Genomic factors contributing to the resilience of *Salmonella enterica* on ready-to-eat muskmelon

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Short Title: Interaction mechanisms of *S. enterica* with ready-to-eat muskmelon

Abstract

Salmonella outbreaks have repeatedly been associated with muskmelons. This fruit poses a food safety risk due to its growth on the soil surface and because it is eaten raw. To identify genes under selection in *S. enterica* growing in this food matrix, barcoded transposon mutant libraries in three serovars of *Salmonella,* Typhimurium, Enteritidis, and Newport, were screened for survival and growth on muskmelon. The role of selected genes was subsequently verified in competition assays. Genes whose disruption caused a fitness effect in all three serovars were enriched for functions related to RNA degradation and ribosome biogenesis. The polyribonucleotide nucleotidyltransferase gene, *pnp,* and the D-3-phosphoglycerate dehydrogenase gene, *serA*, conferred an advantage when growing in muskmelon but not in a nutrition-rich control environment. This study provides a rare insight into genome-wide adaptation mechanisms of multiple *Salmonella* serovars to growth on food matrices, exemplified by muskmelons.

Introduction

Non-typhoidal salmonellosis is a zoonotic disease that has a high impact on the economy and human and animal health. It is among the most relevant foodborne pathogens in the European Union (EU) and worldwide [1].

Ready-to-eat (RTE) melons pose a high infection risk to consumers since pathogen inactivation measures are rarely applied before consumption, and handling may enable the spread of surface contaminants to the inside of the fruit. *S. enterica* foodborne outbreaks associated with the consumption of muskmelons have been reported since 1990 [2, 3], with recent cases in the EU (2023)[4] and the United States (2024)[5]. Especially, netted rind melon varieties offer a favorable niche for bacterial attachment and persistence [6].

To establish infection, *S. enterica* must be able to survive and grow on the food matrix. Quantitative data for the growth of *S. enterica* on muskmelons show that *S. enterica* can grow on muskmelons at 8°C and sustain its population at temperatures as low as 4°C [7-9].

S. enterica responses to food-related stresses have been studied in the past [10, 11] and previous studies revealed fitness effects of gene disruptions vary significantly by food matrix, identifying key survival mechanisms on tomatoes [12, 13], alfalfa sprouts [14], and low-water-activity foods, such as pistachios[15], black peppercorns, almonds, and hazelnuts [16]. Transposon insertion sequencing (TIS) showed matrix-specific effects for *Salmonella* colonization and persistence by the inactivation of genes linked to the alternative sigma factor σ^S (RpoS), DNA recombination, the biogenesis of lipopolysaccharides (LPS)[15], glycolysis, amino acid and LPS production, fatty acid degradation, and purine and pyrimidine biosynthesis pathways [13].

Based on the existing data for other food matrices, we hypothesized that distinct genetic determinants may exist for *S. enterica* fitness on muskmelon. In this study, we used barcoded transposon libraries of *S.* Typhimurium 14028s, *S.* Enteritidis PT4 strain P125109, and *S.* Newport

56 C4.2, to identify genome-wide fitness effects of specific genes and the corresponding metabolic
57 pathways involved in the interaction of these *S. enterica* serovars with RTE muskmelon.

58 Results

59 Growth data

60 Transposon libraries on the food matrix grew 1.0 to 2.0 $\log_{10}$ CFU/g, while *no matrix*
61 samples remained stable at 22°C and decreased slightly at 8°C **(Figure S1)**. SEN libraries showed
62 reduced growth at 8°C compared to SNP and STM. The transposon libraries and their WTs
63 exhibited comparable growth on RTE muskmelons at 8°C and 22°C (**Figure S2**), except for the
64 SNP library, which showed a minor reduction compared to the WT (-0.3 $\log_{10}$ CFU/g, p-adj≤0.01)
65 at d_1, 8°C.

66 Mesophilic aerobic bacteria (MAB) and Enterobacteria on *Salmonella*-free RTE
67 muskmelon increased by approximately 4.0 $\log_{10}$ CFU/g at 8°C and 5.0-6.0 $\log_{10}$ CFU/g at 22°C,
68 with high variability among samples (**Figure S3**).

69 Transposon Insertion Sequencing data analysis

70 TIS screening of STM, SEN, and SNP libraries on RTE muskmelon identified functionally
71 grouped mutants with significant fitness effects. All fitness data of the transposon assay after
72 DESeq2 analysis are presented in **Table S2**, while the subset of genes that fulfilled the strict dual
73 requirement of significance, as detailed in Materials and Methods, are displayed in **Table 2.** The
74 dynamics of mutant representation during incubation are represented in **Figure S4**, while
75 exemplary volcano plots for inoculum/end of storage comparison are shown in **Figure 1**.
76

77 **Table 1.** Genes with a significant fitness effect in *Salmonella enterica* serovars when grown on muskmelon,

78 classified by function.

Function	Gene Number	Gene	STM		SEN		SNP		Comment
			8°C	22°C	8°C	22°C	8°C	22°C	
RNA Degradation	STM14_3962	*deaD*	-	-	-	-	(-)	(-)	
	STM14_0013	*dnaK*	n.d.	n.d.	+ / -				
	STM14_0218	*pcnB*						-	
	STM14_3964.RJ	*pnp*	-	-	-	-	-	-	
	STM14_3066	*ppk*	-		(-)		-		
	STM14_3965	SraG	(-)	-	(-)				
	STM14_5250	*vacB*	-				-		
Ribosome biogenesis	STM14_2082	*rluB*			(-)	-	-	(-)	
	STM14_1359	*rluC*			(-)		-		
	STM14_3263	*rluD*	(-)	(-)	(-)	(-)		-	
	STM14_3240	*srmB*	-	-	-	-	-	-	
	STM14_4639.P	STnc400	-	-	n.d.	n.d.	n.d.	n.d.	
	STM14_4822	*typA*	-	-	-	-	-	-	in SEN: *typA* represented by 50537.33.peg.4013STM14_4822
	STM14_5328+550537.33.peg.4414+108619.9.peg.3873	*yjgA*	-	-	-	-	-	-	in SEN and SNP: STM14_5328 unchanged
Reactive Oxygen Species (ROS) stress response	STM14_4959	*oxyR*	- / +	- / +	- / +	- / +		- / +	
	STM14_4878	*sodA*			- / +	(-)	n.d.	n.d.	
	STM14_4713	*trxA*	-	(-)	(-)		(-)	(-)	
	STM14_1080.J	*trxB*			(-)	(-)	-	(-)	
Lipopolysaccharides, O-antigen and enterobacterial common antigen biosynthesis	STM14_4308	*dcrB*	-				-		
	STM14_4479.J	*rfaI*			(-)		(-)	-	
	STM14_4483	*rfaG*					-	(-)	
	STM14_4484.J	*rfaQ*			(-)			-	
	STM14_4477	*rfaY*					(-)	-	
	STM14_4722	*wecD*	-	(+)	(-)				
	STM14_4723	*wecE*	+ / -	(+)	(-)		(-)	(-)	
	STM14_4725	*wecF*	-	(+)					
	STM14_4004	*yrbI*		(-)	(-)	(-)	(-)	-	
Envelope, membrane transport, and antimicrobial resistance	STM14_2841	*arnF*			(-)	-			
	STM14_5093	*dgkA*				-	(-)	(-)	
	STM14_0267	*hlpA*	-	-	(-)	-		-	
	STM14_0111	*surA*			-		(-)		
Two-component system	STM14_4216	*envZ*					+ / -	(-)	
	STM14_3140	*glnB*			-		n.d.	n.d.	
	STM14_4817	*glnG*	- / +	- / +	(-)	(-)		(-)	
	STM14_4818	*glnL*	(+)	(- / +)	(-)	- / +		(- / +)	
	STM14_2797	*ompC*					+ / -	(+)	
	STM14_4217	*ompR*					+ / -	(-)	
	STM14_1408	*phoQ*	(+)		- / +				
Protein folding, degradation, and secretion system	STM14_3114	*hscA*			(-)	-		(-)	
	STM14_3162	*lepB*					-	-	
	STM14_0162	*secA*	(-)		-		n.d.	n.d.	
	STM14_4461	*secB*			-	-	(-)	-	
	STM14_4780+108619.9.peg.3424	*tatB*			(-)		-	(-)	in SNP: STM14_4780 nearly unchanged
Amino acid metabolism	STM14_1106	*aroA*					-	(-)	
	STM14_2232	*prc*				-			
	STM14_3699	*serA*		(-)		-		-	
	STM14_5499	*serB*		(-)		(-)		-	
	STM14_1105	*serC*		-	(-)	(-)		-	
Translation factors	STM14_2145	*hemK*			(-)	-			

	STM14_5478	*prfC*	-		-	(-)	(-)		
tRNA Biogenesis	STM14_3116	*iscA*			(-)	-	(-)	(-)	
	STM14_3604	*metZ*	(-)				-		
	STM14_2196	*rnd*	-		(-)				
	STM14_0583	*ybaK*		(-)	(-)	-	(-)	(-)	
Mismatch repair	STM14_5372.J	*holC*	-						
	STM14_5475	*holD*	-	-			n.d.	n.d.	
	STM14_3077	*xseA*	-	-					
DNA replication and recombination	STM14_3608	*recD*					(-)	-	
	STM14_0566	*priC*					-		
Cytoskeleton and cell division	STM14_0159	*ftsZ*					-	(-)	
	STM14_3095	*yfgA*			(-)		-	-	
Transcription and transcriptional regulators	STM14_4399	*cspA*					-	(-)	in SNP: *cspA* represented by STM14_4399STM14_4399.J
	STM14_4991	*rpoC*	(-)		-		(-)		
	STM14_2310	*yebC*				-			
Purine and pyrimidine metabolism	STM14_3076.LR	*guaB*				-			
	STM14_5248	*purA*	(-)	(-)	(-)	-		(-)	
	STM14_3050	*purC*		(-)		-			
	STM14_5017	*purH*		(-)		-		(-)	
	STM14_0623	*purK*				-			
	STM14_1200	*pyrD*				(-)		-	
Phosphotransferase system	STM14_2990	*ptsI*			(-)	(-)	-	(-)	
Carbohydrate metabolism and Pentose phosphate pathways	STM14_2289	*eda*		-	(-)	-	n.d.	n.d.	
	STM14_2118	*galU*	n.d.	n.d.			-	(-)	
	STM14_4444	*mtlD*				- / +		(-)	
	STM14_3124	*suhB*	n.d.	n.d.	n.d.	n.d.	-	-	in SNP: *suhB* represented by STM14_3124STM14_3123.J
Bacterial defense and phage	STM14_3172	Phage genes					+ / -	(-)	
	STM14_1150.JSTM 14_3224				n.d.	n.d.	+ / -	+ / -	
	STM14_1193	*sseI*					+ / -	(+ / -)	
	STM14_0340.J	putative RHS-like protein	(-)	-	n.d.	n.d.	n.d.	n.d.	
Uncharacterized proteins	STM14_2205	*yoaH*	-				(-)		
	STM14_4069	hypothetical protein					-		
	STM14_3096.J	hypothetical protein					-		
	STM14_2704	hypothetical protein						-	
	STM14_1197	hypothetical protein			(-)		+ / -	(+ / -)	
	108619.9.peg.3873	FIG138315 - putative alpha-helix protein	n.d.	n.d.	n.d.	n.d.	-	-	
	STM14_1530	putative cytoplasmatic protein			n.d.	n.d.	-		
	STM14_1076	putative inner membrane protein	(-)		(-)		-		
	STM14_1194	Pseudogene					+ / -	(-)	

Mutants listed met two criteria: (i) a two-fold change (i.e., $\log_2$-fc>|1.0|) with a $p_{adj} \leq 0.01$ for the matrix versus *no matrix* comparison, AND (ii) a two-fold change with a $p_{adj} \leq 0.01$ for the inoculum versus matrix comparison at the same sampling time point.

-, conditions for which a statistically significant negative fitness effect was observed.

+/-, positive fitness effect at the first sampling time point, followed by a negative fitness effect later.

-/+, negative fitness effect at the first sampling time point, followed by a positive fitness effect later.

(), mutants with a two-fold fitness effect at one OR the other criterion of the dual requirement, at $p_{adj} \leq 0.1$.

n.d., absence of mutants in the transposon library.

RHS - rearrangement hotspot.

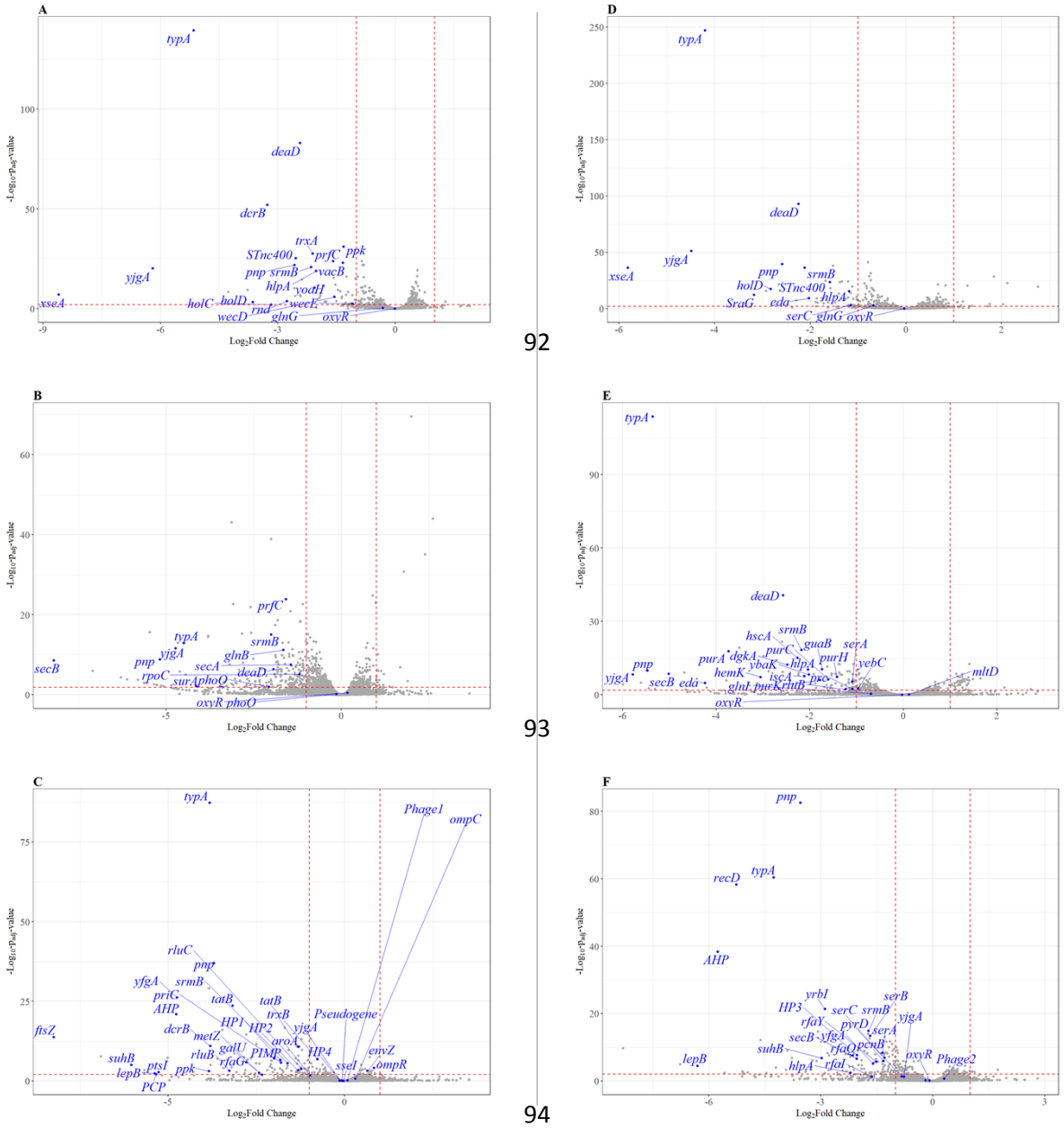

Figure 1. Change in the mutant abundances after growing the random transposon libraries on RTE muskmelons. Volcano plots depict the fold change of relative abundance of mutants and their adjusted p-values between inoculum and growth on muskmelon for 24 h at 22˚C for S. Typhimurium 14028s (**A**), S. Enteritidis PT4 strain P125109 (**B**), and S. Newport C4.2 (**C**); and the growth on muskmelon for 4 days at 8˚C for S. Typhimurium 14028s (**D**), S. Enteritidis PT4 strain P125109 (**E**), and S. Newport C4.2 (**F**). Genes with significantly changed representation (at least 2-fold, $p \leq 0.01$) are labelled in blue. Intergenic regions (IRs) with a significant fitness effect were excluded from the graph. Plots showing changes for all other sampled time points are shown in **Supplementary Figure S4**.

104 Three core genes (*pnp, typA,* and *srmB*) contributed to fitness in all three serovars at both
105 temperatures, while *hlpA* and *oxyR* mutants were found to be underrepresented in all three serovars
106 at 22°C. For STM, mutants in 24 genes showed a significant fitness effect on RTE muskmelon,
107 including 21 genes at 8°C and 15 genes at 22°C, with 11 overlapping. Most mutations had a
108 negative fitness effect, except for *oxyR* and *glnG,* that switched from early negative to late positive
109 fitness effects, and *wecE,* which showed the opposite trend. In SEN, mutants in 32 genes exhibited
110 a significant fitness on RTE muskmelon. At 8°C, 14 genes were identified, while at 22°C, mutants
111 in 26 genes showed fitness effects. Mutants in seven of these genes were significantly affected in
112 both temperatures (*pnp, deaD, yjgA, srmB, typA, secB, oxyR)*. In SNP, mutations in 24 genes
113 affected fitness on RTE muskmelon at 22°C, while 35 genes were implicated at 8°C. Temperature-
114 independent fitness effects were observed for eight of these genes (*pnp, srmB, typA, yfgA, yjgA,*
115 *suhB, lepB,* and FIG138315; **Table 2**).

116 In all three serovars, mutants in RNA degradation genes, ribosome biogenesis, envelope
117 and membrane transport, and tRNA biosynthesis showed a statistically significant difference after
118 growth on muskmelon. For some categories displayed in **Table 2**, statistical significance, as
119 defined in our criteria, was only observed for genes in one or two serovars. As an example, mutants
120 in genes of the purine and pyrimidine pathways were statistically significantly affected in growth
121 in SEN, but not in STM or SNP, and cytoskeleton and cell division gene mutants cleared the
122 significance threshold in SNP but not in the other two serovars (**Table 2**). However, the number
123 of transposon mutants in each gene and their abundance in the library differ among strains, so
124 these differences should not be misinterpreted as confirmation of a difference in the role of that
125 pathway between strains.

126 We performed KEGG enrichment analyses to gain a better insight into pathways significant
127 for the growth of *Salmonella* on muskmelon. Significantly enriched KEGG pathways ($q \leq 0.01$) and
128 associated GO terms ($p \leq 0.01$) are summarized for all three serovars in **Table 3**, with full results
129 in **Table S3.**

130 **Table 2.** Significantly enriched KEGG pathways and associated GO terms for *S.* Typhimurium 14028,
131 *S.* Enteritidis P125109, and *S.* Newport C4.2 on RTE muskmelon.

KEGG Pathway	Serovar	Temp.	*q*-value	Selected Genes	Associated GO Terms
Cellular Processes Cell motility Flagellar assembly	STM	8°C	2.5 * E-10	*flgBDFGI, flhAB, fliCFHIQR*	GO:0044780, GO:0040011, GO:0051674, GO:0071978, GO:0044781, GO:0071973
	SNP		0.005	*flgABCDEK*	GO:0040011, GO:0071978, GO:0051674
Genetic Information Processing Folding, sorting, and degradation RNA degradation	STM	8°C	0.004	***deaD, pnp, ppk, vacB***	GO:0006402, GO:0010501
	SNP		0.001	***deaD, pcnB, pnp, ppk, vacB***	GO:0010501, GO:0000455
	STM	22°C	0.007	***deaD, pcnB, pnp, vacB***	GO:0010501, GO:0006402
Genetic Information Processing Translation Ribosome	STM	22°C	0.007	***rrlABCDEGH, rrsG***	-
Genetic information processing Folding, sorting and degradation Protein export	SNP	8°C	0.001	***lepB, secB, tatABC***	GO:0043953, GO:0065002
Metabolism Glycan biosynthesis and metabolism Biosynthesis of nucleotide sugars	STM	8°C	0.004	***rfbK,*** *wecBC,* ***wecDE***	GO:0009246
Metabolism Nucleotide metabolism Purine metabolism	SEN	22°C	0.001	***guaB, purACDEGHK***	GO:0006189, GO:0046040

132 Gene lists were based on p_{adj}<0.05 in at least one matrix/*no matrix* and inoculum/timepoint comparison.
133 Genes in **bold** were under negative selection between sampling time points, while underlined genes were
134 positively selected.

135 Competition assays confirm genes with a fitness effect

136 A total of 25 genes with a fitness effect in STM, SEN, or SNP on RTE muskmelon were
137 selected from the *S.* Typhimurium 14028 SGD mutant collection [17] for competition assays
138 (**Table 4)**. Four of these genes (*serA*, *serB, ybaK*, and *purA*) displayed changes in STM that were
139 not as significant as in SEN or SNP but were tested to verify whether the results of the transposon
140 assays were, in fact, a representation of the true diversity between *Salmonella* serovars. Two genes
141 that exhibited temporal abundance changes in STM, *oxyR* and *glnG*, were also included in these
142 competition assays, along with *rfbI_2*, which showed no significant changes in any of the three
143 strains analyzed. A final gene, *wecF [D]*, showed opposite effects in the two different incubation
144 temperatures in STM. However, significance (p_{adj}<0.01) was only achieved at 8˚C, while at 22°C
145 only the inoculum/t_{24} comparison was significant.

146 For 18 of these 25 genes, we had two single-gene deletion mutants available for each gene,
147 one where a kanamycin resistance (Kan^R) cassette replaced the gene in the sense orientation and
148 one where a chloramphenicol cassette (Cm^R) replaced the gene in the antisense orientation. For

149 five genes (*yjgA, hlpA, holC*, *purA,* and *holD*), we only had a Kan^R mutant available, while for
150 *wecE*, our collection only harbored a Cm^R mutant. Competition assays were performed at the
151 temperature where transposon data suggested a stronger fitness effect. Assays were performed on
152 muskmelon and rich LB media, to verify whether any fitness effects occurred in any rich nutrient
153 environment or were potentially specific to growth on muskmelon. For selected genes, the fitness
154 effect observed in muskmelon or LB was also tested in PBS, where the nutrient-restricted
155 conditions only confer a growth difference due to differential death. The results are summarized
156 in **Table 4**. To confirm a negative fitness effect, both SGD mutants were required to display a
157 competition index where CI+STDEV<1. In contrast, for a confirmed positive fitness effect, both
158 mutants needed to adhere to the CI–STDEV>1 threshold. If only one SGD mutant was available,
159 an expected single mutant behavior as outlined above was sufficient to confirm the phenotype
160 observed in the transposon assay.

161 As shown in **Table 4**, the observed fitness effects in the transposon assays were confirmed
162 for 15/24 genes. Notably, *serA* and *pnp* deletions showed a negative fitness effect during the
163 competition with the WT on muskmelon that was not detected in LB. Similarly, the positive fitness
164 effect of transposon insertions in *glnG*, observed in STM at 8°C on d_5, was confirmed in
165 competition assays on muskmelon and not on LB, although with high standard deviations. Further
166 replicates would be needed to illuminate this difference further. Mutants in *xseA* exhibited a strong
167 polar effect in LB and RTE muskmelon, where one single-gene deletion mutant confirmed the
168 expected debilitated phenotype on muskmelon, while the other suggested a growth advantage. This
169 may be due to the different orientations of the constitutive promoter introduced into the gene
170 during the generation of the two mutant constructs. For four genes (*wecD, trxA, typA*, and *serB*),
171 one SGD mutant confirmed the expected phenotype while the other showed no significant change.
172

173 # Discussion

174 This study investigated *S. enterica* fitness determinants on RTE muskmelon using TIS in
175 three serovars: *S.* Typhimurium ATCC 14028, *S.* Enteritidis PT4 strain P125109, and *S.* Newport
176 C4.2. The growth potential was comparable to previous studies with *S. enterica* on muskmelons
177 [7, 8, 18], with minor variations probably related to strain differences, processing conditions, and
178 time-temperature combinations. The slight reduction in SNP growth at d_1 (8°C) may be due to
179 phage lysis under stress, as phage genes were negatively selected at this temperature.

180 Limited data is available on the background microbiota in muskmelons and their growth
181 during storage. Compared to our data, higher initial MAB counts (3.5-4.0 $\log_{10}$ CFU/g total aerobic
182 bacteria compared to ~2.0 $\log_{10}$ CFU/g in our study) and coliforms (slightly below 2.0 $\log_{10}$ CFU/g
183 compared to 0.5-1.0 $\log_{10}$ CFU/g *Enterobacteriaceae* in our study) were reported in previous
184 studies [7, 19].

185 TIS revealed shared fitness determinants across all three serovars, particularly genes
186 involved in RNA metabolism, ribosome and amino acid biogenesis, oxidative stress response, and
187 cell integrity and membrane transport. Serovar-specific fitness effects were observed for mutations
188 in genes related to protein folding and transport, purine and pyrimidine metabolism, DNA
189 replication, recombination, and mismatch repair. Although these differences have not been further
190 analyzed and may be due to differences in the number of transposon mutants in each gene and
191 their abundance in the library, this approach allowed us to spread a wider net, capturing genes
192 under selection that would have been missed with only one TIS library. Future work may analyze
193 single-gene mutant orthologs to elucidate whether candidate differences between serovars are
194 indeed differences.

195 Mutations in genes involved in **RNA degradation** (*pnp*, *deaD, vacB,* SraG*, ppk, dnaK,*
196 *pcnB)* **and ribosomal homeostasis** (*typA, srmB, yigA, rluBCD, STnc400*) had significant fitness
197 effects across serovars.

198 PNPase (exoribonuclease polynucleotide phosphorylase (*pnp*)), a conserved RNA
199 degradosome component, regulates mRNA degradation, tRNA processing, and small RNA
200 turnover [20]. PNPase is critical for bacterial cold-shock responses, although *S. enterica pnp*
201 mutants exhibit milder cold sensitivity, consistent with its temperature-independent fitness effect
202 on muskmelon. Its relevant role during the interaction on muskmelon was confirmed with a
203 muskmelon-specific negative fitness effect absent in nutrient-rich media in the competition
204 experiments against the WT.

205 DeaD and SrmB are DEAD-box RNA helicases [21, 22]. Both *srmB* and *deaD* mutants
206 exhibit cold-sensitive growth defects in *E. coli* [21], *Pseudomonas syringae* [23], and *Yersinia*
207 *pseudotuberculosis* [24], consistent with our findings, although the effect was observed at both
208 temperatures. Beyond cold stress, DeaD and SrmB participate in post-transcriptional regulation,
209 including the carbon storage regulatory system in *E. coli* [22], which may explain their
210 temperature-independent fitness effects. Co-transcription of *deaD* and *pnp* occurs in certain
211 bacterial species but is absent in others [25, 26]. This is mirrored in our data, where *deaD*
212 disruption affected fitness only in STM and SEN but not SNP. This phenotype was confirmed on
213 both muskmelon and nutrient-rich media in the competition experiments, and is therefore not
214 melon-specific.

215 PPK (Polyphosphate kinase) is highly conserved across bacteria. It catalyzes inorganic
216 polyphosphate (polyP) synthesis, regulates RNA degradation and mRNA biogenesis [27], and
217 contributes to *S. enterica* growth, survival, and virulence [28]. PolyP, a high-energy reservoir,
218 plays a key role in STM and SNP muskmelon interactions but shows no fitness effect in SEN.
219 Serovar-specific differences in *ppk* function were previously observed [28], with *ppk* mutants
220 exhibiting a greater growth defect in *S.* Typhimurium than *S.* Gallinarum, suggesting variations in
221 energy homeostasis or alternative energy use. PolyP synthesis occurs mainly in stationary phase
222 [28], likely balancing metabolism by regenerating ATP through the removal of inhibitory polyP
223 and nucleotide diphosphates [29]. On muskmelon, *ppk* mutants displayed a fitness effect only at

224 8°C and later time points, indicating polyP accumulation is critical under prolonged cold stress,
225 possibly to maintain mRNA degradation homeostasis, a phenotype confirmed for muskmelon and
226 nutrient-rich media. Nevertheless, polyP is also known to be essential for *S. enterica* long-term
227 survival in LB, even at 37°C [30]. Additionally, *vacB* mutants (exoribonuclease R), deficient in
228 RNA degradation and processing as well as in ribosome biogenesis [31], exhibited a fitness effect
229 under the same conditions as Δ*ppk* mutants. Taken together, these findings indicate the importance
230 of RNA and ribosomal homeostasis in *S. enterica* environmental adaptation.

231 In the presence of **reactive oxygen species (ROS)**, OxyR acts as key regulator of oxidative
232 stress. Δ*oxyR* mutants exhibited a dynamic fitness effect that was initially negative but shifted to
233 a strong positive selection over time in SEN and STM at both temperatures and in SNP at 22°C,
234 suggesting *oxyR* disruption initially impairs bacterial adaptation but later provides a selective
235 advantage. Members of the *oxyR*-regulon (e.g., *trxAB*)[32] also showed a negative fitness effect in
236 STM and SNP at 8°C, reinforcing the role of oxidative stress responses in muskmelon adaptation.
237 The competition assays mirrored the transposon results, albeit with high variability, further
238 emphasizing the complex environmental regulation of *oxyR*. While plants generally generate ROS
239 as a defense response [33], cut muskmelon pieces are unlikely to retain this response. Thus, the
240 *oxyR*-effect may relate to alternative metabolic adaptations [34, 35] that shall be further
241 investigated in future studies.

242 **Cell wall integrity** and **membrane transport** components were also identified. Several
243 genes involved in LPS and membrane biosynthesis (*rfaG, dcrB*) and enterobacterial common
244 antigen (ECA) biosynthesis (*rfaG, wecDEF, dcrB*) exhibited fitness effects, particularly at 8°C,
245 although for SNP, the negative fitness effect was prevalent at 22°C. While the fitness effect at 8°C
246 may represent an adaptation to altered membrane fluidity at cold temperatures [36], a special need
247 for the membrane LPS or specifically for the ECA in SNP during its interaction with muskmelon
248 at room temperature may be of importance. LPS biosynthesis was shown to be important for *S.*

249 *enterica* survival on pistachios [15], tomatoes, and mouse colonization [12], reinforcing their
250 broad role in environmental adaptation.

251 Disruptions in membrane transport (*secAB, lepB, hlpA, hscA, surA, tatB*) impaired bacterial
252 fitness on muskmelon, indicating that efficient protein translocation is critical for survival. The
253 chaperone SecB, required for protein targeting to the SecYEG transport system, was particularly
254 important in SEN and SNP. The outer membrane protein HlpA also exhibited a fitness effect at
255 22°C, although competition assays did not confirm this phenotype. Nevertheless, the enrichment
256 of membrane transport genes suggests that maintaining protein export balance is essential; either
257 the accumulation of metabolites in the cytoplasm or the depletion of functional proteins in the
258 periplasm may be detrimental for *S. enterica* growth on muskmelons.

259 **Two-component systems (TCSs)** and **motility** genes were also overrepresented in the TIS
260 data. Mutants in various TCSs showed fitness effects at 8°C, suggesting a role under cold stress.
261 Nitrogen assimilation TCS mutants (*glnG* in STM, *glnBL* in SEN) showed significant fitness
262 effects, while *phoQ* mutants (sensing antimicrobial substances and Mg^{2+} starvation) appeared to
263 be affected only in SEN. In SNP, osmolarity regulators (*envZ, ompR, ompC*) exhibited fitness
264 effects. Positive selection for *glnG, phoQ, envZ*, and *ompR* mutants during part of the interaction
265 period suggests a fitness advantage.

266 Positive selection was also observed for mutations in flagellar assembly genes, likely
267 aiding adaptation to the plant environment [37]. This correlates with the results of a previous study,
268 where *S.* Typhimurium 14028 largely downregulated flagellin production, while a subset of cells
269 exhibited high flagellin expression inside the plant [38].

270 **Protein synthesis** was relevant for the interaction of *S. enterica* with muskmelons, as
271 inactivation of genes involved in the serine (*serABC, prc)*, aromatic amino acids (*aroA),* and
272 glutamine metabolism (*glnB)* resulted in serovar- and temperature-dependent fitness effects.
273 Additionally, genes related to tRNA (*rnd, iscA, ybaK, metZ*) and translational regulation (*prfC,*
274 *hemK*) were also identified. Both *serA* and *glnB* were previously identified as necessary for tomato

275 colonization [12], suggesting a critical role for scavenging serine and glutamine from the food
276 matrix. The positive fitness effect observed at later time points for *glnG* mutants, a gene coding
277 for the nitrogen regulation protein NR(I), was confirmed in the competition assays to be
278 muskmelon-specific, suggesting that a switch to alternative nutrient sources or alternative
279 regulation mechanisms of the nitrogen metabolism may be beneficial for growth on muskmelon.
280 Perhaps nitrogen assimilation is unnecessary for the fitness of *S. enterica* on muskmelons.

281 *ΔaroA* mutants have demonstrated a fitness effect in SNP and SEN under desiccation stress
282 on low-moisture foods [15]. Since *aroA* mutants only showed an effect at 8°C, a role in cold stress
283 could be hypothesized, with a potential link to TypA as a tyrosine-based enzyme with a role in
284 cold-shock response [39]. A growth defect for *E. coli met*-mutants was previously demonstrated
285 [40, 41]. While the *de novo* biosynthesis of amino acids was also found to be critical for the
286 persistence of *S. enterica* on tomatoes, *S. enterica* appeared to prioritize amino acid scavenging
287 during animal infections [12, 13].

288 Mutants in genes with a role in **purine metabolism** were strongly repressed in SEN at
289 22°C. Notably, *ΔpurA* exhibited nearly four $\log_2$-fc reductions, suggesting *purA* may be essential
290 for growth. The purine biosynthesis was previously implicated in *S.* Typhimurium colonization of
291 tomatoes [12, 13], and competition experiments confirmed a negative fitness effect for STM
292 Δ*purA* mutants on ready-to-eat muskmelon and nutrient-rich media.

293 The *pyrD* transposon mutants, deficient for this gene involved in the *de novo* pyrimidine
294 metabolism and linked to both purine and pyrimidine *de novo* synthesis [42, 43], exhibited a fitness
295 effect in SNP. Pyrimidine metabolism is required by *S.* Typhimurium infection in animals [12,
296 44], and proliferation in tomatoes [12]. However, our study found fitness effects only in SNP,
297 although associated GO terms were also enriched for SEN and SNP. This might suggest an ability
298 to scavenge pyrimidines from the environment for certain *S. enterica* strains [13].

299 Mutants in genes related to **Fe-S-cluster biosynthesis** showed fitness defects in SEN and
300 SNP. These clusters play essential roles in electron transfer, substrate binding, Fe-S storage, gene

301 expression, and enzyme activity [45]. Disruption in *hscA* and *iscA*, involved in Fe-S cluster
302 assembly [46, 47], as well as *aroA,* required for siderophore synthesis and iron uptake, were
303 detrimental during muskmelon interaction. Similar iron uptake genes were previously linked to *S.*
304 *enterica* proliferation in tomatoes [48], and *iscA* was relevant during alfalfa sprouts' colonization
305 [14], suggesting a shared iron requirement for fresh produce colonization.

306 Our analyses during the competition assays revealed that the fitness effects observed during
307 the interaction of several STM mutants with muskmelon were similar to those observed in LB
308 medium, suggesting that muskmelon provides conditions resembling a rich nutrient medium for
309 *S. enterica*.

310 An important limitation of our study was the high inoculation loads required to maintain
311 the complexity of the transposon library, which was mitigated to some extent by conducting direct
312 competition assays between individual candidate mutants and the WT. Future studies could focus
313 on individual genes, allowing lower inoculation levels and a more precise assessment of growth
314 potential. A further limitation is that several genes identified in the transposon screen were either
315 unavailable as single mutants in the collection (*serC, secAB, vacB)* or not confirmed in competition
316 assays (*hlpA, serB, ybaK)*. The latter may be due to sensitivity differences between the two
317 methods: TIS detects subtle fitness effects within a mutant library, whereas competition assays
318 measure fitness relative to the WT, potentially overlooking small but biologically relevant
319 differences. Moreover, hitchhiking mutations within the mutant pool may obscure individual gene
320 contributions, as complete mutant purity cannot always be ensured.

321 This study identified key fitness determinants enabling *S. enterica* survival on muskmelon,
322 revealing both conserved and serovar-specific adaptation mechanisms. RNA metabolism, *de novo*
323 amino acid and ribosome biogenesis, oxidative stress response, and cell wall integrity emerged as
324 central survival factors, with additional metabolic adaptations influencing fitness under specific
325 conditions. Many of these mechanisms align with known plant-endophyte interaction pathways,
326 including ROS detoxification, nitrogen fixation, flagella, and cell wall components such as LPS,

327 surface-associated proteins, or transporters [49]. Future studies should investigate the metabolic
328 interplay between *S. enterica* and plant-derived nutrients to further elucidate bacterial survival
329 strategies in fresh-produce environments.

330 Material and Methods

331 Bacterial strains and culture media

332 *S. enterica* wild type (WT) strains used in this study and their sources are listed in **Table**
333 **1**. All strains were preserved at −80°C in Luria-Bertani broth (LB; Carl Roth GmbH & Co. KG,
334 Karlsruhe, Germany) with 20% glycerol (Th. Geyer GmbH & Co. KG, Renningen, Germany).
335 Strains were grown in LB broth or 1.5% LB agar (Oxoid Deutschland GmbH, Wesel, Germany).
336 Where necessary, culture media were supplemented with 60 µg/ml kanamycin (LB^{kan}; Carl Roth),
337 20 µg/ml chloramphenicol (LB^{cm}, Carl Roth) or 15 µg/ml tetracycline (LB^{tet}; Carl Roth).

338 **Table 3.** *Salmonella enterica* wild type strains used for the barcoded transposon libraries.

Strain	Abbreviation	Source
Salmonella Typhimurium strain ATCC 14028	STM	Isolated from 4-week-old chickens in 1960 [50] NCBI accession no. CP001363.1/ CP001362.2
Salmonella Enteritidis PT4 strain P125109	SEN	Clinical strain associated with poultry outbreak in the United Kingdom [51] NCBI accession no. CP063700.1/ CP063701.1
Salmonella Newport C4.2	SNP	Isolated from a tomato field in Virginia, United States [13] NCBI accession no. GCA_006821125.1

339

340 Library construction

341 The construction of the barcoded Tn*5*-based mutant libraries has been previously described
342 [12, 13]. Briefly, a library of Tn*5* insertion mutants was constructed for each strain with a mini-
343 Tn*5* derivative into which an N_{18} random barcode was inserted via PCR. This construct was
344 randomly introduced into the genome with the EZ-Tn*5* <T7/KAN-2> promoter insertion kit

345 (Epicentre Biotechnologies, Madison, WI, United States). Transformed cells were recovered on
346 LB^{kan} agar after overnight culture at 37°C.

347 The methods for mapping the barcoded transposons to specific locations in the genome
348 have also been previously described [12]. The *S.* Typhimurium ATCC 14028 transposon mutant
349 library consisted of about 230,000 insertion mutants [12], the *S.* Enteritidis PT4 strain P125109
350 transposon mutant library consisted of about 140,000 insertion mutants [16], and the *S.* Newport
351 C4.2 transposon mutant library consisted of about 80,000 insertion mutants [13].

352 Comparative growth analysis

353 Growth curves on RTE muskmelon for all three barcoded transposon libraries were
354 assessed (eight replicates) and compared to those of the WT (five replicates) at 8°C and 22°C.
355 Inoculation and sampling were performed as described for the screenings below. The growth data
356 was subjected to an ANOVA test, where an adjusted *p*-value below 0.05 was considered
357 significant.

358 Library screening on RTE muskmelon

359 For screenings on RTE muskmelon, 300 µl frozen stocks of the *S. enterica* barcoded mutant
360 libraries were thawed and grown in LB^{kan} (Carl Roth; 60 µg/ml) at 37 °C and 200 rpm until OD_{600}
361 1.0 was reached. One ml of this culture was diluted 1:10 in phosphate-buffered saline (PBS; Carl
362 Roth) to reach approximately 8.0 $\log_{10}$ CFU/ml, centrifuged at 4.500 rcf for 5 min, the supernatant
363 discarded, and the pellet resuspended in 5 ml PBS to create the inoculum.

364 Galia muskmelons (*Cucumis melo* L. *reticulatus*) were purchased at local grocery stores.
365 For inoculation, muskmelon cubes were cut with sterile knives, and 10 g were weighed into sterile
366 filter Stomacher® bags (0.4 L, Avantor VWR International GmbH, Darmstadt, Germany). *No*
367 *matrix* samples containing only the inoculum without the muskmelon were included as negative
368 controls. RTE muskmelon samples and *no matrix* samples were inoculated with 5 ml of the

369 inoculum to reach an initial concentration of approximately 7.0 $\log_{10}$ CFU/g or ml to ensure that
370 the complexity of the library was maintained. The inoculum was evenly distributed over the
371 muskmelon samples by manually massaging the bags, ensuring the muskmelon cubes were
372 completely covered. Samples were incubated at 22°C and 8°C for 24 h and 5 days, respectively.
373 No macroscopic changes were observed in the appearance of the muskmelon cubes during the
374 incubation period.

375 Additionally, uninoculated RTE muskmelon samples were examined for total mesophilic
376 aerobic bacteria (MAB; ISO 4833-2:2013-12) and *Enterobacteriaceae* (ISO 21528-2:2004;
377 anaerobic incubation: O_2 <0.1%, CO_2 7.0-15.0%) at every sampling time point. The absence of
378 pre-existing *Salmonella* spp. in the purchased melons was confirmed by qualitative
379 microbiological analysis according to ISO 6579-1:2017.

380 Mutant libraries were recovered after 1 h (t_1), 7 h (t_7) and 24 h (t_{24}) for experiments at 22°C,
381 and after 1 h (d_1), 48 h (d_3) and 96 h (d_5). For this, the full RTE muskmelon and *no matrix* samples
382 were transferred into 90 ml LB^{kan} (Carl Roth) in 100 ml-Erlenmeyer flasks and incubated at 37°C
383 and 200 rpm for 30 min to detach the *Salmonella* from the matrix. After this, *Salmonella* spp.
384 populations were quantitatively assessed by preparing serial dilutions in PBS and plating on XLT-
385 4 agar (Oxoid) that was then incubated at 37°C for 24 h. The RTE muskmelon samples in 90 ml
386 LB^{kan} were then centrifuged at 4.500 rcf for 5 min to eliminate muskmelon juice residues, and the
387 pellet was resuspended in 90 ml LB^{kan}. Samples were then incubated for a further 7.5 h at 37°C
388 and 200 rpm before genomic DNA extraction for subsequent sequencing. Five biological
389 replicates were performed.

390 Sequencing library preparation and data analysis

391 Aliquots of 40 µl from each sample were washed three times in sterile water, pelleted and
392 then lysed with proteinase K (Sigma-Aldrich; 100 µg/ml) in 20 µl 10 mM Tris [pH 8.0] with 1 mM
393 EDTA (Carl Roth) and 0.1% Triton X-100 (Carl Roth) for 2 h at 55°C. After enzyme inactivation

394 at 95°C for 10 min, 5 µl of extracts were subjected to PCR with indexing primers targeted to the
395 regions directly adjacent to the barcode on the transposon (0.4 µM Illumina-barcoded Primers L
396 and V, listed in **Table S1**). The PCR was carried out using the following thermal cycling
397 conditions: an initial denaturation step at 98°C for 2 min, followed by 10 cycles of denaturation at
398 98°C for 10 seconds, annealing at 65°C for 10 seconds, and extension at 72°C for 20 seconds. This
399 was followed by an additional 20 cycles consisting of denaturation at 98°C for 10 seconds and
400 extension at 72°C for 20 seconds. A final extension was performed at 72°C for 2 minutes. The
401 reaction was subsequently held at 20°C. The Q5™ Hot Start High-Fidelity 2x Master Mix (Item
402 number M0494L; New England Biolabs, Frankfurt am Main, Germany) was added at a final
403 concentration of 1x. Equal volumes of PCR products were pooled and purified using the QIAquick
404 PCR Purification Kit (Qiagen, Item number 28106), following the manufacturer's instructions.
405 Illumina sequencing was performed as a dual-indexed paired-end 50, 100 or 150-base run on a
406 NovaSeq 6000 with at least one million reads/sample.

407 Raw reads were binned based on their indexes and analyzed using custom Python scripts
408 to identify and enumerate barcodes bordered by the expected invariable DNA sequence. Barcode
409 insertions were compiled into an aggregated count for each disrupted gene. The differences in the
410 aggregated abundances of the different mutants between time points, as well as between the matrix
411 and *no matrix* samples for each sampling time point, were statistically analyzed using DESeq2
412 [52] and $\log_2$-fold changes (fc) were reported (**Table S2**).

413 Mutations were considered to have a significant fitness effect for *S. enterica* on
414 muskmelons if they met a dual requirement for at least one sampling time point: (i) they showed
415 a two-fold change (i.e., $\log_2$-fc >|1.0|) with an adjusted *p*-value ≤0.01 in the comparison of the
416 matrix versus *no matrix* samples, and (ii) fulfilled the same criteria for a comparison between the
417 inoculum and the matrix sample at the same sampling time point. If criterion (i) was achieved at
418 the final timepoint (t_{24} or d_5), criterion (ii) was also deemed fulfilled if in comparisons the first
419 timepoint (t_1 or d_1) was used instead of the inoculum (i.e. t_1 matrix versus t_{24} matrix; d_1 matrix

420 versus d_5 matrix), reflecting interaction mechanisms *after* the adaptation to the matrix. This strict
421 set of criteria was implemented to identify the genes with the most significant role during bacterial
422 growth on muskmelon.

423 To generate gene lists for the KEGG [53]- and GO [54-56] analysis input, the above *p*-
424 value criterion of $p_{adj} \leq 0.01$ was relaxed to $p_{adj} < 0.05$. GO enrichment analysis was carried out using
425 TopGO [57] and KEGG enrichment analysis used enrichKEGG from ClusterProfiler [58] in R
426 (v.4.4.1). For KEGG results, pathways with *q*-values≤0.01 were considered significant. Resulting
427 GO terms with classic Fisher *p*-values≤0.01 were considered significant.

428 Competition assays

429 To confirm the phenotype of candidate genes identified using TIS, single-gene deletion
430 (SGD) mutants in *S*. Typhimurium 14028s were used in direct competition with the WT strain.
431 These mutants were obtained from an existing SGD library [17] in STM that includes two mutants
432 for a majority of all genes, one mutant harboring a kanamycin resistance gene in the sense direction
433 of the deleted gene (SGD-Kan^R) and one mutant harboring a chloramphenicol resistance gene in
434 the antisense direction (SGD-Cm^R). The WT (*S.* Typhimurium 14028s) used in the competition
435 experiments was isogenic except for a tetracycline resistance cassette inserted in the neutral *malXY*
436 genome location (WT_{STM}-Tet^R).

437 SGD mutants and the WT_{STM}-Tet^R were grown in 5 ml LB^{kan}, LB^{cm}, and LB^{tet}, respectively,
438 at 37°C with 200 rpm shaking until approximately OD_{600} 0.9 was reached. For each selected gene,
439 the different SGD cultures were mixed in a 1:1:1 ratio (or 1:1 if only one SGD mutant was available
440 in the collection). The SGD-WT_{STM}-Tet^R mix was then pelleted, resuspended in PBS, and 5 ml
441 were inoculated onto 10 g RTE muskmelon at a concentration of approximately 7.0 $\log_{10}$ CFU/g
442 and screened as previously described. Post-screening, samples were plated on LB^{kan}, LB^{cm}, and
443 LB^{tet} agar and incubated at 37°C for 24 h to assess changes in population ratios. All mutants were
444 similarly tested in competition assays in LB broth to investigate whether any genes played a role

445 specific to growth on muskmelon or whether their role was universal in a nutrient-rich
446 environment. Furthermore, some mutants were also tested in competition assays in PBS to confirm
447 lack of differential death in the absence of nutrients. Competition assays in PBS and LB were
448 inoculated at approximately 7.0 $\log_{10}$ CFU/ml. All cultures were tested for cross-resistance before
449 being combined into a mixed inoculum.

450 The mutant phenotype was confirmed by comparing the changes in colony counts between
451 samplings in the respective sense and antisense mutants and the WT_{STM}-Tet^R. If the competitive
452 index (the ratio of mutant/WT ratios of timepoint/inoculum) plus standard deviation from at least
453 three experiments remained below 1, a negative fitness effect was deemed confirmed. If the
454 competitive index minus standard deviation remained higher than 1, a positive fitness effect was
455 deemed confirmed. Competition assays were performed in triplicate, except when no fitness effect
456 was observed in the first two replicates.

457 **Table 4.** Fitness effect in selected *S.* Typhimurium 14028s gene deletion mutants in competition with the
458 wild type.

Condition	STM Gene Number	Gene	Gene Function	Transposon assays#			Competition assays														
							muskmelon					LB					PBS				
				STM	SEN	SNP	CI sense	stdev sense	CI antisense	stdev antisense	outcome*	CI sense	stdev sense	CI antisense	stdev antisense	outcome*	CI sense	stdev sense	CI antisense	stdev antisense	outcome*
22°C, 24h	STM14_3962	***deaD***	ATP-dependent RNA helicase DeaD	-	-	(-)	0.28	0.45	0.03	0.02	-	0.34	0.54	0.04	0.02	-	1.01	0.30	1.34	0.14	O
	STM14_3964	***pnp***	Polynucleotide phosphorylase/polyadenylase	-	-	-	0.04	0.03	0.04	0.03	-	1.58	2.67	1.53	2.60	O	0.77	0.45	0.52	0.20	O
	STM14_5328	***yjgA***	Ribosome associated protein	-	-	-	0.08	0.08			-	0.11	0.12			-	1.27	0.46			O
	STM14_4959	*oxyR*	DNA-binding transcriptional regulator OxyR	(- / +)	(- / +)	(- / +)	0.55	0.33	0.23	0.35	-	0.19	0.24	0.01	0.01	-	0.74	0.29	0.38	0.24	O
	STM14_3699	***serA***	Phosphoserine phosphatase	(-)	-	-	0.43	0.17	0.35	0.07	-	1.21	0.51	1.29	0.77	O	1.25	0.67	1.20	0.97	O
	STM14_5499	*serB*	Phosphoserine phosphatase	(-)	(-)	-	0.86	0.47	0.57	0.24	O	0.76	0.38	0.67	0.29	O	1.29		0.59		O
	STM14_0583	*ybaK*	Putative cytoplasmic protein	(-)	-	(-)	1.30	1.88	1.06	1.68	O	0.97	0.31	0.69	0.14	O	2.16	1.13	1.50	1.23	O
	STM14_2587	*rfbI_2*	CDP-6-deoxy-delta-3,4-glucose reductase				1.58	0.30	0.96	0.17	O	1.36	0.24	0.96	0.15	O					
8°C, 5d	STM14_3066	***ppk***	Polyphosphate kinase	-	(-)	-	0.05	0.03	0.10	0.10	-	0.07	0.00	0.11	0.04	-	2.56	2.54	1.87	1.44	O
	STM14_3240	***srmB***	ATP-dependent RNA helicase SrmB	-	-	-	0.20	0.08	0.17	0.10	-	0.22	0.09	0.19	0.13	-	1.37	0.27	0.93	0.05	O
	STM14_4639	**STnc400**	sRNA	-	n.d.	n.d.			0.15	0.06	-			0.18	0.11	-			1.15	1.13	O
	STM14_4822	*typA*	GTP-binding protein	-	-	-	0.18	0.28	0.40	0.64	O	0.07	0.10	0.12	0.19	-	0.93		0.92	0.08	O
	STM14_4713	*trxA*	Thioredoxin	-	(-)	(-)	0.52	0.20	0.95	0.33	O	0.35	0.01	0.63	0.18	-					
	STM14_4722	*wecD*	TDP-fucosamine acetyltransferase	-	(-)		1.16	0.60	0.55	0.21	O	0.72	0.26	0.74	0.43	O					
	STM14_4723	*wecE*	TDP-4-oxo-6-deoxy-D-glucose transaminase	+ / -	(-)	(-)			2.61	1.42	+			1.58	0.23	+					
	STM14_4725	***wecF [D]***	4-alpha-L-fucosyltransferase	(-)			0.34	0.29	0.32	0.25	-	0.31	0.12	0.29	0.10	-	1.54	0.75	1.05	0.49	O
	STM14_0267	*hlpA*	Periplasmic chaperone	-	(-)		1.94	1.18			O	1.26	0.81			O					
	STM14_4817	***glnG***	Nitrogen regulation protein NR(I)	(- / +)	(-)		2.39	1.38	1.96	0.55	+	1.58	1.15	1.28	0.50	O					
	STM14_5478	***prfC***	Peptide chain release factor 3	-	-	(-)	0.32	0.11	0.40	0.23	-	0.25	0.16	0.30	0.17	-	2.09	1.64	2.23	1.08	O
	STM14_2196	***rnd***	Ribonuclease D	-	(-)		0.42	0.17	0.32	0.10	-	0.23	0.06	0.22	0.06	-	0.74	0.01	1.05	0.28	O
	STM14_5372.J	***holC***	DNA polymerase III subunit chi	-			0.05	0.05			-	0.01	0.01			-	1.05	0.12			O

STM14_5475	***holD***	DNA polymerase III subunit psi	-		n.d.	0.01	0.00			-	0.01	0.01			-	0.90	0.43			○
STM14_3077	*xseA*	Exodeoxyribonuclease VII large subunit	-			1.37	0.11	0.02	0.02	○	0.87	0.70	0.02	0.02	○	1.68	1.28	0.64	0.19	○
STM14_5248	***purA***	Adenylosuccinate synthetase	(-)	(-)		0.41	0.25			-	0.61	0.12			-					
STM14_2205	***yoaH***	Hypothetical protein in *fadD-pabB* IR	-		(-)	0.16	0.05	0.15	0.03	-	0.08	0.01	0.05	0.04	-	0.77	0.49	1.42	1.16	○

From two to four separate experiments. Genes in **bold** are where the STM transposon assay was confirmed in the competition assays. Genes in **bold** and <u>underlined</u> were important for the growth of *Salmonella* on muskmelon but not in LB.

CI = $(\text{mutant/wild type})_{\text{condition}}$ / $(\text{mutant/wild type})_{\text{inoculum}}$.

#, Fitness effects, as described in **Table 2**.

* Fitness effects: -, single-gene deletion mutants with a significant growth disadvantage in competition assays compared to wild type (CI + stdev < 1); +, advantage in competition assays compared to wild type (CI - stdev > 1); ○, no effect or contradicting Tn data.

Sense – single-gene deletion mutant with a kanamycin resistance cassette inserted in the sense orientation of the gene; antisense – single-gene deletion mutant with a chloramphenicol resistance cassette inserted in the antisense orientation of the gene.

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Supporting Information

Supplementary Figure S1. Growth of the *Salmonella enterica* barcoded transposon mutant libraries (lib) and the corresponding wild type strains (wt) on ready-to-eat muskmelon at 22°C (A) and 8°C (B).

Supplementary Figure S2. Quantitative assessment of the *Salmonella enterica* barcoded transposon mutant libraries.

Supplementary Figure S3. Quantitative assessment of the background microbiota on the ready-to-eat muskmelon samples.

Supplementary Figure S4. Changes in the mutant abundances after growing the random transposon libraries on ready-to-eat muskmelons.

Supplementary Table S1. Primers used for library preparation prior Illumina sequencing.

Supplementary Table S2. DESeq2 statistical analysis for the transposon insertion sequencing experiments of the three *Salmonella enterica* libraries on muskmelon.

Supplementary Table S3. Results of the KEGG- and GO- enrichment analysis.

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