADVGTAVL	ELMANGVDM-----LCWADPFFPDS

Y3555	54	VLPE--HISQAVIKSM--ENG	SASHYTMPIGNPDLKEKIALKLRKNNISVDAQRNIIIT
Y2760_AspC	47	KTPVLTSSVKKAEQYL--ENE	ATKNYLGIDGLPFVFACTQELLFGANSALITADKRRTAQT
Y0579_TyrB	42	EIPQMQAVDAAEAQLSTQ	PHGTPVYLPMEGLQSYRTAQQQLFGHDHPMLVQQRVATIQT
STM0998_AspC	42	KTPVLTSSVKKAEQYL--ENE	TTKNYLGIDGIPFARCTQELLFGKGSALINDKRRTAQT
STM4248_TyrB	42	IIPQLKTVAEAEARLNAQ	PHGASLYLPMEGLNTYRHTIAPLLFGADHPVLQQRVATIQT
07704	54	VLPE--HISQAVIKSM--KNG	NASHYTMPIGNPDLKEKIALKLRKNNISVDAQRNIIIT
072382	54	VLPE--HISQAVIKSM--ENG	NASHYTMPIGNPDLKEKIALKLRKNNISVDAQRNIIIT
093506	54	VLPE--HISQAVIKSM--ENG	NASHYTMPIGNPDLKEKIALKLRKNNISVDAQRNIIIT
Se_AspB	54	VLPE--HISQAVIKSM--ENG	NASHYTMPIGNPDLKEKIALKLRKNNISVDAQRNIIIT

Y3555	110	PGSDSGLLFAMMPFIENN---	DDEVLIHSPSYPSNFLNVELLGGKPIISVELKAENNFOIEI
Y2760_AspC	106	PGGT--GGLRIAADFIAHQ	TSAKRVWVSNPSWPNHKNVFEAAGLEVVEYAYYDAANHALDF
Y0579_TyrB	102	VGGG--GALKVGADEFLNH	YFPDSQVWVSDPTWENHVAIFSGAGFKVNTYPYFDNDKLVKFK
STM0998_AspC	101	PGGT--GALRIAADFIAK	NTPVKRVWVSNPSWPNHKSVFNAAGLEVREYAYYDAENHTLDF
STM4248_TyrB	102	LGGS--GALKVGADEFIK	RYPDAGVWVSDPTWENHIAIFAGAGFEVSTYPWYDITATNGIRF
07704	110	PGSDSGLLFAMMPFIENN---	GDEVLIHSPSYPSNFLNVELLGGTPVSVELKAENNFOIEI
072382	110	PGSDSGLLFAMMPFIENN---	GDEVLIHSPSYPSNFLNVELLGGTPVSVELKVENNFOIEI
093506	110	PGSDSGLLFAMMPFIENN---	GDEVLIHSPSYPSNFLNVELLGGTPVSVELKVENNFOIEI
Se_AspB	110	PGSDSGLLFAMMPFIENN---	GDEVLIHSPSYPSNFLNVELLGGTPVSVELKVENNFOIEI

Y3555	167	KDFENKITEK--TKMVILT	N--PNNPTGTVLRRESLQALADFIIAHDLLVVDQAFED---
Y2760_AspC	165	DGLLNSLSEAQAGDVVLF	HGCCHNPTGIDPTETQWSQLAEFLSVAKGWLPIDDFAYQGFAN
Y0579_TyrB	161	DPMLATLQQLPARSIV	LLHPCCHNPTGSDITNAQWDRIIDVVKERELIPFDIAYQGFGA
STM0998_AspC	160	EALQASLSEAQAGDVVLF	HGCCHNPTGIDPTLEQWQVLAFLSVEKGWLPIDDFAYQGFAR
STM4248_TyrB	161	NDLLATLNTLPARSIV	LLHPCCHNPTGADLTPSQWDAMIEIVKARDLIPFDIAYQGFGA
07704	167	SDFEEKITEK--TKMVILT	N--PNNPTGTVLRRESLQALADFVIDHDLMLVVDQAFED---
072382	167	SDFEEKITEK--TKMVILT	N--PNNPTGTVLRRESLQALADFVIDHDLMLVVDQAFED---
093506	167	SDFEEKITEK--TKMVILT	N--PNNPTGTVLRRESLQALADFVIDHDLMLVVDQAFED---

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325 Se_AspB 167 SDFEEKITEK-TKMVILTN--PNNPTGTVLRRESLQALADFVIDHDLMVLVVDQAFED---
326
327
328 Y3555 221 AIFDEIEFISIASLPGMWERTISVFSFSKMGGLSGFRVGYL---VADAQIIVDVLIFGCTVN
329 Y2760_AspC 225 GIEEDAQG--LRIFAATHQELIVCSSYSKDNFGLYNERVGACTLVAADSNVADTAFISQVKA
330 Y0579_TyrB 221 GINEDAYA--IRAMAAGLPCILISNSFSKIFSLYNERVGGISVVCESDEAAGRVLGQLKA
331 STM0998_AspC220 GIEEDAEG--LRAFAALHKELIVASSYSKDNFGLYNERVGACTLVAADAETVDRAFISQVKA
332 STM4248_TyrB221 GMDDDAYV--IRATASAGLPAIVSNFSKIFSLYGERVGGISVVCEDAETIARVLGQLKA
333 07704 221 AIFDNIEFISIASLPGMWERTISVFSFSKMGGLSGFRVGYL---VADDHIMDALYGCTVN
334 072382 221 AIFDNIEFISIASLPGMWERTISVFSFSKMGGLSGFRVGYL---VADDHIMDALYGCTVN
335 093506 221 AIFDNIEFISIASLPGMWERTISVFSFSKMGGLSGFRVGYL---VADDHIMDALYGCTVN
336 Se_AspB 221 AIFDNIEFISIASLPGMWERTISVFSFSKMGGLSGFRVGYL---VADDHIMDALYGCTVN
337
338
339 Y3555 278 VVGATNTSSQ---AGM-----IAALDEPSFMGEYTIQIFERRRKVVFEMMN--AIPG
340 Y2760_AspC 283 VTRANYSNPPAHGASVATILSNAALRAIWEQELTDMRCRIQMRQLFVNTLQEKGAQQD
341 Y0579_TyrB 279 TVRRNYS SPPNFGAQVSKVLNDTVLRAQWQAEVEQMLRLRILDMRHTLVEALKASLPERN
342 STM0998_AspC278 ATRANYSNPPAHGASIVATILSNDALRAIWEQELTDMRCRIQMRQLFVNTLQEKGANRD
343 STM4248_TyrB279 TVRRIYS SPPCFGAQVATVLGDEALKAGWLAEVDAMRNRIISMRQTLVKEIKAE MPDRN
344 07704 278 VVGATNTSSQ---AGM-----IAALNDEPSFIDTYNQIFDHRRKVVFEMMN--AIPG
345 072382 278 VVGATNTSSQ---AGM-----IAALNEPSFIDTYNQIFDHRRKVVFEMMN--AIPG
346 093506 278 VVGATNTSSQ---AGM-----IAALNEPSFIDTYNQIFDHRRKVVFEMMN--AIPG
347 Se_AspB 278 VVGATNTSSQ---AGM-----IAALNEPSFIDTYNQIFDHRRKVVFEMMN--AIPG
348
349 Y3555 324 VSMVMPESGFLSWIDISKLGSSAFICDYLLQHAHVMVNSGVVPYGE GEGGYIRLVHGCIYKD
350 Y2760_AspC 343 FSFLINQNGMFSFSGLTKQVLRRL----RDEFVAVYAVNSGR-----VN-VAGMTPD
351 Y0579_TyrB 339 FDYLLQQRGMFSYTGFSQAQVESL----REEFGVYLIASGR-----MC-MAGVNHQ
352 Se_AspC 338 FSFLIKQNGMFSFSGLTKDQVLRRL----REEFGVYAVASGR-----VN-VAGMTPD
353 Se_TyrB 339 FDYLLQQRGMFSYTGFSQAQVDRRL----RDEFVYLIASGR-----MC-VAGLNAS
354 07704 324 VSMVMPESGFLSWIDISKLGSSAFICDYLLQHAHVMVNSGVVPYGE GEGGYIRLVHGCIYKD
355 072382 324 VSMVMPESGFLSWIDISKLGSSAFICDYLLQHAHVMVNSGVVPYGE GEGGYIRLVHGCIYKD
356 093506 324 VSMVMPESGFLSWIDISKLGSSAFICDYLLQHAHVMVNSGVVPYGE GEGGYIRLVHGCIYKD
357 Se_AspB 324 VSMVMPESGFLSWIDISKLGSSAFICDYLLQHAHVMVNSGVVPYGE GEGGYIRLVHGCIYKD
358
359
360 Y3555 384 D-EKLYAVLTRIQQALMQLAKEKGLDHE
361 Y2760_AspC 389 NMAPLCEATVAVL-----
362 Y0579_TyrB 385 NVEQVAVAFAAVQ-----
363 Se_AspC 384 NMAPLCEATVAVL-----
364 Se_TyrB 385 NVHRVAKAFAAVM-----
365 07704 384 D-EKLFVAVLTRIQSALTM LAKEKGLDHE
366 072382 384 D-EKLCVAVLTRIQSALTM LAKEKGLDHE
367 093506 384 D-EKLCVAVLTRIQSALTM LAKEKGLDHE
368 Se_AspB 384 D-EKLCVAVLTRIQSALTM LAKEKGLDHE
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373 **Figure S3. Alignments of Y3555 and 07704, 072382, 093506, AspB from CDC isolates**
374 **of *S. enterica*.** *denotes the glutamate instead of aspartate that is predicted to coordinate
375 the pyridine nitrogen of PLP in the active site.

376

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