New insights into food preservation stress responses in *Listeria monocytogenes* using comparative genomics

Conor O'Byrne,
Teagasc Ashtown, May 24th 2023

Thanks to…

Jialun Wu

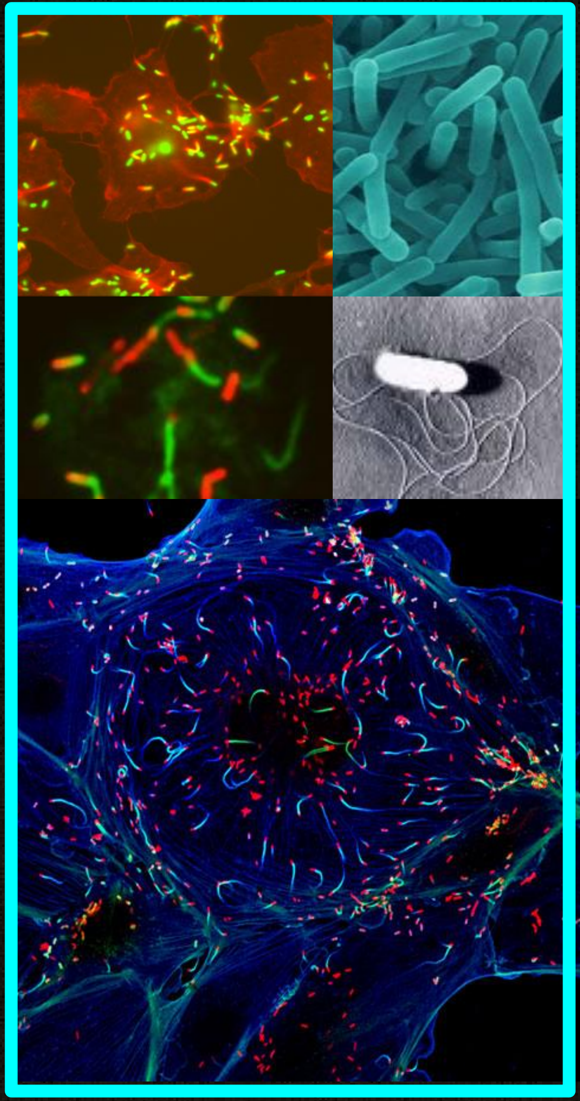
- Kieran Jordan
- Olivia McAuliffe

Teagasc
Agriculture and Food Development Authority

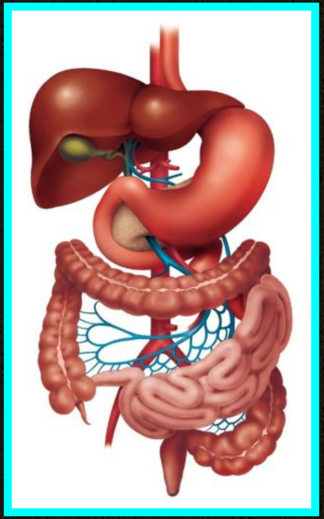
- Good luck to Jialun for his PhD defence – next Friday!
- And thanks to his examiner Prof Fabian Commichau!

Talk Overview

- *Listeria monocytogenes*
- Background on acid resistance
- Characterisation of strain 1381
  - Role of Mn^{2+} in low pH growth
  - Identification of a new regulator of acid resistance
- Summary/conclusions



Listeria monocytogenes: A Pathogen Adapted for Environmental Survival



- Listeria belongs to phylum Bacillota (formerly the Firmicutes) – found widely in the environment
- Important food-borne pathogen that can invade and replicate in human cells
- Grows over a wide range of pHs (4.3-11.0) and survives down to pH 2.0
- Grows at refrigeration temperatures (as low as 0ºC)
- Grows at salt concentrations of up to 2M
- Serious problem for RTE-food producers

Teagasc escapee visits Unilever!

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0099-2240/99/$04.00+0
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Survival of Low-pH Stress by *Escherichia coli* O157:H7: Correlation between Alterations in the Cell Envelope and Increased Acid Tolerance

KIERAN N. JORDAN,[1,2] LYNN OXFORD,[1] and CONOR P. O'BYRNE[1]*

Microbiology Department, Unilever Research Colworth, Sharnbrook, Bedfordshire MK44 1LQ, England,[1] and TEAGASC, National Dairy Products Research Centre, Moorepark, Fermoy, County Cork, Ireland[2]

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Adaptive Acid Tolerance: Two Landmark Papers!

Microbiology (1996), **142**, 2975–2982

Printed in Great Britain

Acid tolerance in *Listeria monocytogenes*: the adaptive acid tolerance response (ATR) and growth-phase-dependent acid resistance

Mark J. Davis, Peter J. Coote and Conor P. O'Byrne

Author for correspondence: Conor P. O'Byrne. Tel: +44 1234 222377. Fax: +44 1234 222277.

Microbiology Department, Unilever Research, Colworth Laboratory, Sharnbrook, Bedfordshire MK44 1LQ, UK

Listeria monocytogenes **acquired increased acid tolerance during exponential growth upon exposure to sublethal acid stress, a response designated the acid tolerance response (ATR). Maximal acid resistance was seen when the organism was exposed to pH 5·0 for 1 h prior to challenge at pH 3·0, although intermediate levels of protection were afforded by exposure to pH values**

Applied and Environmental Microbiology, May 1996, p. 1693–1698

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Adaptive Acid Tolerance Response in *Listeria monocytogenes*: Isolation of an Acid-Tolerant Mutant Which Demonstrates Increased Virulence

BRID O'DRISCOLL, CORMAC G. M. GAHAN, AND COLIN HILL*

Department of Microbiology and National Food Biotechnology Centre, University College Cork, Cork, Ireland

Received 4 December 1995/Accepted 2 March 1996

The ability of *Listeria monocytogenes* to tolerate low-pH environments is of particular importance because the pathogen encounters such environments in vivo, both during passage through the stomach and within the

Summary of what we know about Acid tolerance/Resistance in *L. monocytogenes*

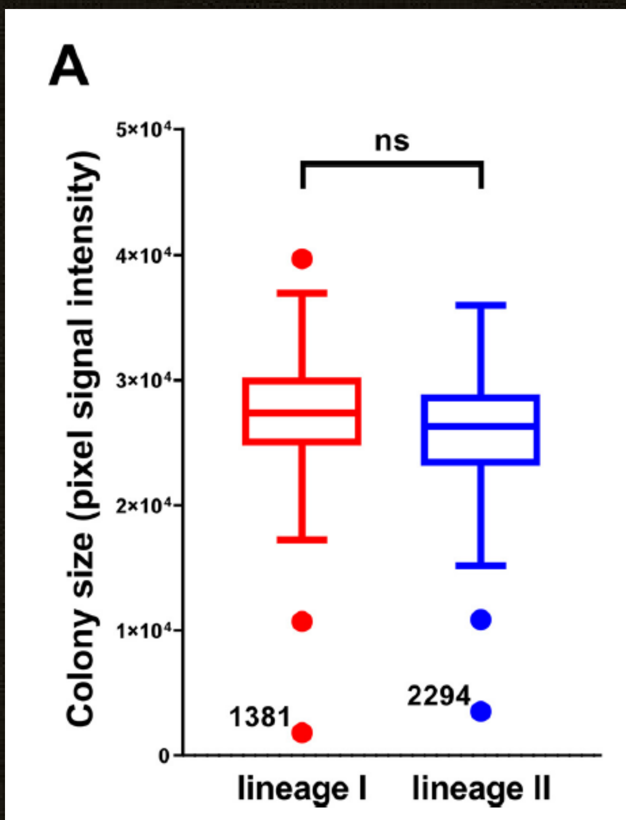
- *L. monocytogenes* can adapt to sub-lethal acid stress to produce highly acid resistant cells (the ATR)
- Stationary phase cells are more acid resistant than exponential cells
- The alternative sigma factor SigB plays a key role in acid resistance
- But the ATR can still occur in a mutant lacking *sigB*
- Amino acid decarboxylation and deamination/deimination are important determinants for acid resistance
- Of these the glutamate decarboxylase (GAD) system appears to be particularly significant – *L. mono* has 2 GAD systems

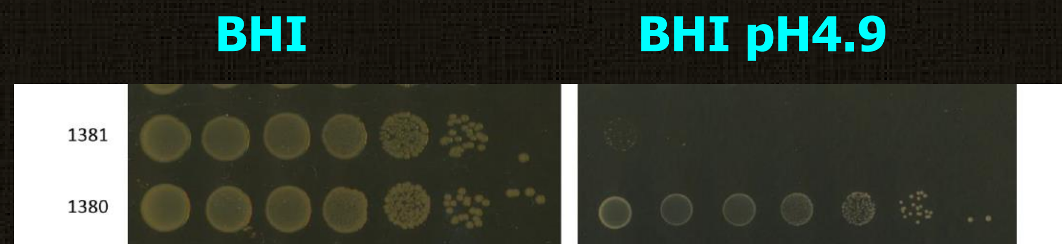
Phylogenetically Diverse Collection of Food & Clinical *L. monocytogenes* Isolates

- Through an earlier DAFM funded project we generated a large collection of food isolates
- Combined with some clinical isolates and EURL strains we phylogenetically compared 168 strains in total
- The collection is phylogenetically diverse – with strains spanning multiple clonal complexes (CC)
- Growth at low pH (acid tolerance) and survival at extreme low pH (acid resistance) was measured for the full set of strains
- Both phenotypes were highly variable across the collection

One CC2 strain (1381) was highly sensitive to growth at low pH

Colony size following 4 d growth on BHI agar at pH 4.9





Growth at 1 d

Wu *et al.*, AEM, 2022

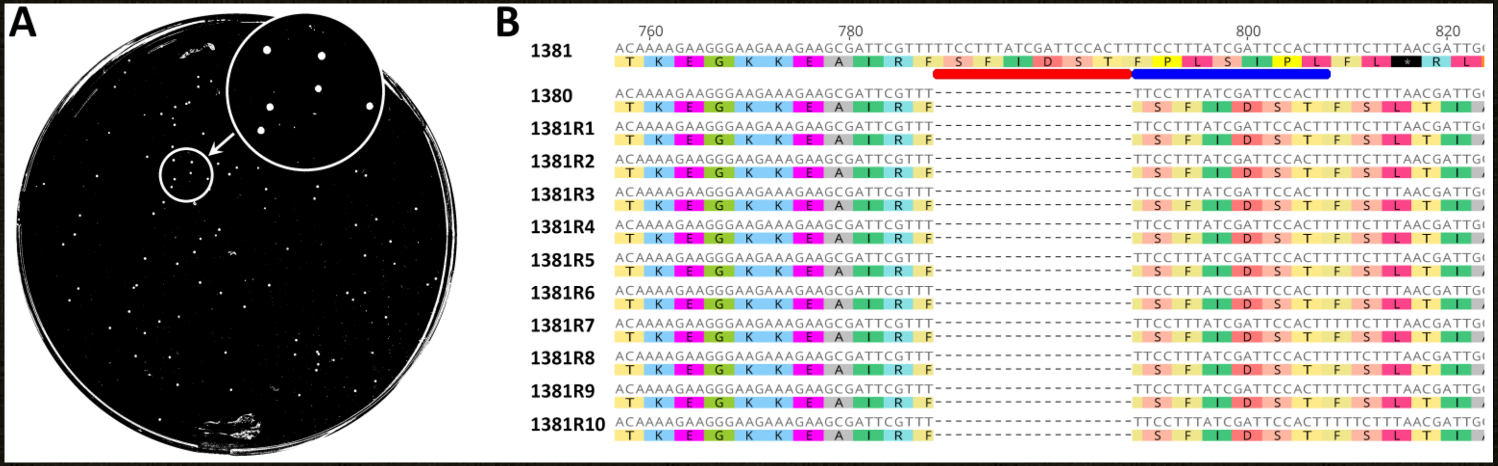
Genetic Differences between CC2 strains 1381 and strain LI0521: Where to start?!

Locus	Polymorphism	Protein Effect	Details	Full length (aa)	Note	Annotation
lmo2365/2364	Deletion	Frame Shift	91fs	504		
lmo1666	SNP	Truncation	Y177*	1718		*lapB*
lmo1424	Insertion	Frame Shift	263fs	448		*mntH*
lmo0835	Deletion	Frame Shift	255fs	335		
lmo2640	SNP	Substitution	G149V	180		
lmo2201	SNP	Substitution	S132P	413		*fabF CDS*
lmo2035	SNP	Substitution	G80S	363		*murG CDS*
lmo0857	SNP	Substitution	R44H	237		
lmo0786	SNP	Substitution	V754I	937		*manR*
lmo0782	Deletion		del F207	271		*mpoD*
lmo2287	SNP	Substitution	D1235G	1788		
lmo1100 :: hypothetical protein	Insertion					
lmo2131 :: lmo2132	Insertion					*crp/fnr :: crp/fnr*
lmo1145	SNP	Synonymous				*eutP*
lmo1596	SNP	Synonymous				
lmo0689 :: lmo0690	Deletion					*FliC/FljB :: cheV*
lmo0631	SNP	Synonymous				
lmo1855	Insertion	Frame Shift			*	
lmo1780	Insertion	Frame Shift			*	*pepT*
lmo1244	Insertion	Frame Shift			*	
lmo0785	Insertion	Frame Shift			*	*manR*
lmo0714	Insertion	Frame Shift			*	*filG*

- SNPs & indels across 22 genetic loci

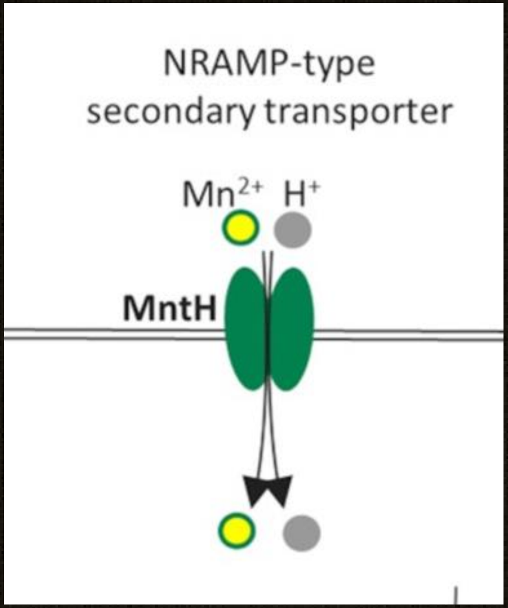
Reversion mutants on low pH agar plates revealed a mutation in *mntH* in 1381

BHI agar
pH 4.9



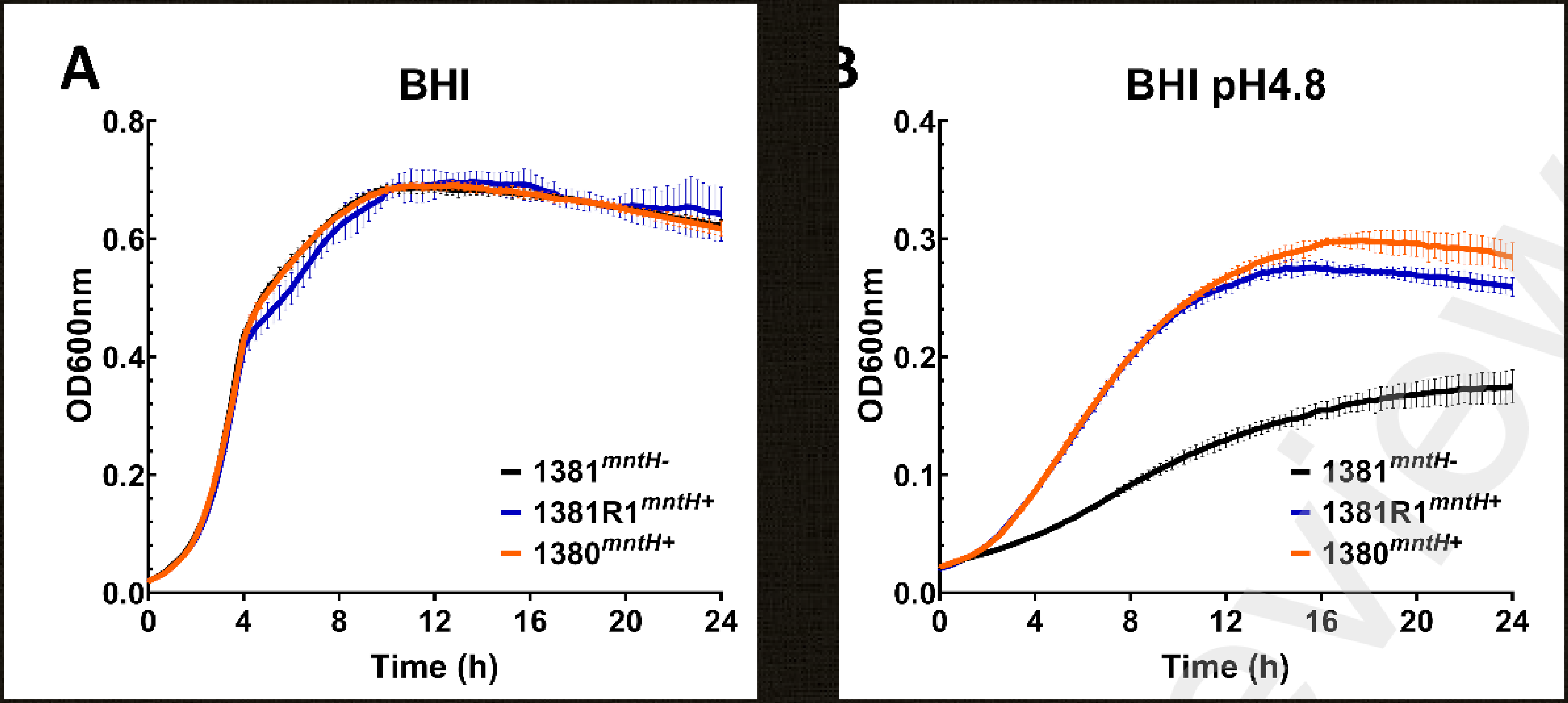
Wu *et al.*, IJFM, 2023

- *mntH* encodes a secondary Mn^{2+} transporter
- Suggested that Mn^{2+} transport is required for low pH growth



Bosma *et al.*,
FEMS Micro Rev 2021

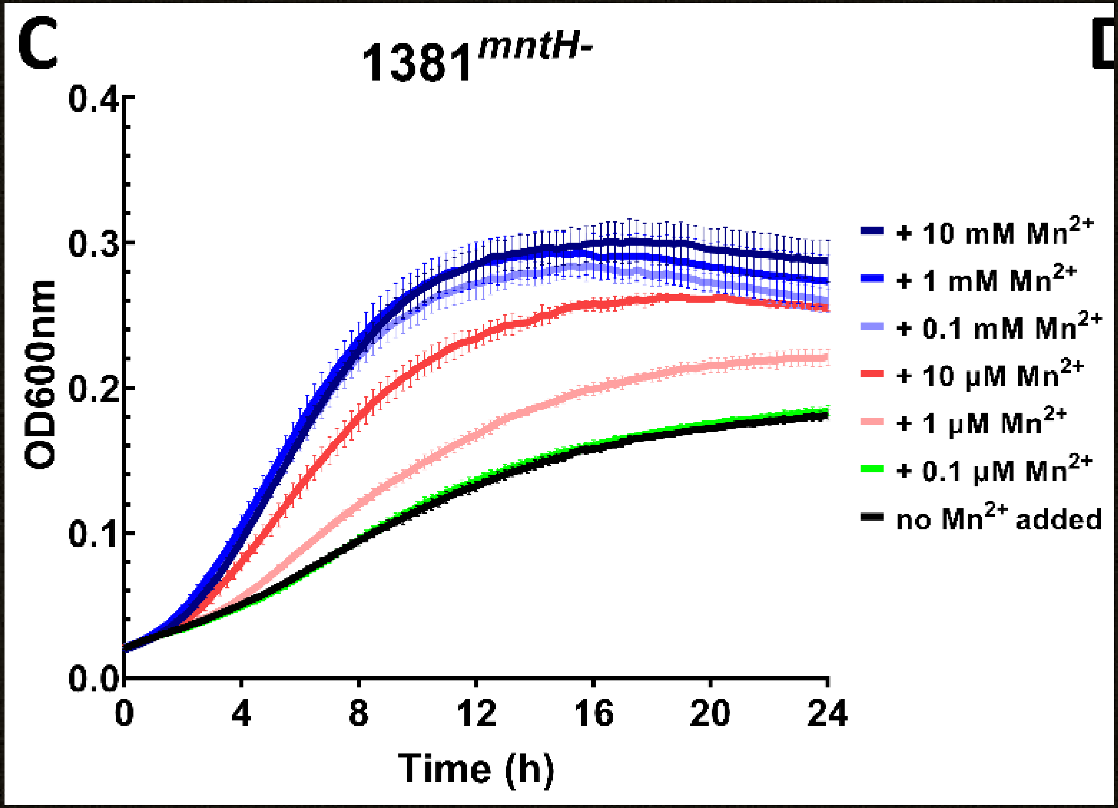
Reversion mutant restoring *mntH* ORF restores growth at low pH



Wu *et al.*, IJFM, 2023

Mn^{2+} can restore the growth of 1381 at pH 4.8

BHI pH 4.8

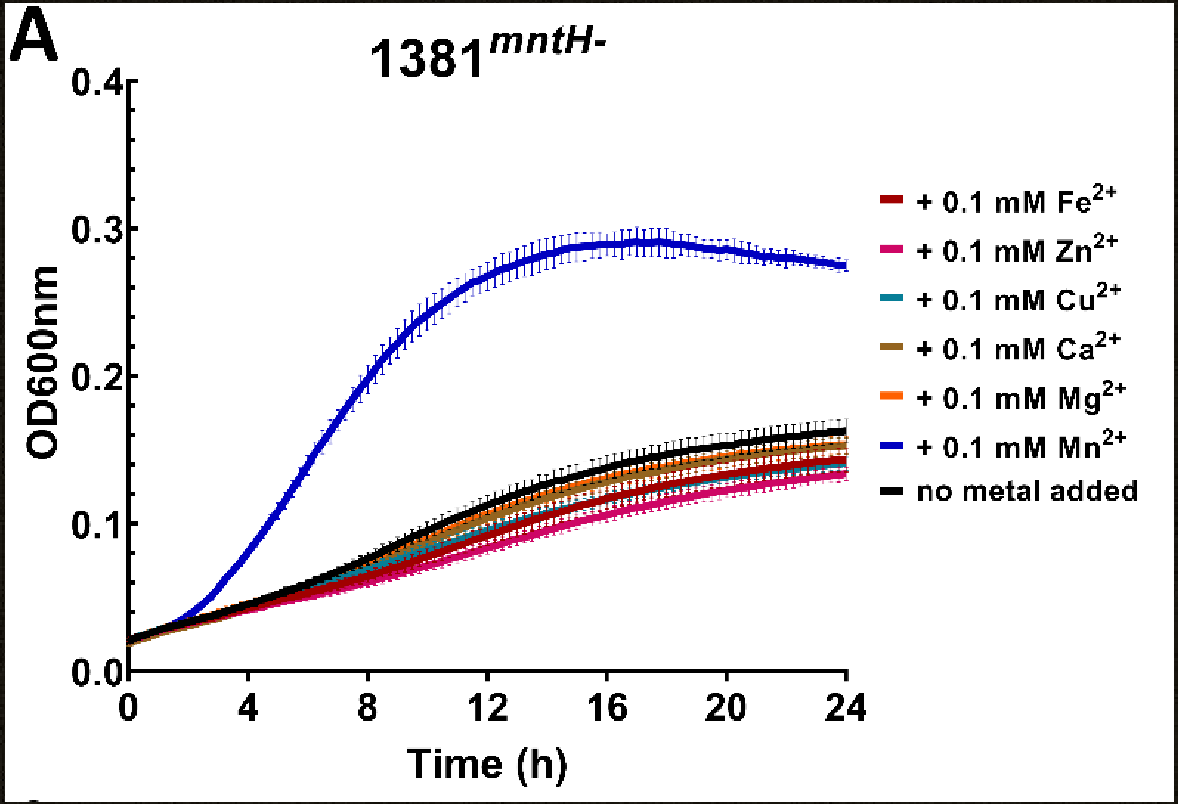


- Suggests that the Mn^{2+} deficiency is limiting growth at low pH

Wu *et al.*, IJFM, 2023

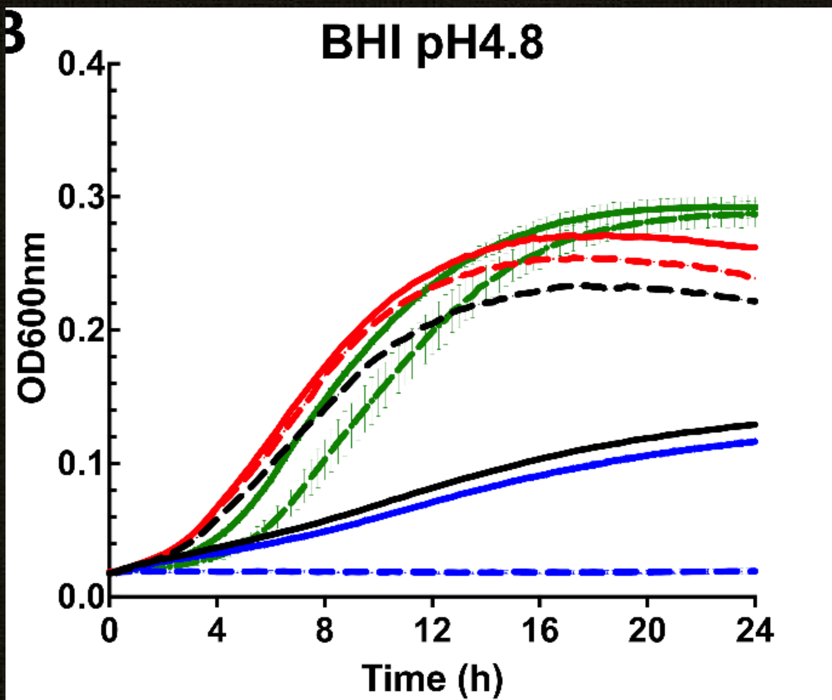
Other divalent cations do not restore the growth of 1381 at pH 4.8

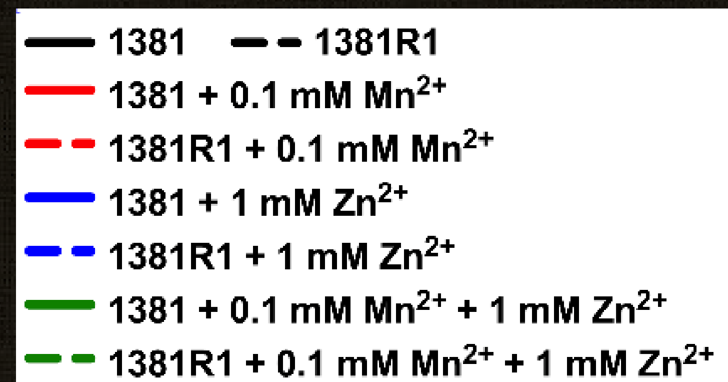
BHI pH 4.8



Wu *et al.*, IJFM, 2023

Mn^{2+} protects against Zn^{2+} toxicity at low pH

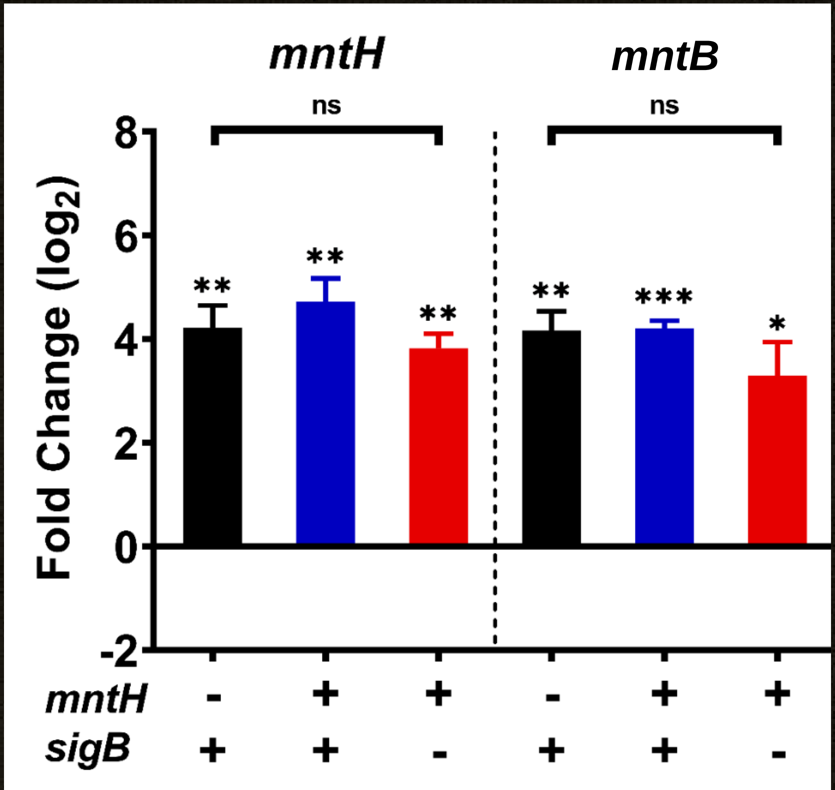




Wu *et al.*, IJFM, 2023

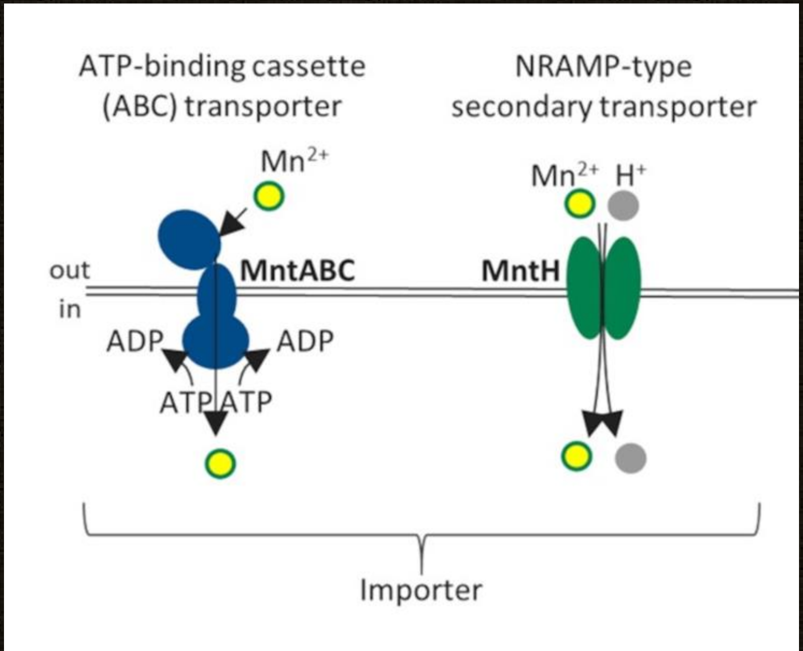
- Suggests that MntH can also transport Zn^{2+}
- Could this provide a selective advantage for loss of MntH function?

Transcription of both Mn^{2+} transporters are induced at low pH



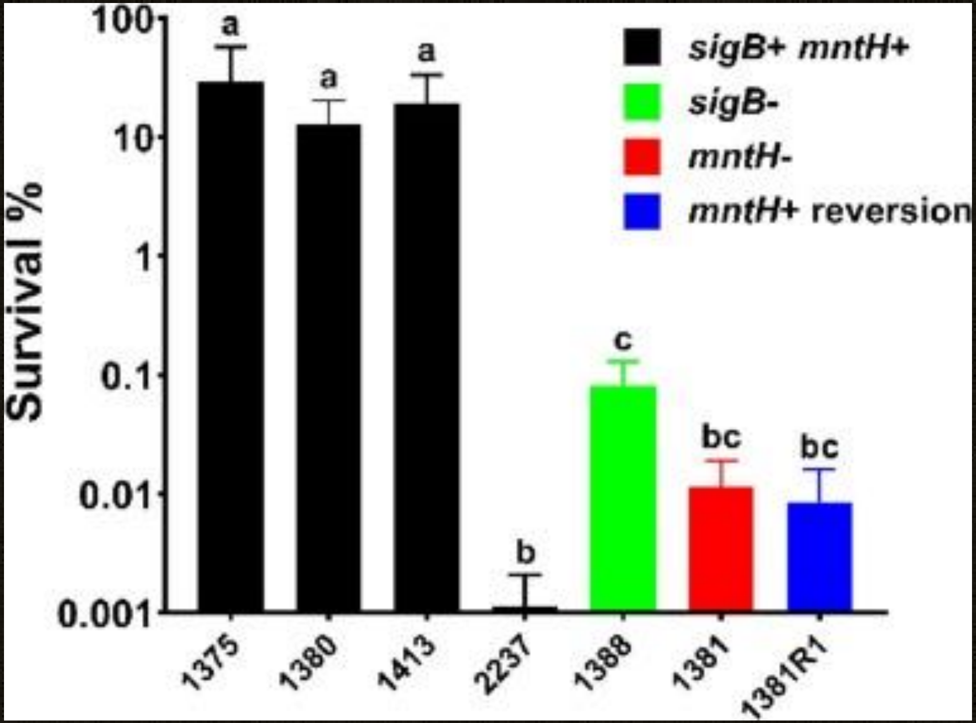
Wu *et al.*, IJFM, 2023

Exp. Phase,
pH 5.0 for 15 min



Bosma *et al.*,
FEMS Micro Rev 2021

mntH genotype doesn't affect acid resistance



1h @ pH 2.3

- Suggests that in strain 1381 sensitivity to lethal acid stress is independent of *mntH*

Ollscoil na Gaillimhe
University of Galway

Thanks For Listening!

Jialun Wu

Duarte Guerreiro

COLLABORATORS

- Kieran Jordan
- Olivia McAuliffe

- Jörgen Johansson
- Francisco García-del Portillo
- Bert Poolman
- Birgitte Kallipolitis
- Christine Ziegler

Department of Agriculture, Food and the Marine
An Roinn Talmhaíochta, Bia agus Mara

Science Foundation Ireland sfi For what's next

MARIE CURIE ACTIONS