SUPPLEMENTAL MATERIAL FOR PUBLICATION

Contribution of asparagine catabolism to *Salmonella* virulence

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Supplemental Table 1. Primers used in this study.

ΔSTM1294-F	5'-AATCATTAATACGTCCTTCGCGACACGACTGAACATTATCGTGTAGGCTGGAGCTGCTTC-3'
ΔSTM1294-R	5'-GCCCGGTTTGTCATTATCTTCTCCTTGTCGTCGGGATTTTATGGGAATTAGCCATGGTCC-3'
ΔSTM3997-F	5'-TTACAATTAACGCCAATGTATTAATCGGAGAGAGTTGATCGTGTAGGCTGGAGCTGCTTC-3'
ΔSTM3997-R	5'-AACATCTTATAAAAACGCCGGTCAGTGACCGGCGTTCTTTATGGGAATTAGCCATGGTCC-3'
STM1294-F	5'-ACTAGCTAGCCGAGGGGGATAAACTGGCGGC-3'
STM1294-R	5'-CTAGTCTAGACCTGATGCTGGCTGGCGAAG-3'
STM3993-F	5'-AAGTGCTAGCCATGATACCGCTACTCG-3'
STM3993-R	5'-GGACTCTAGAAGTGAAGAAGCCCATCCTGA-3'
STM3994-F	5'-AAGTGCTAGCACACGGTAGCAGGGTCAATC-3'
STM3994-R	5'-GGACTCTAGAAGCAGTGTTGTTTTTCCCGT-3'
STM3995-F	5'-AAGTGCTAGCGATTGACCCTGCTACCGTGT-3'
STM3995-R	5'-CCTGTCTAGAGCGTCTGGAAAGTAAAAGCG-3'
STM3996-F	5'-AAGTGCTAGCTCGCCACGAAATGATATCCTG-3'
STM3996-R	5'-CCTGTCTAGAGGCGCTAAAAGCTAAAACCA-3'
STM3997-F	5'-AAGTGCTAGCCGGAGAGAGTTGATCATG-3'
STM3997-R	5'-CCTGTCTAGACGATATGCTAAGCGGTAGGC-3'
STM3998-F	5'-AAGTGCTAGCTCACTCAGCGCCATCATTAG-3'
STM3998-R	5'-GGACTCTAGACGTGGTGAAATCACTGGTTG-3'
q*STM3106*-F	5'-CTCTGGTAATGGGTTTCAGCGGCG-3'
q*STM3106*-R	5'-TGCTCGCCTTTCACTACCGCAATG-3'
q*STM3835*-F	5'-GGCGTTCTCCTCGATAAAGGCACG-3'
q*STM3835*-R	5'-TGGCTGGTGAAAGAATCACGTCGC-3'
*STM3106.*C99A-F	5'-CGAAAAAAATCAATACCGAGGCGGACAGCACCGACGGTTTCG-3'
*STM3106.*C99A-R	5'-CGAAACCGTCGGTGCTGTCCGCCTCGGTATTGATTTTTTTCG-3'
*STM3106.*C127A-F	5'-CTCGATCTGACCGTGAAAGCGAATAAACCGGTGGTACTG-3'
*STM3106.*C127A-R	5'-CAGTACCACCGGTTTATTCGCTTTCACGGTCAGATCGAG-3'

Supplemental Figure 1, related to Figure 2. Relative expression of *STM3106* (*ansB*) normalized to *STM3835* (*gyrB*) as determined by use of quantitative real-time PCR (2^(-ΔΔCt) method). Data show mean with SEM obtained from three independent experiments and were analyzed by repeated measures one-way ANOVA with Tukey's multiple comparisons posttest (n.s. not significant).

Supplemental Figure 2, related to Figures 3 and 4. Alignment of the primary structure of L-Asparaginase II of *S*. Typhimurium with the primary structure of L-Asparaginase II of *E. coli* (pdb:3ECA) (1) by use of ESPript 3.0 server (http://espript.ibcp.fr/) (2), which renders sequence similarities and secondary structure information from aligned sequences. L-Asparaginase II of *S*. Typhimurium is 92.82% identical to L-Asparaginase II of *E. coli* at the amino acid level. Black boxes with white bold characters indicate conserved residues. White boxes with bold characters indicate similar residues. Arrows above the aligned sequences indicate beta sheets. Loops above the aligned sequences indicate alpha and pi helices. Alpha and beta turns are indicated by (TT) or (TTT) above the sequence. Amino acid residues predicted or previously shown to be involved in the formation of a structural disulfide bond are indicated by (*1*) below the sequence. The location of the predicted signal peptide cleavage site as determined by use of SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/) is indicated by (^) below the sequence.

Supplemental Figure 3, related to Figure 5. (***A***) Alignment of the primary structure of L-Asparaginase I of *S*. Typhimurium with the primary structure of L-Asparaginase I of *E. coli* (pdb:2P2D) (3) by use of ESPript 3.0 server (2). L-Asparaginase I of *S*. Typhimurium is 94.67% identical to L-Asparaginase I of *E. coli* at the amino acid level. Black boxes with white bold characters indicate conserved residues. White boxes with bold

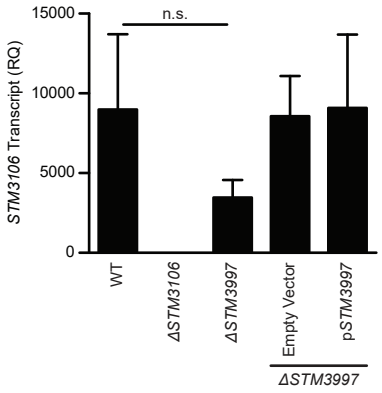
characters indicate similar residues. Arrows above the aligned sequences indicate beta sheets. Loops above the aligned sequences indicate alpha and pi helices. Alpha and beta turns are indicated by (TT) or (TTT) above the sequence. (***B***) Growth of wild-type *S*. Typhimurium (WT; circles) and *ΔSTM1294 S*. Typhimurium (*ΔSTM1294*; triangles) in M9 minimal medium with ammonium chloride (NH_4Cl) as the sole nitrogen source. (***C***) Growth of wild-type *S*. Typhimurium (WT; circles) and *ΔSTM1294 S*. Typhimurium (*ΔSTM1294*; triangles) in M9 minimal medium with asparagine (Asn) as the sole nitrogen source. Data show means with SEM obtained from at least three independent experiments (B and C). Data were analyzed by repeated measures two-way ANOVA with Sidak multiple comparisons posttest; observed differences were not statistically significant.

Supplemental Figure 4, related to Figure 6. (***A***) Survival and growth of wild-type *S*. Typhimurium (WT; black), *ΔSTM1294 S*. Typhimurium (*ΔSTM1294*; grey), *ΔSTM3106 S*. Typhimurium (*ΔSTM3106*; white) or *ΔSTM1294 ΔSTM3106 S*. Typhimurium (*ΔSTM1294 ΔSTM3106*; dashed) inside bone marrow-derived macrophages cultured from 129X1/SvJ mice. (***B-D***) Total bacterial loads per gram of mesenteric lymph node tissue (MLN; B), liver tissue (C) and spleen tissue (D) harvested from 129X1/SvJ mice (n = 6-8 per group per time point) at various times after intragastric inoculation with 1×10^8 CFU of a 1:1 mixture of differentially marked wild-type and *ΔSTM1294 ΔSTM3106 S*. Typhimurium. Data were analyzed by repeated measures two-way ANOVA with Tukey's multiple comparisons posttest (A) or one-way ANOVA with Dunnett's multiple comparisons posttest (with data compared to the total bacterial load per gram of tissue harvested on day 15 after inoculation). *P* values < 0.05 were considered to be statistically significant. Asterisks indicate statistically significant differences for

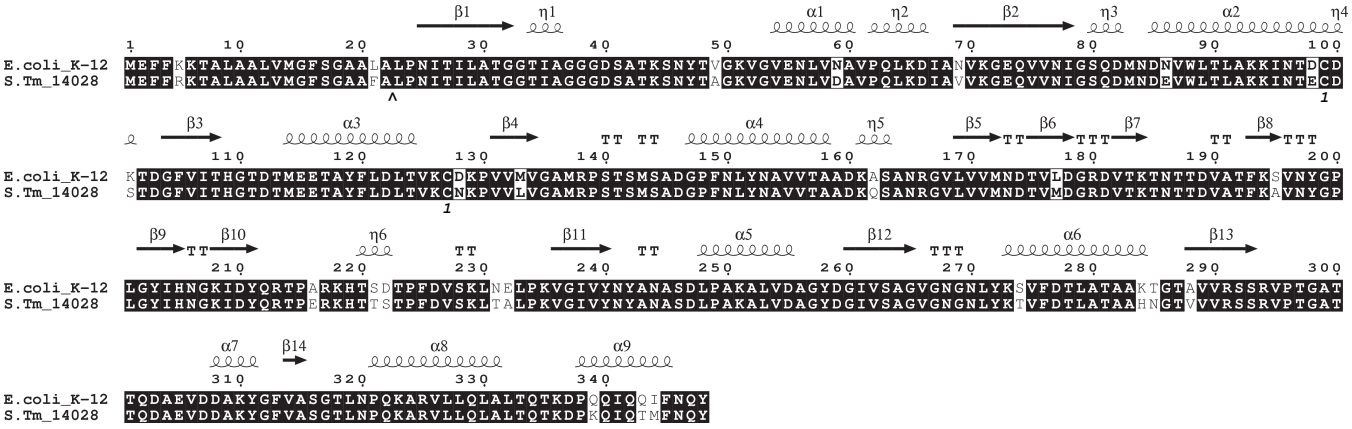
designated posttest comparisons (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.01$, n.s. not significant).

SUPPLEMENTAL REFERENCES

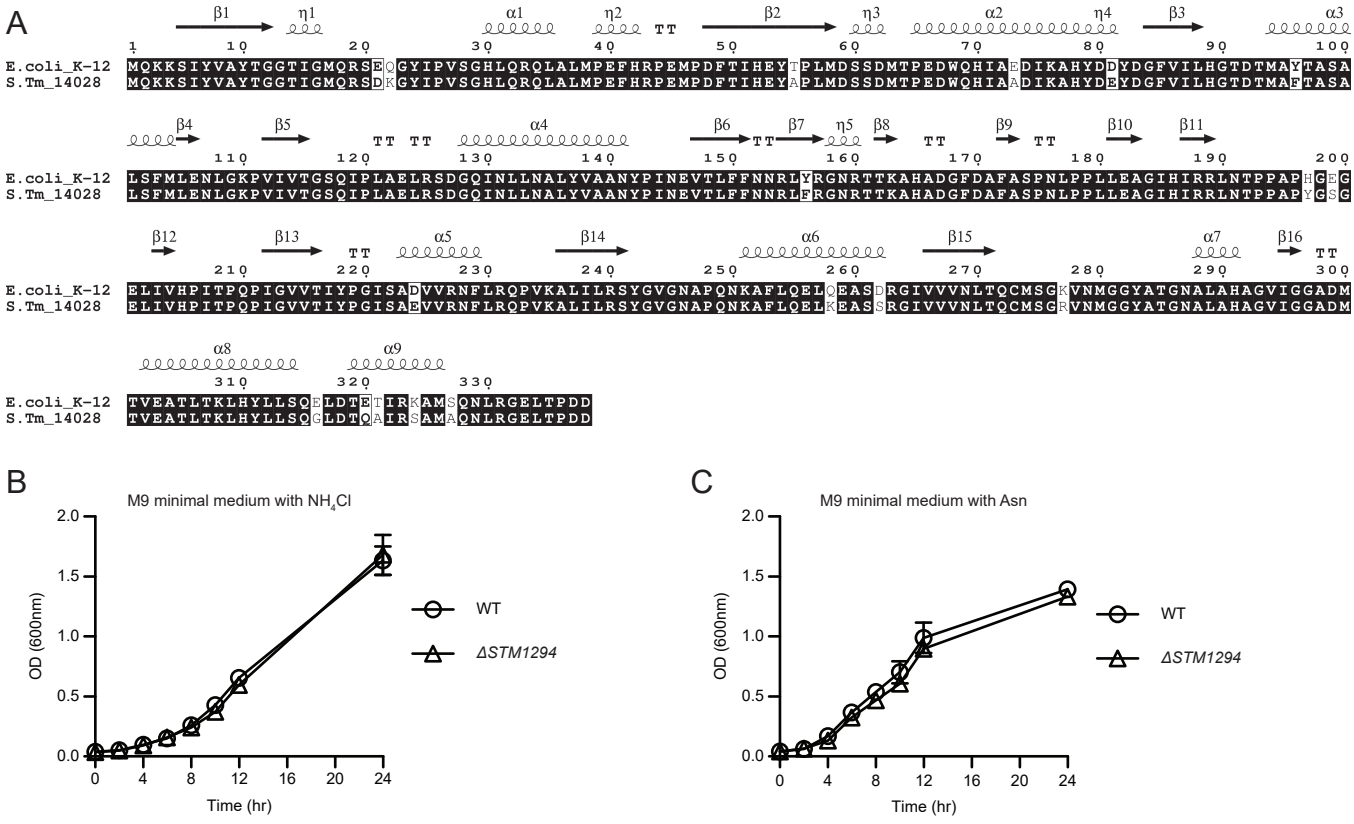
1. **Swain AL, Jaskolski M, Housset D, Rao JK, Wlodawer A.** 1993. Crystal structure of Escherichia coli L-asparaginase, an enzyme used in cancer therapy. Proc Natl Acad Sci U S A **90:**1474-1478.
2. **Robert X, Gouet P.** 2014. Deciphering key features in protein structures with the new ENDscript server. Nucleic Acids Research **42:**W320-W324.
3. **Yun MK, Nourse A, White SW, Rock CO, Heath RJ.** 2007. Crystal structure and allosteric regulation of the cytoplasmic Escherichia coli L-asparaginase I. Journal of Molecular Biology **369:**794-811.



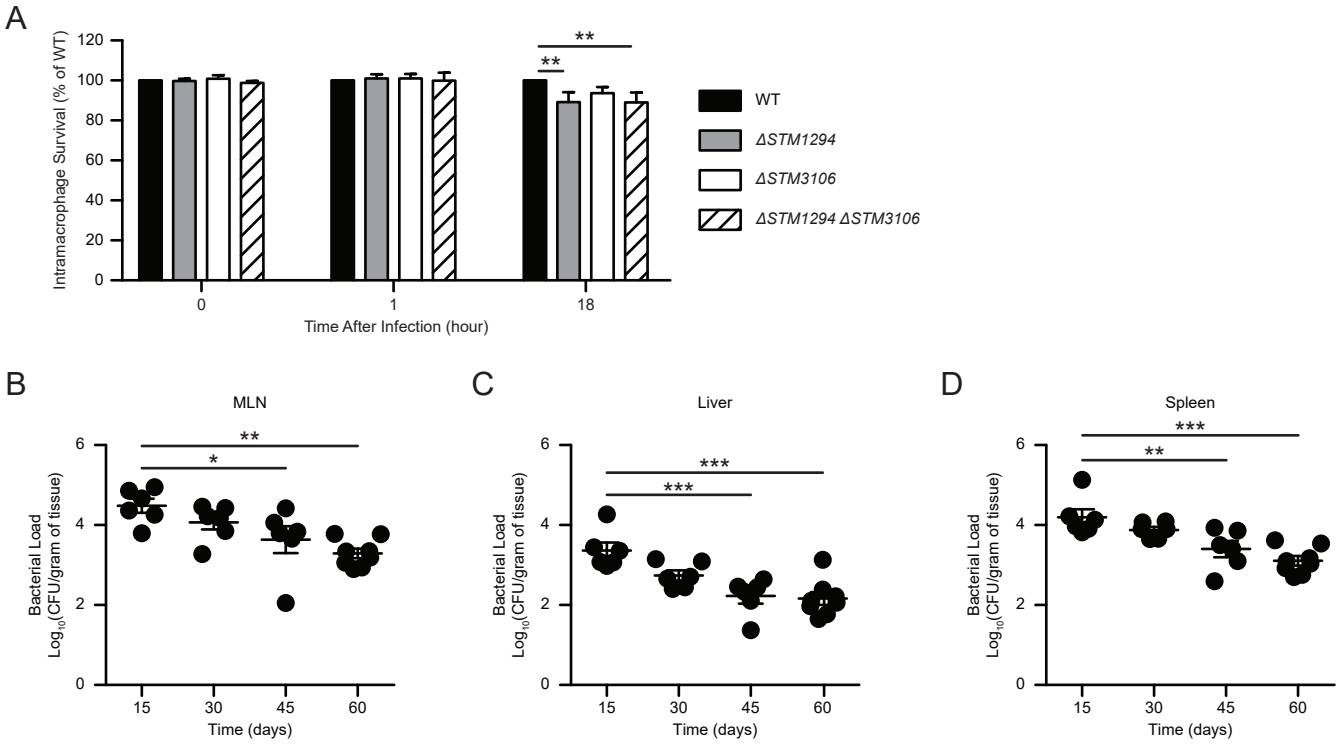
McLaughlin et al., Supplemental Figure 1



McLaughlin et al., Supplemental Figure 2



McLaughlin et al., Supplemental Figure 3



McLaughlin et al., Supplemental Figure 4