

# Deciphering the Interaction of *Salmonella enterica* subsp. *enterica* with Ready-to-eat Melon

Irene Esteban-Cuesta<sup>1</sup>, Steffen Prowollik<sup>2</sup>, Weiping Chu<sup>2</sup>, Michael McClelland<sup>2</sup>, Claudia Guldimann<sup>1</sup>

<sup>1</sup> Chair of Food Safety and Analytics, LMU Munich, Oberschleissheim, Germany

<sup>2</sup> Department of Microbiology and Molecular Genetics, University of California, Irvine, Irvine, USA



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Dr. Irene Esteban Cuesta, 1<sup>st</sup> Year Resident, Food Safety Subspecialty

Chair of Food Safety and Analytics, Veterinary Faculty, LMU Munich, Germany



- Background
- Methodology
- Results
- Outlook

# *Salmonella enterica* subsp. *enterica*

- *Salmonella enterica* is one of the most important foodborne pathogens worldwide and salmonellosis was the second most commonly reported zoonosis in 2020.
- Infection mainly results from the consumption of contaminated food.
- Generalists and host-adapted serovars



Source: BAV Institut

# *Salmonella enterica* subsp. *enterica*

- Foodborne outbreaks mainly linked to egg products, and poultry
- Other sources of concern: Ready-to-eat
  - No inactivation measures



# *Salmonella enterica* subsp. *enterica*

## ■ The aim of the project:

To understand the molecular mechanisms behind the resilience of *S. enterica* subsp. *enterica* towards the stress conditions in ready-to-eat (RTE) melon.

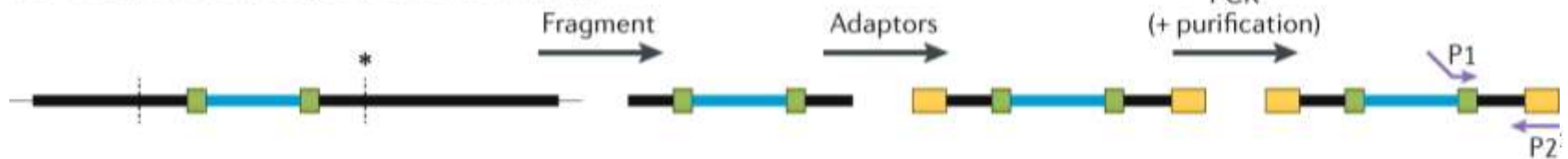
Find the genetic determinants for survival on this specific food matrix.



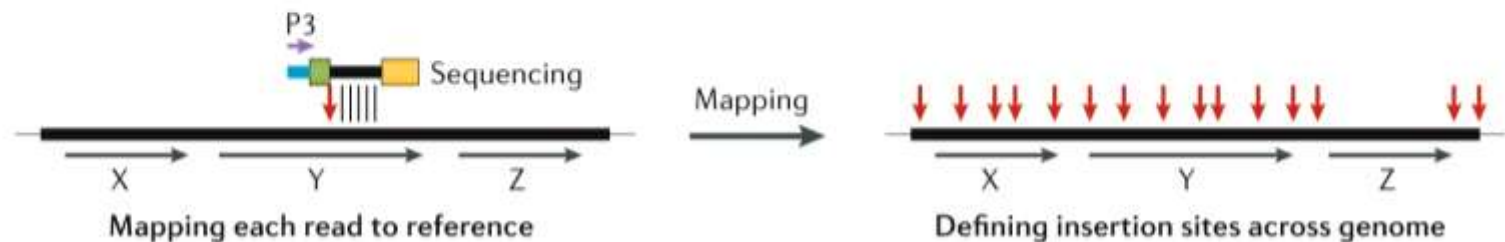
Source: BAV Institut

# Transposon Direct Insertion Sequencing

## Ac Fragment, add adaptors and PCR amplify



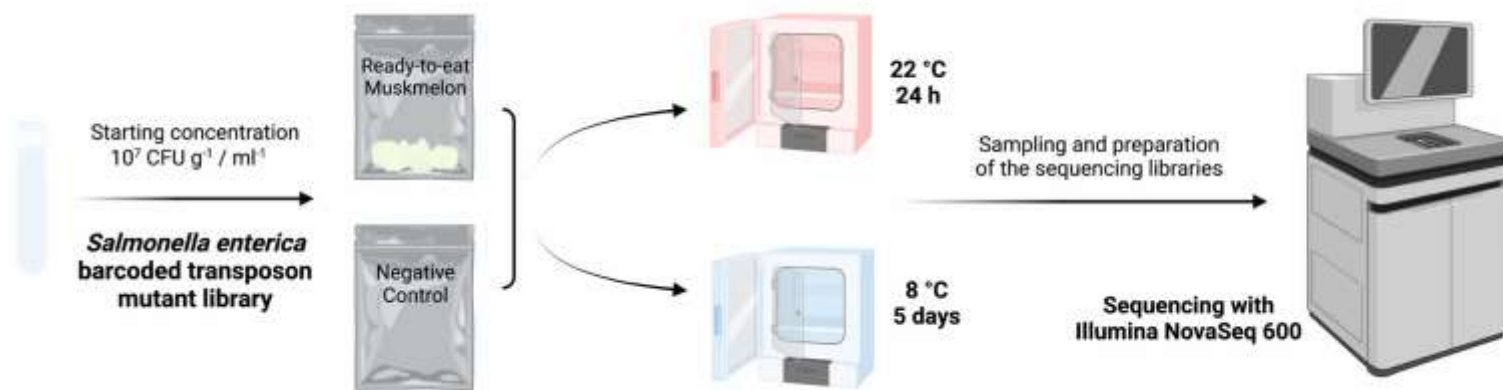
## Ad Sequencing and mapping



Cain, A. K., Barquist, L., Goodman, A. L. et al. A decade of advances in transposon-insertion sequencing. *Nat Rev Genet* **21**, 526–540 (2020). <https://doi.org/10.1038/s41576-020-0244-x>

# Screening of the Libraries on RTE Melon

TraDIS Libraries on *S. enterica* serovars Typhimurium 14028s, Enteritidis PI25109 and Newport C4.2 were screened on RTE melon.



Figures created with biorender.com

# Results of the screening of the *S. enterica* serovars on ready-to-eat melon

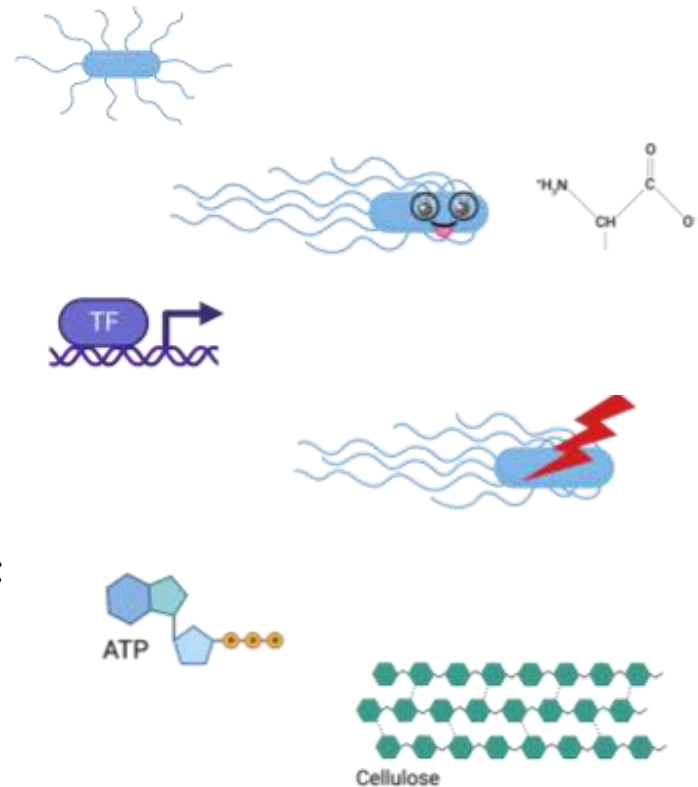
DNA strand	Gene	Gene function	No of barcodes	8 °C			22 °C		
				IM STM Inoc vs d5	IM SEN Inoc vs d5	IM SNP Inoc vs d5	IM STM Inoc vs t24	IM SEN Inoc vs t24	IM SNP Inoc vs t24
+	ppk	polyphosphate kinase; catalyzes the reversible transfer of the terminal phosphate of ATP to form a long chain polyphosphate	99	x	x	x		x	x
-	cpsA	two-component sensor protein; part of two-component CpsA/CpsR system; senses envelope stress; upregulates a number of periplasmic folding and trafficking factors	82	x	x		x	x	x
+	hspA	periplasmic chaperone; (SW:DMPH_SALTY); histone-like protein	18	x	x	x		x	x
+	flgG	Flagellar basal body rod protein FlgG; makes up the distal portion of the flagellar basal body rod	35	x	x	x	x		
-	speA	arginine decarboxylase; catalyzes the formation of agmatine from arginine in putrescine and spermidine biosynthesis	20				x	x	x
-	yjgA	hypothetical protein; similar to Escherichia coli putative alpha helix protein (AACF79.)	19	x				x	x
+	dsbA_2	periplasmic protein disulfide isomerase I; thiol-disulfide interchange protein DsbA precursor (SW:DSBA_SALTY)	32	x		x	x		
-	serA	D-3-phosphoglycerate dehydrogenase; catalyzes the formation of 3-phosphonoacetylpyruvate from 3-phospho-D-glycerate in serine biosynthesis; can also reduce alpha ketoglutarate to form 2-hydroxyglutarate	52		x		x	x	x
-	prc	carboxy-terminal protease; involved in the cleavage of a C-terminal peptide of residues from the precursor form of penicillin-binding protein 3	39				x	x	x
-	ebcC [D1_2]	hypothetical protein; protein YbaK (SW:YBAK_SALTY)	10				x	x	x
+	serS_2	phosphoserine phosphatase; catalyzes the formation of serine from D-phosphoserine	40				x	x	x
+	asnA	asparagine synthetase AsnA; catalyzes the formation of asparagine from aspartate and ammonia	50				x		x
+	flhI	flagellar assembly protein H; binds to and inhibits the function of flagella specific ATPase FlhI	24	x	x		x		
+	argG	argininosuccinate synthase; catalyzes the formation of argininosuccinate from citrulline and aspartate in arginine biosynthesis	15				x	x	x
+	cydG	siroheme synthase; multifunction enzyme consisting of uroporphyrin-III C-methyltransferase, precorrin-2 dehydrogenase and sirohydrochlorin ferrochelatase; catalyzes the methylation of uroporphyrinogen III to form precorrin-2, then catalyzes formation of sirohydrochlorin from precorrin-2 and finally catalyzes the formation of siroheme from sirohydrochlorin	28				x	x	x
+	argH	argininosuccinate lyase; catalyzes the formation of arginine from (N-L-arginino) succinate	43				x	x	x
+	emrR	transcriptional repressor MprA, DNA-binding transcriptional repressor of microcin B7 synthesis and multidrug efflux; negative regulator of the multidrug operon emrAB	7				x		x
-	yhjR	putative cytoplasmic protein	46	x			x		x
-	yhjR	cellulose synthase regulator protein; binds the cellulose synthase activator, bis-(3'-5')cyclic diguanylic acid (c-di-GMP)	73	x				x	
-	nagB	glucosamine-6-phosphate deaminase; catalyzes the reversible formation of fructose 6-phosphate from glucosamine 6-phosphate	33				x	x	x
-	glnG	nitrogen regulation protein NR(G); response regulator of a two-component regulatory system involved in the activation of nitrogen assimilation genes; interacts with sigma-54	48	x			x		
-	ecrK	putative regulatory protein; LuxR family	15	x	x		x		x
+	oxyR	DNA-binding transcriptional regulator OxyR; Activates the expression of a regulon of hydrogen peroxide-inducible genes such as katG, gor, ahpC, ahpF, oxyS, fop, fur and grxA	95	x	x		x	x	x
+	metL	homofunctional aspartate kinase II/homoserine dehydrogenase II; multifunctional homodimeric enzyme that catalyzes the phosphorylation of aspartate to form aspartyl-4-phosphate as well as conversion of aspartate semialdehyde to homoserine; functions in a number of amino acid biosynthetic pathways	25	x		x		x	x

red means underrepresented; green means overrepresented; Inoc: Inoculum; STM: *S. enterica* Typhimurium; SEN: *S. enterica* Enteritidis; SNP: *S. enterica* Newport; WT: wildtype; IM: individual mutants; x: genes selected for further analysis; +: sense DNA strand; -: antisense DNA strand; d5: day five; t24: time 24 hours

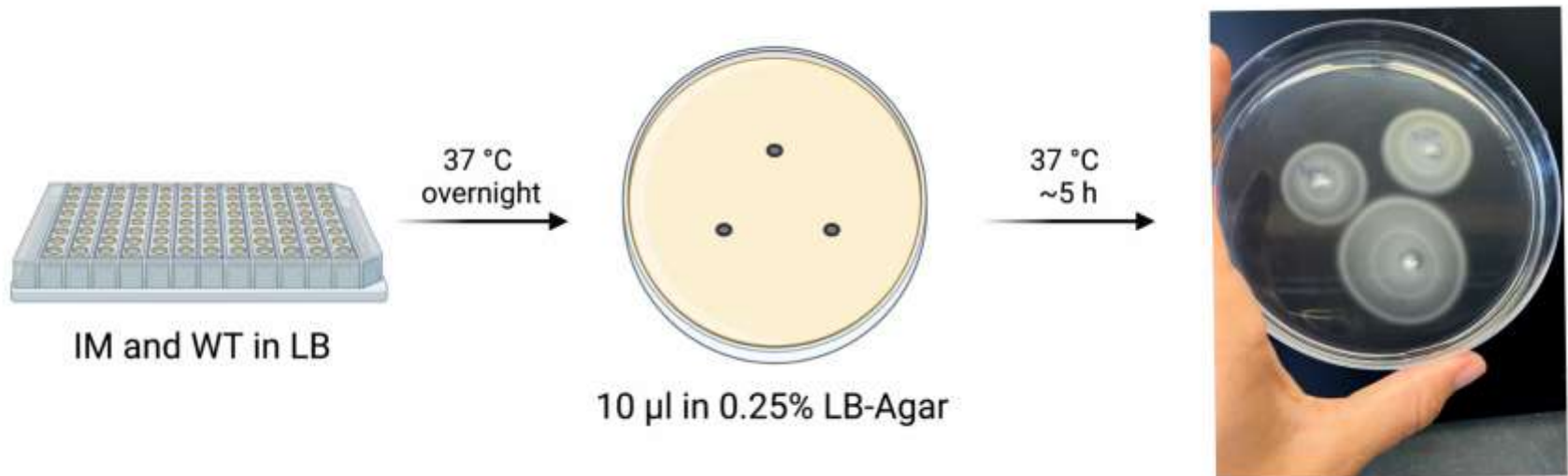


# Conclusions and Outlook

- Genes involved in flagellar biosynthesis:  
cpxA, hlpA, flgG, fliH, speA, dsbA, emrR
- Genes involved in amino acid biosynthesis:  
serA, asnA
- Genes that are transcriptional regulators:  
emrR, oxyR
- Genes involved in the stress response:  
cpxA, speA, dsbA, prc, ppk
- Genes involved in ATP production and homeostasis:  
argG, argH, asnA, ppk
- Genes involved in cellulose production:  
yhjN, yhjR



# Screening of the Libraries on RTE Melon



Figures created with biorender.com

## Conclusions and Outlook

No known virulence genes were significantly affecting the resilience of *Salmonella enterica* on RTE melon.

Based on these results, competition assays with single gene deletions are currently ongoing in our lab.

New Libraries were created

New screenings are planed





*thank you*



**Chair of Food Safety and Analytics, LMU Munich, Oberschleissheim, Germany**  
**Department of Microbiology and Molecular Genetics, University of California, Irvine, Irvine, USA**

Chair of Food Safety and Analytics, Veterinary Faculty, LMU Munich

