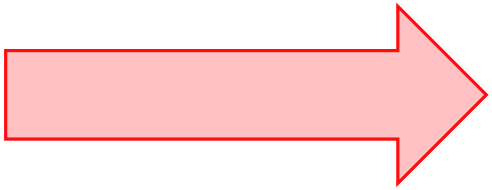
That which does not kill us makes us stronger.

-Friedrich Nietzsche

Characterization of Bacteriophage Insensitive *E .coli* O157:H7 Mutants

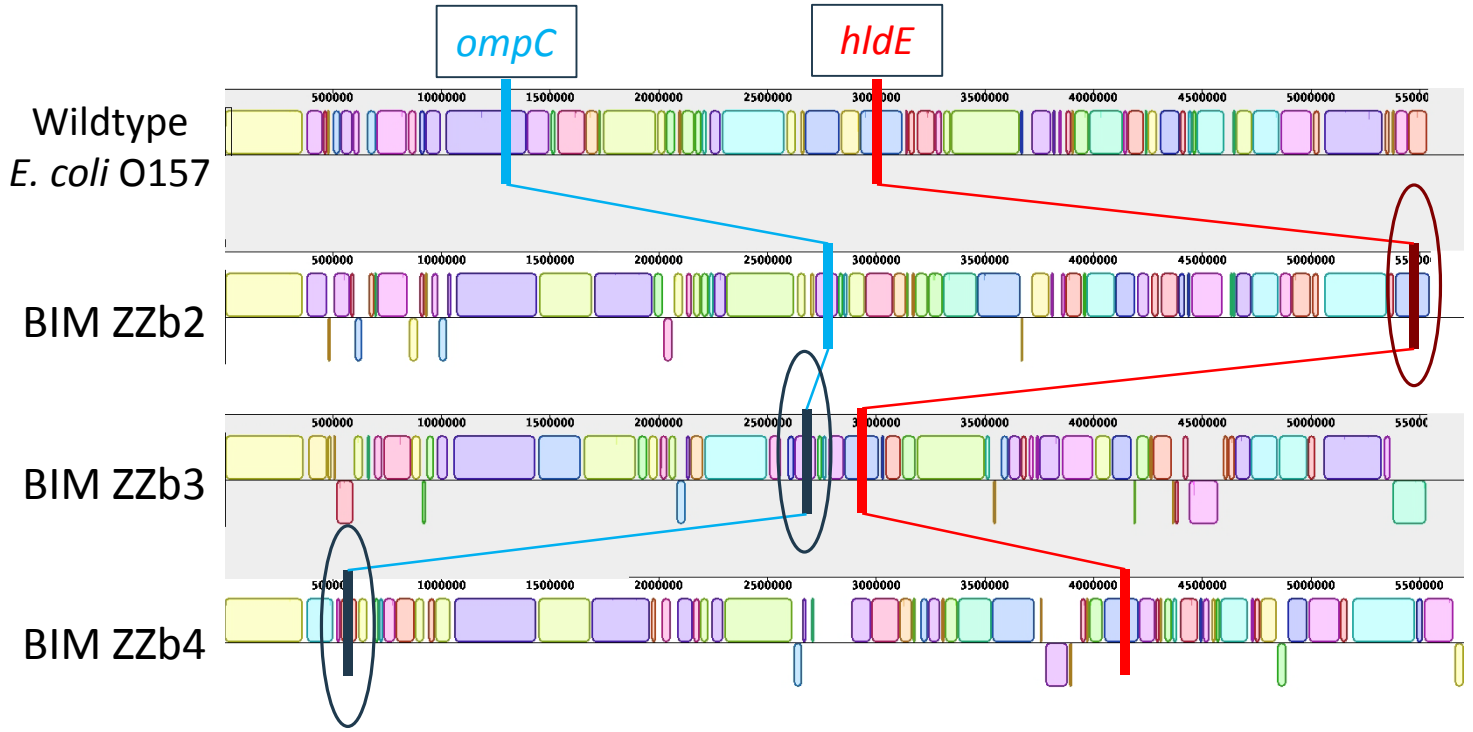
Emergence of bacteriophage insensitive mutants (BIMs)



"Stronger"??

Zeyan Zhong
McGill University

Genomic Comparisons



- Three *E. coli* O157:H7 BIMs isolated from phage AR1 infection
- Mutations found in phage receptors related gene *ompC* and *hldE*, resulting in altered membrane permeability

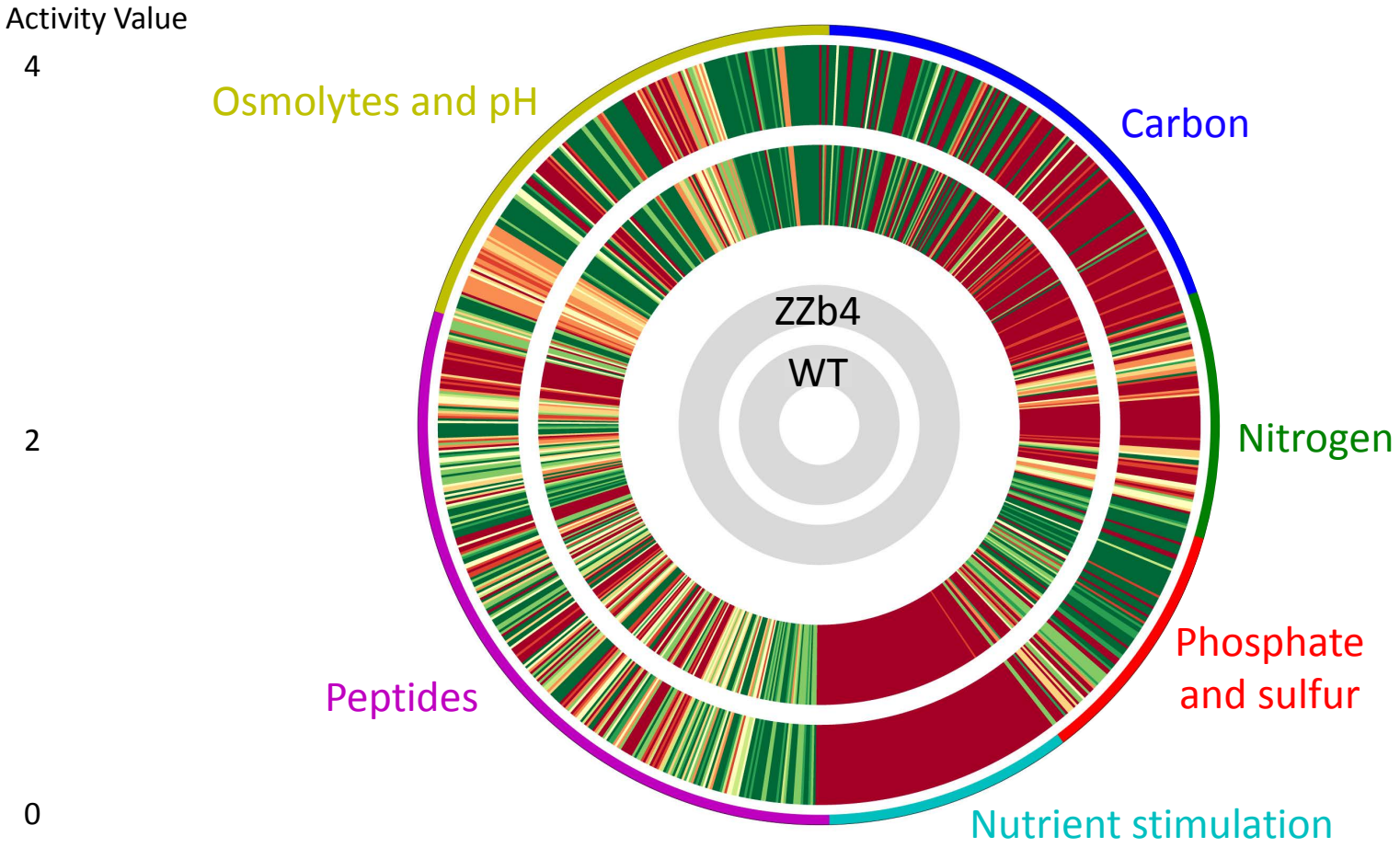
MICs to Various Substrates

	Substrates	Wildtype	ZZb2 (*hldE*)	ZZb3 (*ompC*)	ZZb4 (*ompC*)
OmpC-mediated	Ampicillin (ug/ml)	2.8	1.7*	2.7	1.7*
	Cefotaxime (ug/ml)	0.125	0.125	0.125	0.0625*
LPS-mediated	Novobiocin (ug/ml)	100	25*	100	100
	EDTA (mg/ml)	25	12.5*	12.5*	12.5*
	SDS (mg/ml)	>200	0.125*	>200	>200

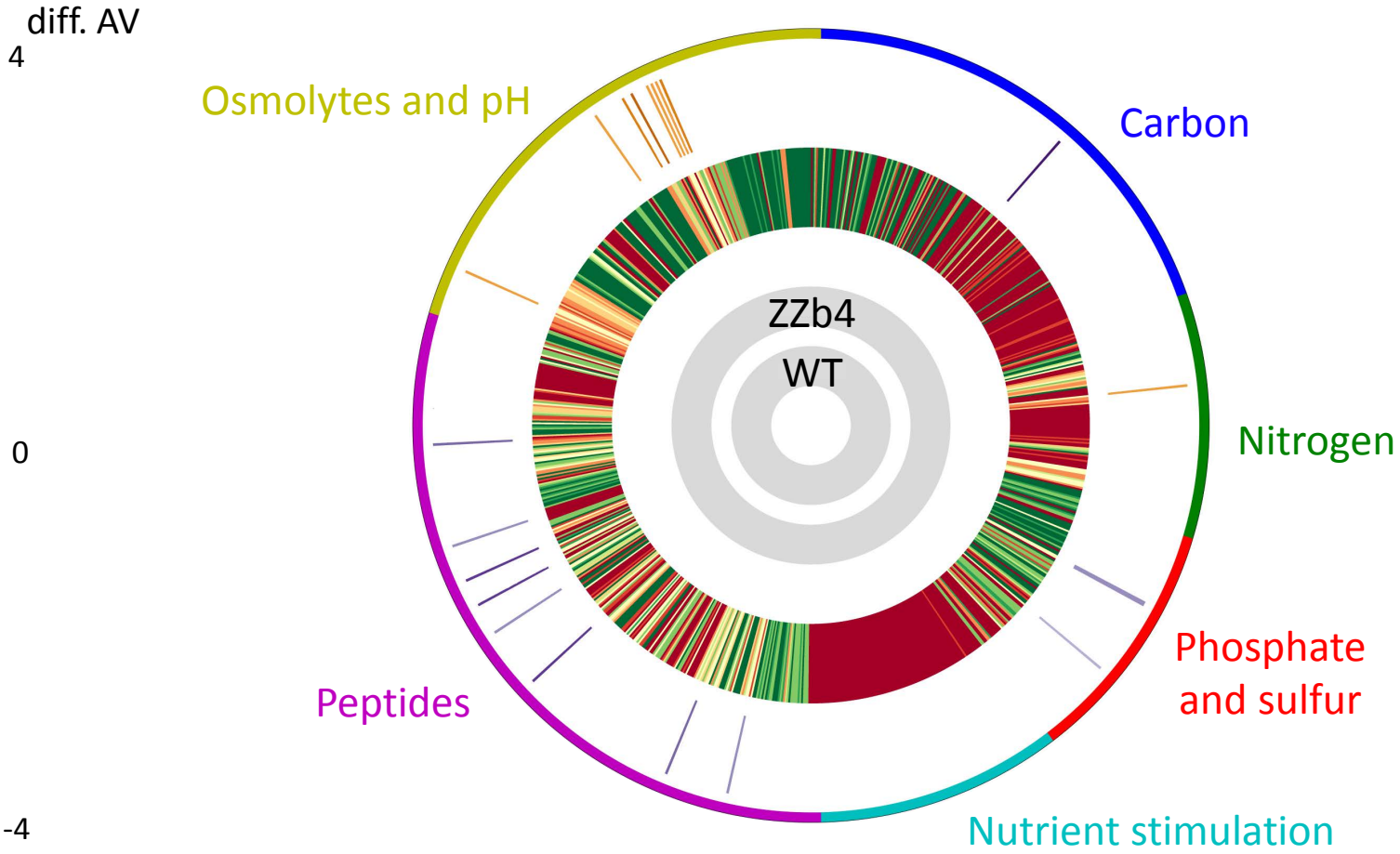
*. P-value<0.05

- Deep rough *E. coli* BIMs showed hypersensitivity to SDS (supported by unpublished data)
- Mutations in *ompC* confer to diversified membrane permeabilities. (Delcour, 2008)

Phenotypic Comparison



Phenotypic Comparison



Differences in Metabolisms

PM plate	Well#	Compound	Category	WT AV	ZZb4 AV	diff. AV
PM02	B02	N-Acetyl-Neuraminic acid	carbon	0	3.5	3.5
PM03	C08	D-Serine	nitrogen	2	0	2
PM04	C03	D-Glucose-1-Phosphate	phosphate & sulphur	2	4	2
PM04	C04	D-Glucose-6-Phosphate	phosphate & sulphur	2	4	2
PM06	C10	Asp-Glu	nitrogen peptides	0.5	2.5	2
PM06	E12	Gly-Ser	nitrogen peptides	1.5	4	2.5
PM07	C06	Met-Pro	nitrogen peptides	0	3	3
PM07	E07	Ser-Pro	nitrogen peptides	1	3	2
PM07	F08	Trp-Asp	nitrogen peptides	0	3	3
PM07	G07	Tyr-Glu	nitrogen peptides	0	3	3
PM07	H10	Val-Tyr	nitrogen peptides	0	2	2
PM08	D04	Pro-Ser	nitrogen peptides	0.5	3	2.5
PM09	B04	6% NaCl + Sarcosine	osmolytes & pH	2	0	2
PM10	A03	pH 4.5	osmolytes & pH	3	1	2
PM10	B04	pH 4.5 + L-Asparagine	osmolytes & pH	2.5	0	2.5
PM10	B08	pH 4.5 + Glycine	osmolytes & pH	3	0	3
PM10	C03	pH 4.5 + L-Proline	osmolytes & pH	2	0	2
PM10	C05	pH 4.5 + L-Threonine	osmolytes & pH	2	0	2
PM10	C07	pH 4.5 + L-Citrulline	osmolytes & pH	2	0	2
PM10	C09	pH 4.5 + Hydroxy-L-Proline	osmolytes & pH	3	0.5	2.5

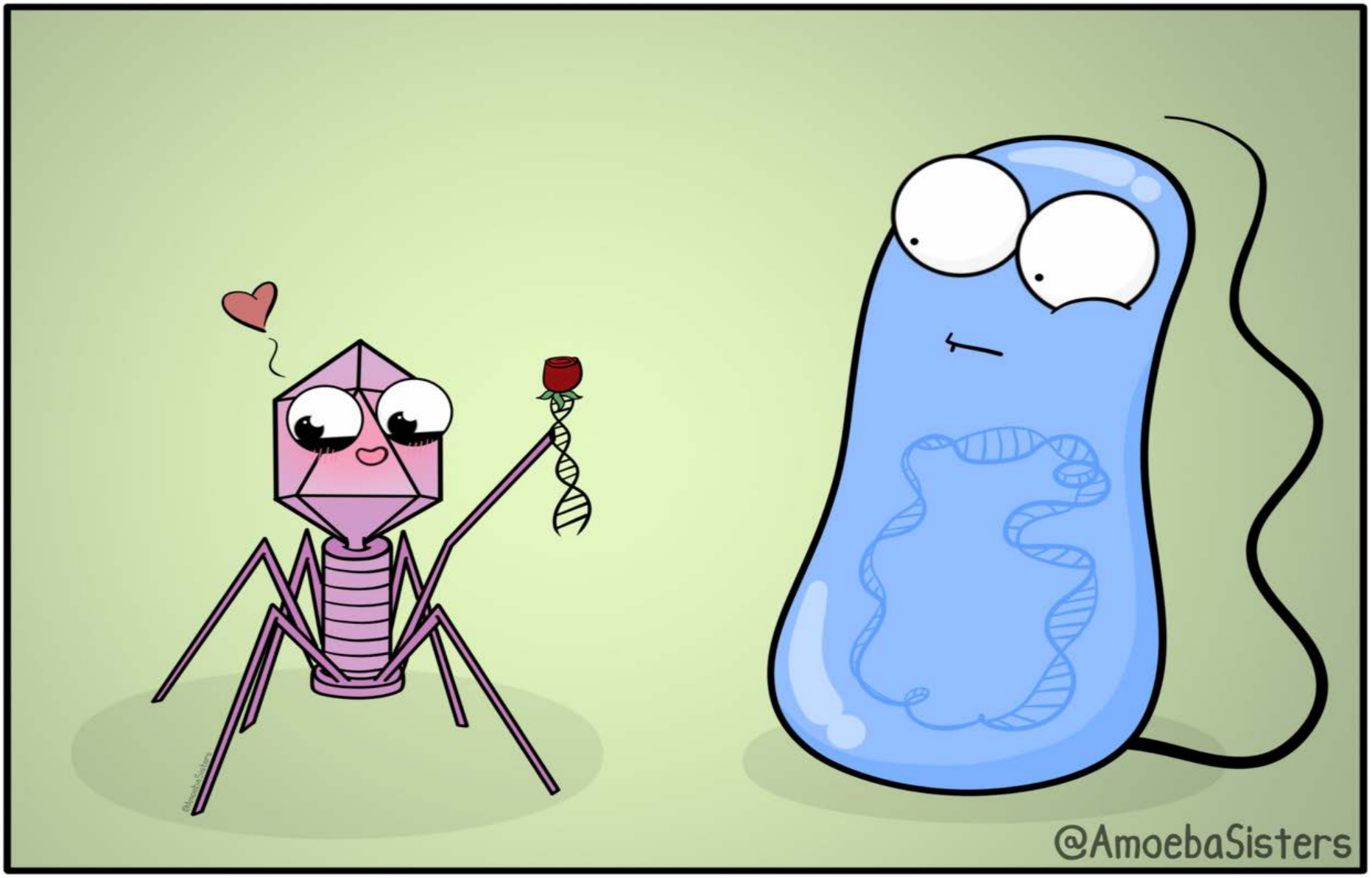
- Disrupted outer membrane might increase the permeability for dipeptides. (Delcour, 2008)
- Lower tolerance in low acid might due to the loss of OmpC-conferred acid resistance. (Bekhit et al., 2011)

Summary

- Mutations in genes *hldE* and *ompC* of *E. coli* O157:H7 mutants confer resistance to phage AR1, along with subsequent fitness changes that affect bacterial susceptibility to antibiotics and detergents.

- Altered OmpC of BIM ZZb4 likely results in lower tolerance to acid conditions, suggesting that bacteriophage insensitive mutants may less likely survive in low acid foods.

Paramecium Parlor



Billy the bacterium had to quickly consider the etiquette for declining a bacteriophage's valentine.