Microbe : host : food interactions

implications for food safety.

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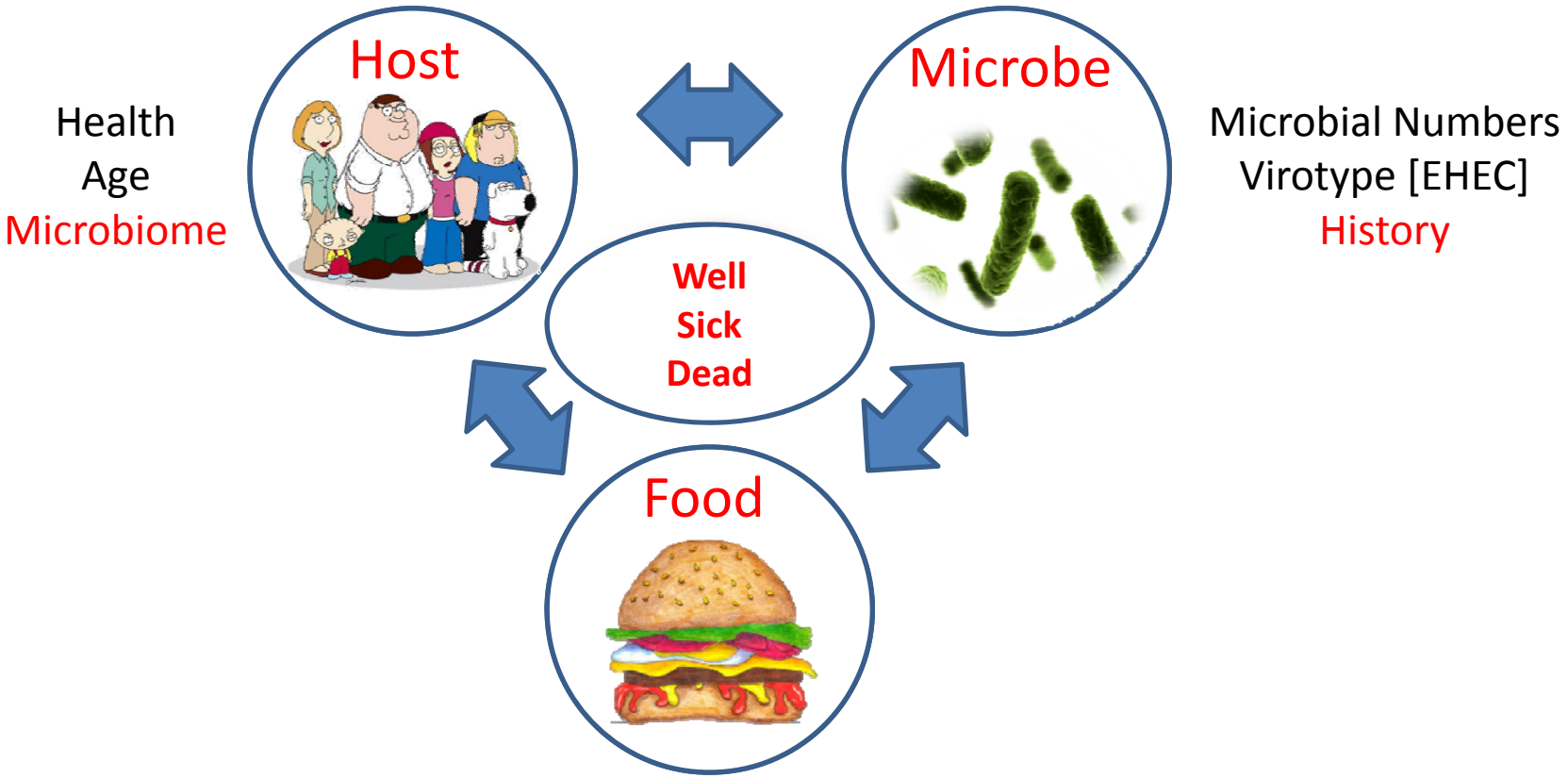
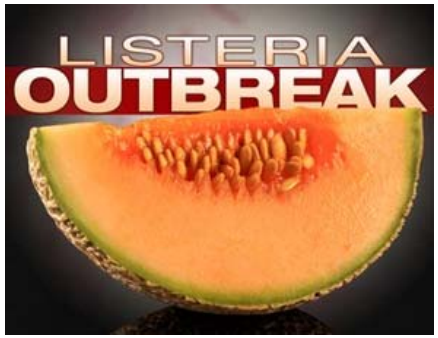
Many factors determine the outcome of bacterial host interactions



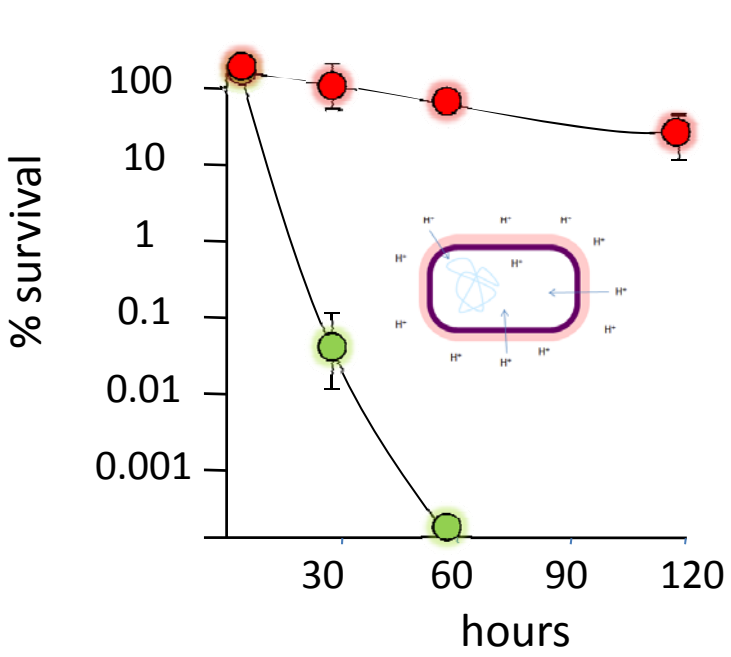
Listeria monocytogenes

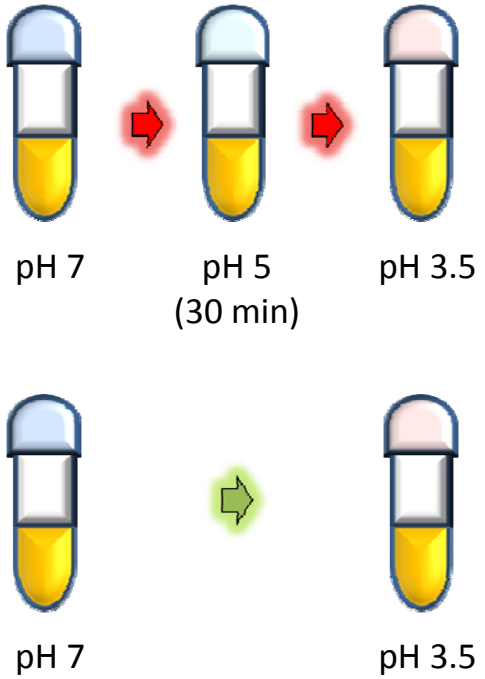
- Causes a rare but often fatal illness, listeriosis (Increasing in EU).
- Associated with dairy products, delicatessan meats and other ready-to-eat foods
- Can grow in the fridge, survive high temperatures, resist high salt and low pH, withstand high pressures
- Certain strains more likely to cause epidemic outbreaks (serotype 4b)
- Regulatory confusion? 100cfu/g appropriate?

EC regulation 2073 / 2005



Immediate history

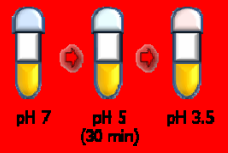




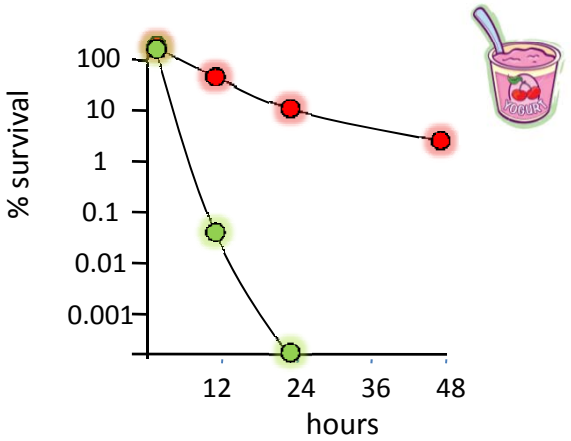
The acid tolerance response

O' Driscoll et al. 1996. Appl. Environ. Microbiol. 62: 1693-1698.

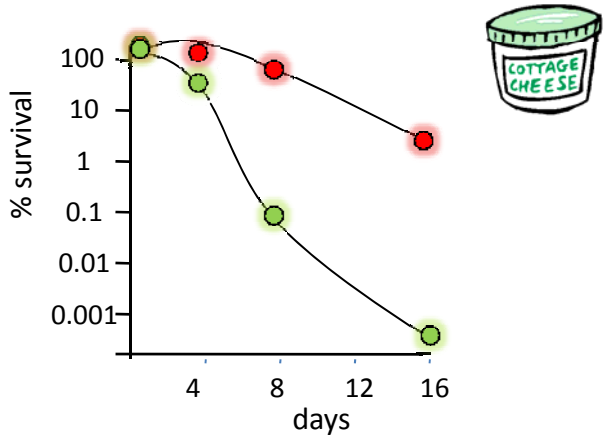
Immediate history



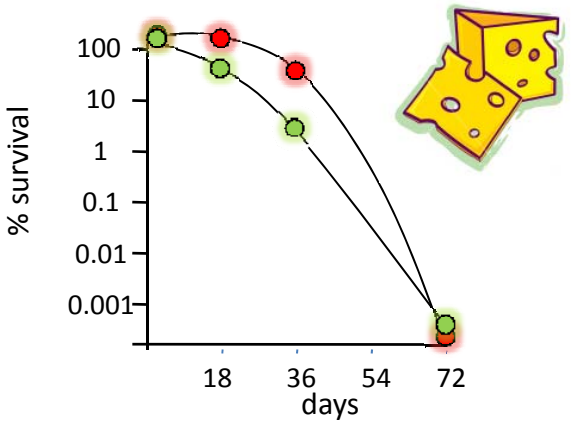
Yoghurt (pH3.9)



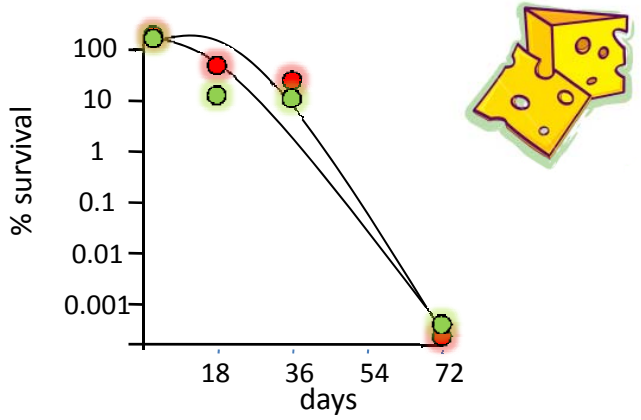
Cottage cheese (pH4.7)



Cheddar (pH5.1)

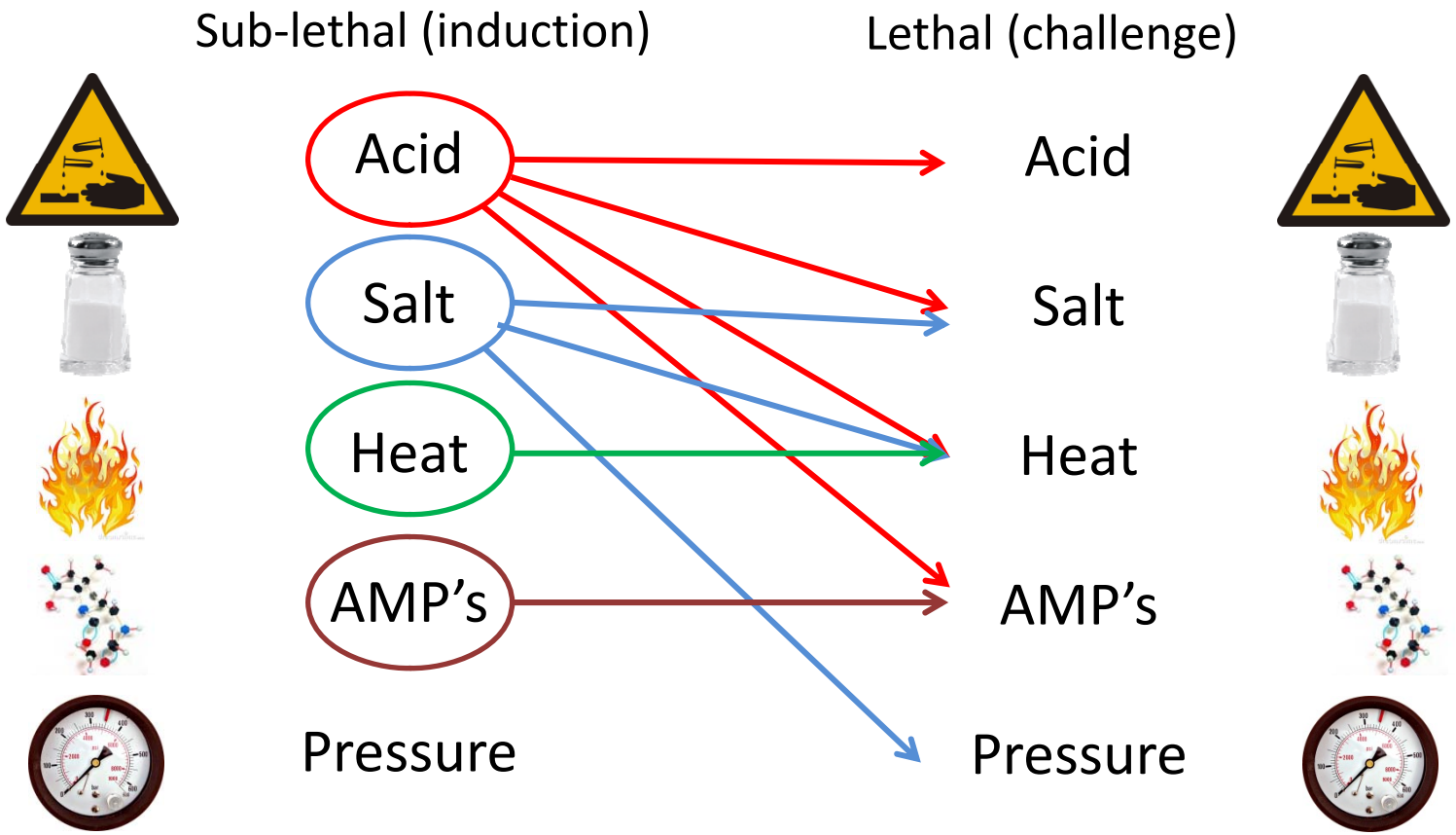


Low-fat Cheddar (pH5.2)



Gahan et al. 1996. Appl. Environ. Microbiol. 62:3128-3132.

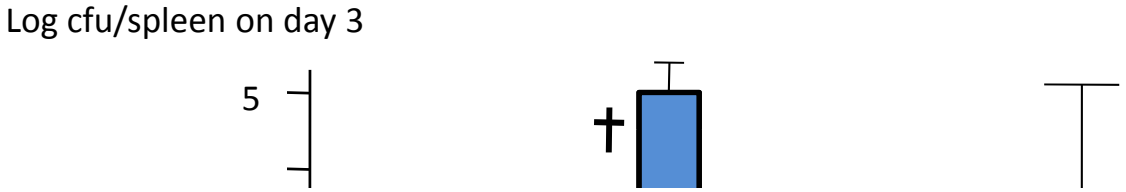
Cross adaptation between stresses



3% NaCl increases pressure resistance >1000-fold

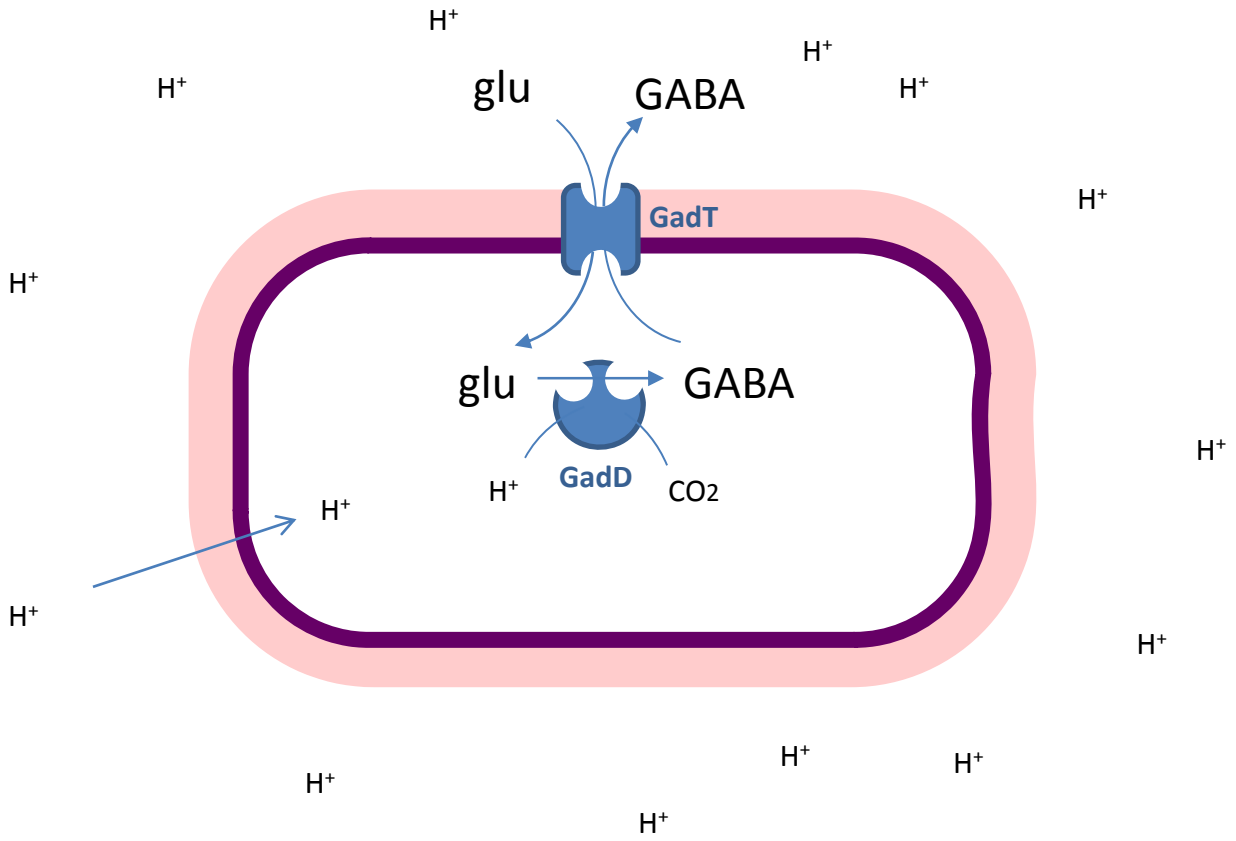
Smiddy et al. 2005. Applied Environ. Microbiol. 70: 7555-7557.

The ability to adapt affects virulence



The **immediate history** of a bacterium can significantly influence it's survival and virulence potential

Mechanism of adaptation– GAD system

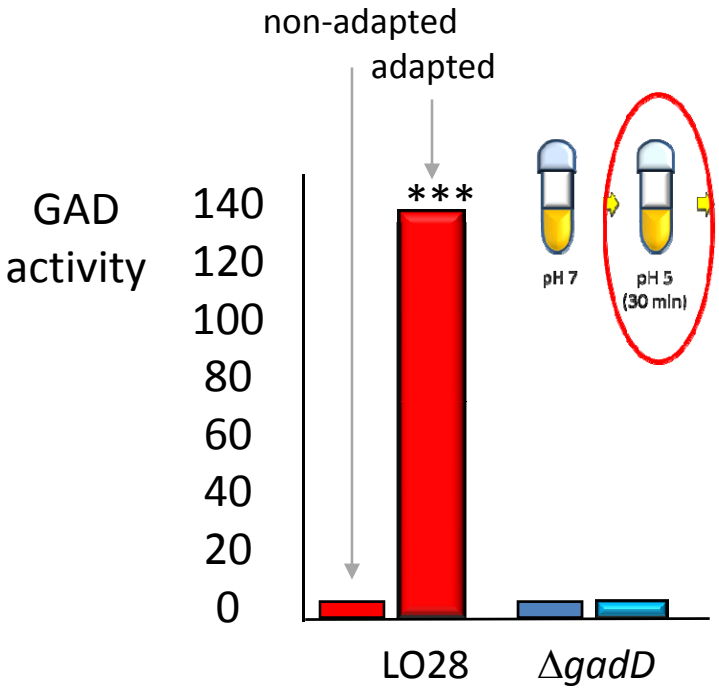


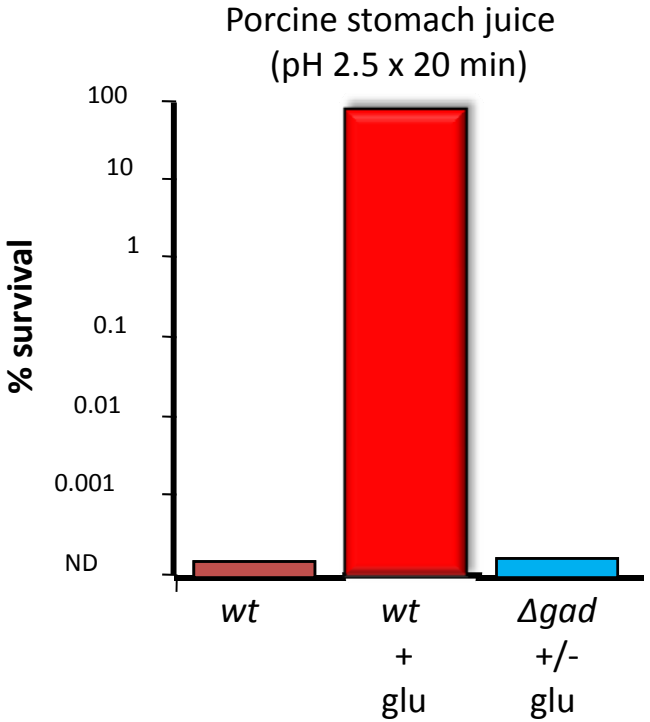
Glutamate is exchanged for GABA (outside the cell)

A proton is consumed (inside the cell)

Cotter et al. 2001. Mol. Microbiol. 40:465-75.

History and constituents combine



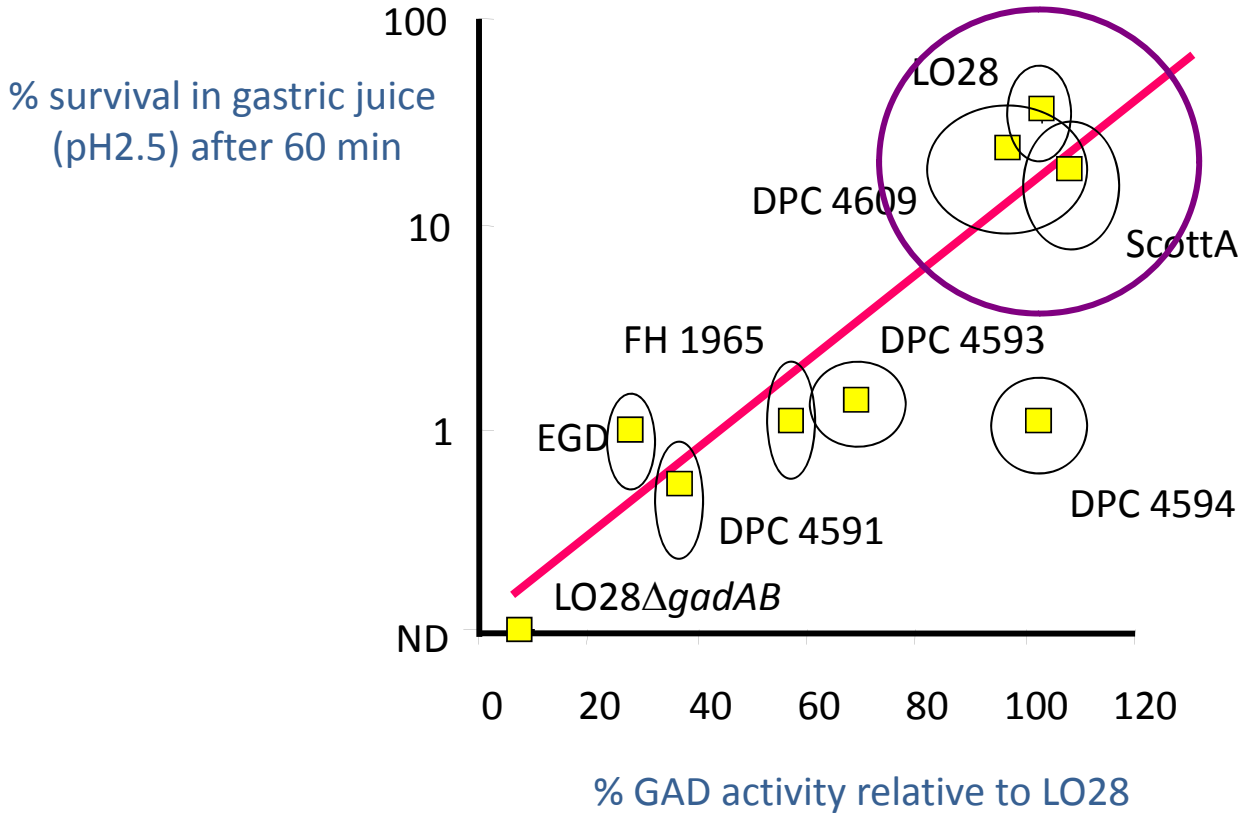


GAD is a major player in the Acid Tolerance Response

Can dramatically affect acid tolerance - and gastric transit

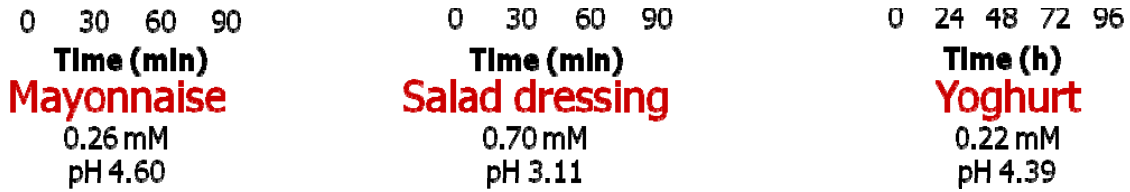
Supporting Evidence

Clinical strains have high GadBC activity and survive better in gastric acid



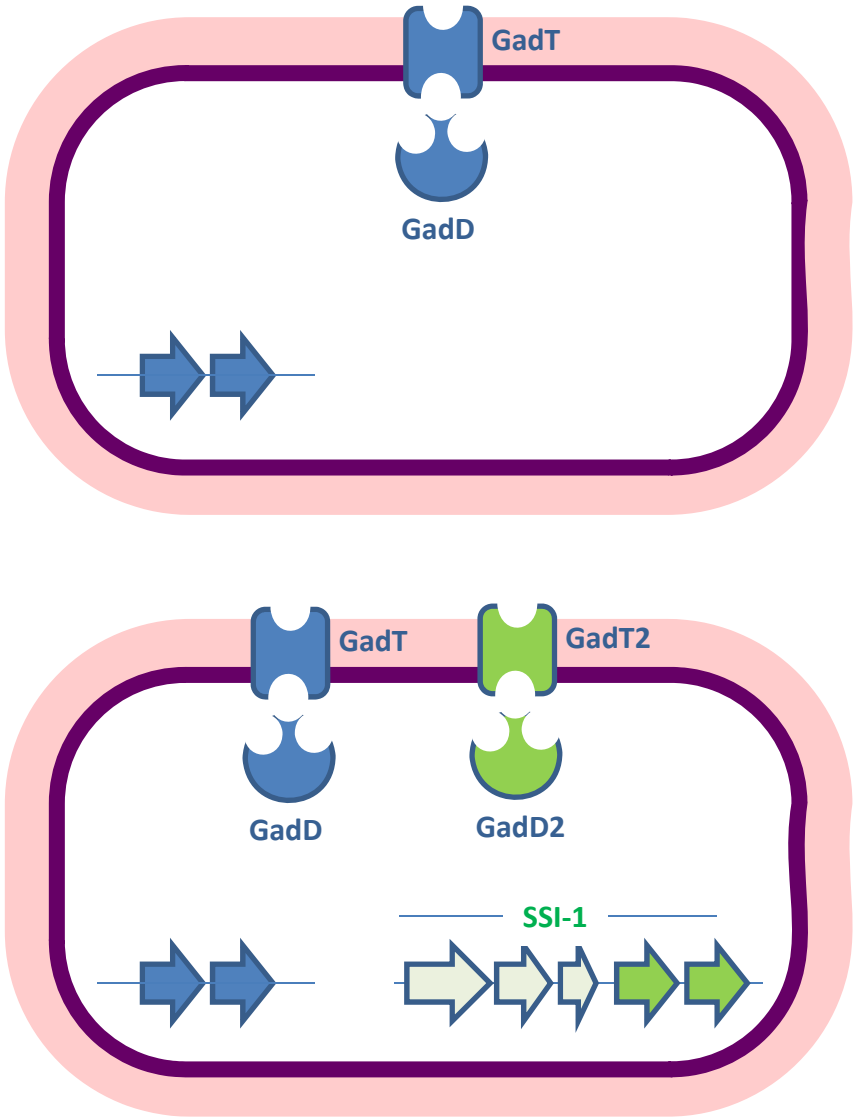
Role of GAD in survival in food

- The **immediate history** of a bacterium can significantly influence it's survival and virulence potential
- **Food constituents** can significantly influence growth and survival (and infectious dose?)



Cotter et al. 2001. Mol. Microbiol. 40:465-75.

Strain diversity

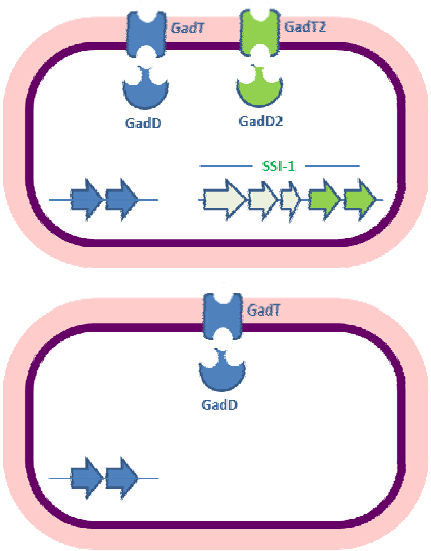


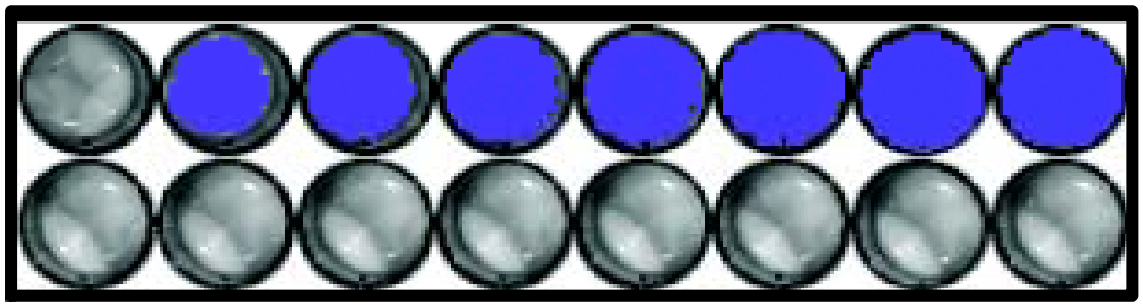
Some strains have two GAD systems

The second is encoded on a Stress Survival Island [SSI-1]

Ryan, et al. 2010. J. Appl. Microbiol. 109:984-995.

Strain diversity

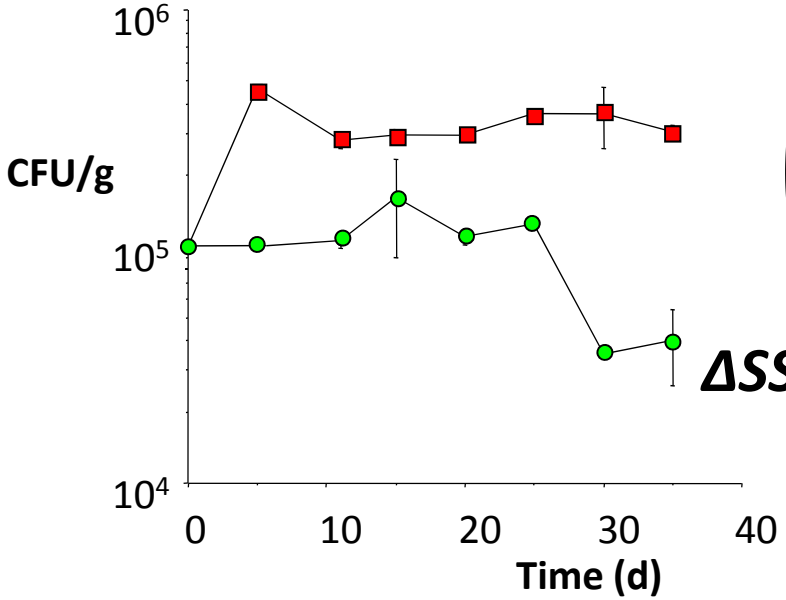


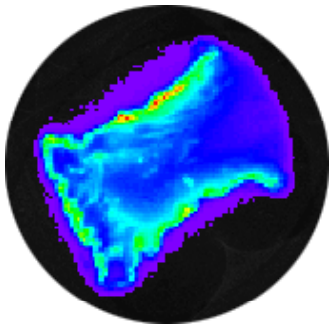


0 1 2 3 4 5 6 8 days

No 4b strains (epidemic strains) contain SSI-1

[of >20 tested]





ΔSSI-1

Ryan, et al. 2010. J. Appl. Microbiol. 109:984-995.

Strain diversity and virulence

Lineage I
(Serotype 4b)
'epidemic strains'

"The overriding *Listeria* conundrum relates to the fact that there is little genomic difference between high- and low-virulence isolates"

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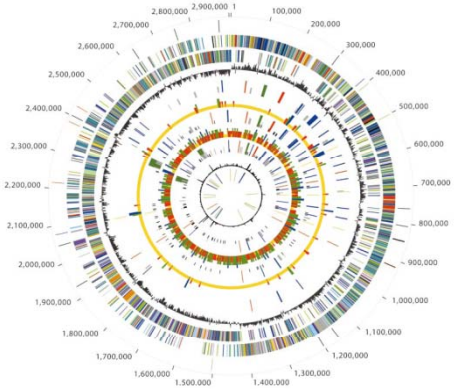
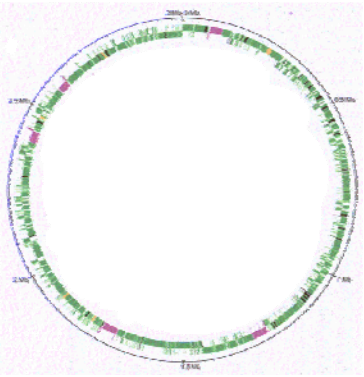
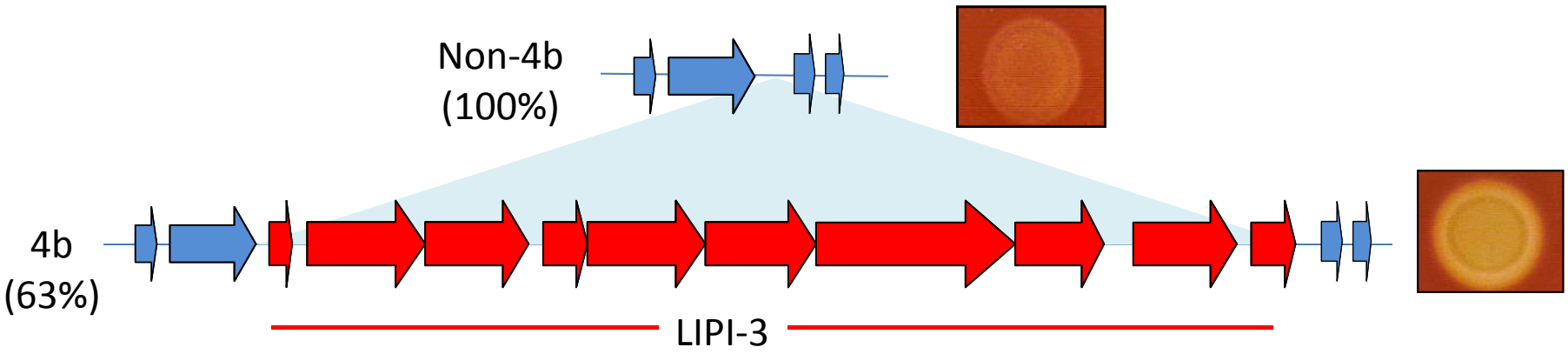


Only 51 genes unique to 4b strains
Nelson *et al*, 2004



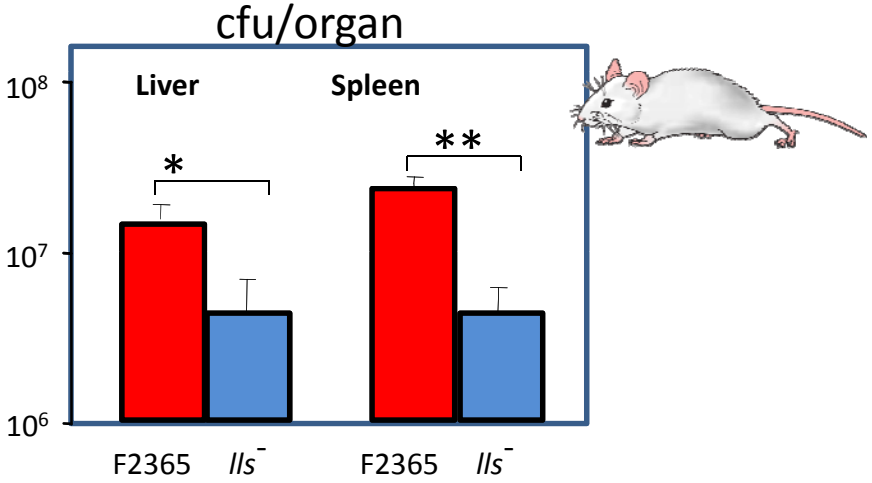
Lineage II, III
'food & environmental strains'

Listeriolysin S



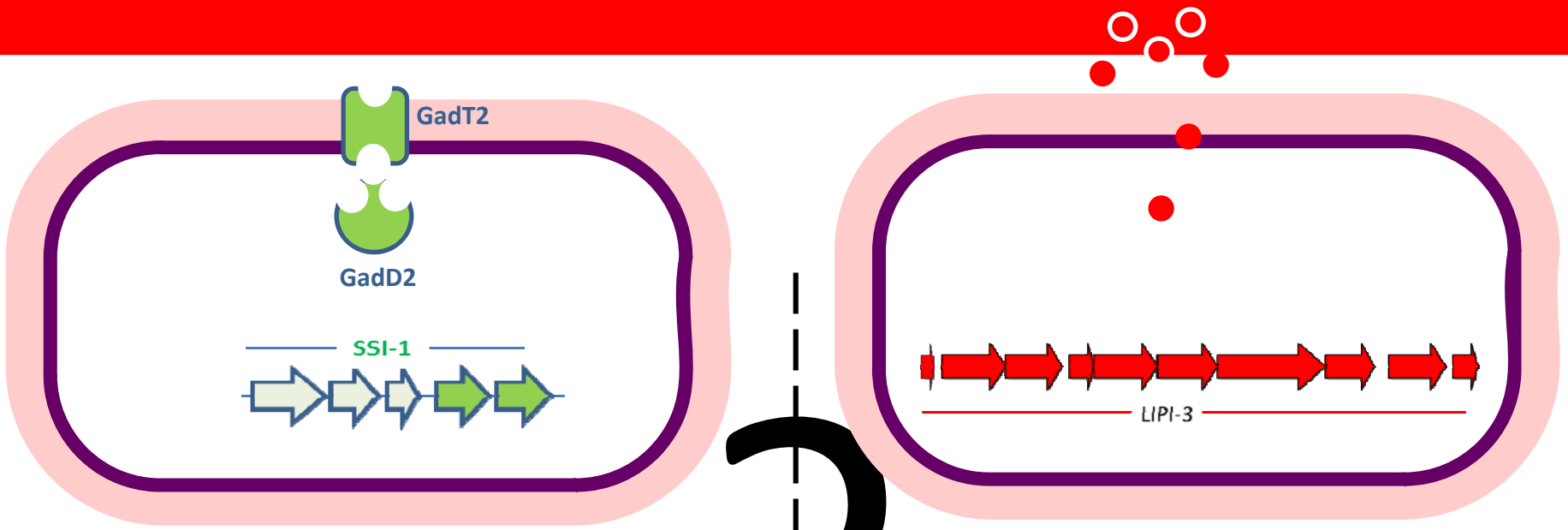
LlsA is a haemolysin (only expressed *in vivo*).

Knockouts are less virulent in murine models and in human PMN's.



- The **immediate history** of a bacterium can significantly influence it's survival and virulence potential
- **Food constituents** can significantly influence growth and survival (and infectious dose?)
- **Strain diversity** (genetic complement) may significantly affect survival and virulence potential

‘Food’ v ‘Disease’ strains?



‘Food’ strains

Lineage 1 (non-4b), lineage II and III

SSI-1 +ve LIPI-3 -ve

Grow well in food, no 2nd hemolysin – less virulent?

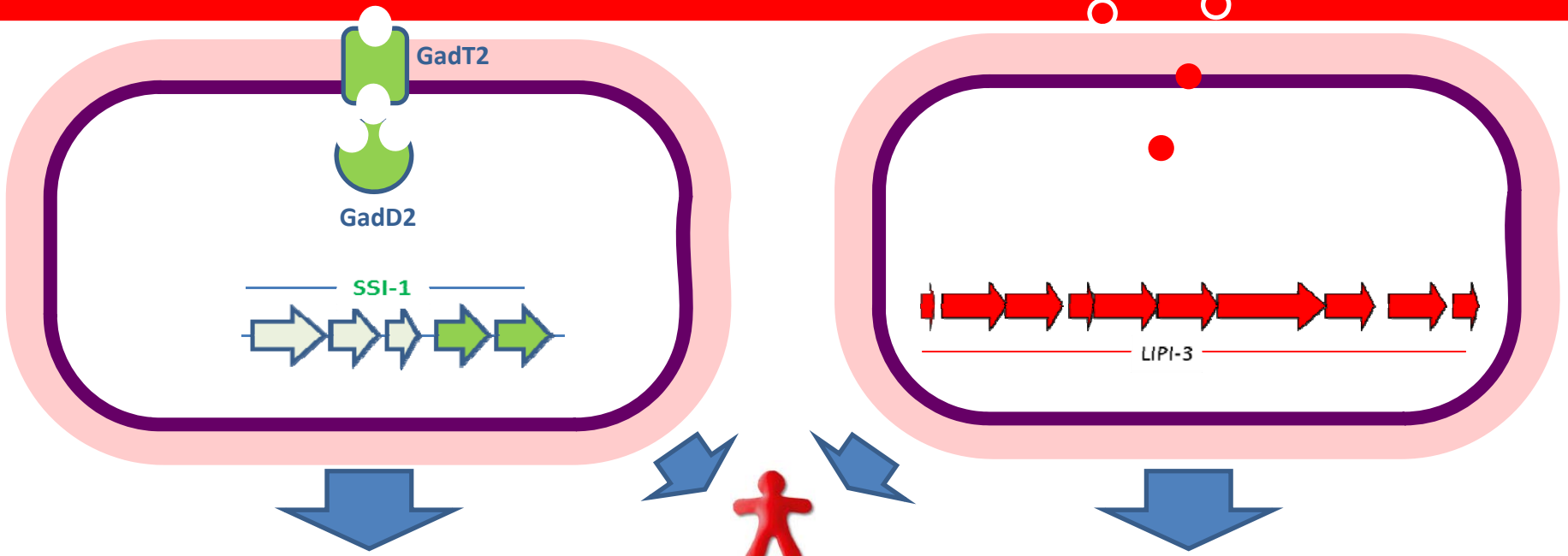
‘Disease’ strains

Lineage 1 (esp. 4b)

SSI-1 -ve LIPI-3 +ve

Grow poorly in food, 2nd hemolysin – more virulent?

35 strain survey



CD1078
FSL N1 017
CD1066 CD1742
CD1198 CD1038
CD748 CD1028
CD1061 CD749
CD1032 CD1121

CD243
CD246
CD83
C241

FSL J1 208
FSL J2 071

CD878
H7858
FSL J1-194

CD147
F2365

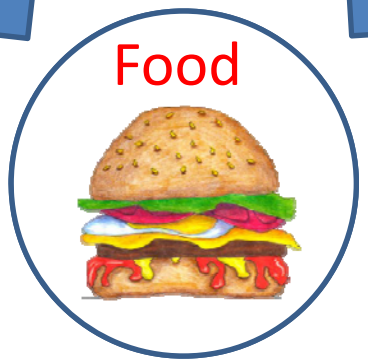
F6854

In order to predict outcomes more accurately …

Health
Age
Microbiota

Microbial Numbers
Species or Strain
History

Well
Sick
Dead

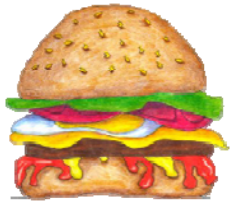


Food type
Processing
Constituents

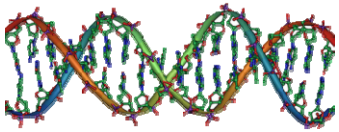
We need to assess risk based on:

- Genetic complement
- Stress history
- Food constituents
- Host parameters

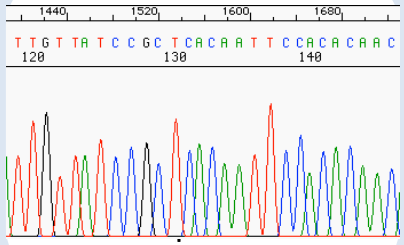
the future .....



Food



Extract DNA/RNA



Bioinformatics

16S: Complete quantitative microbial profile
Bacterial, viral, fungal
[spoilage, commensal, pathogenic]

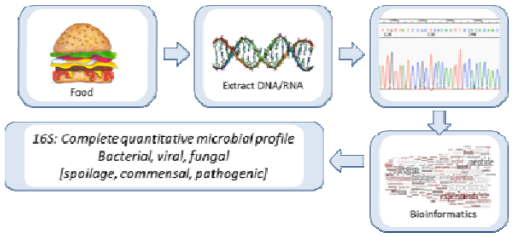
Total DNA: gene-based risk assessment
[pathogenicity islands, survival islands, metabolic potential]

RNA: Viability assessment, strain history, stress adaptation status

Output: Quantitative risk assessment
Epidemiological certainty
Therapeutic options
Emerging pathogens
Rapidity (minutes/hours)

Metagenomics of IMF

Infant milk formula



culture dependent

Strain number	Closest homologue (accession number)
IMF1	*Bacillus licheniformis strain LQ98 (Ef472268)*
IMF2	*Bacillus cereus strain APB2 (Hm046583)*
IMF3	*Bacillus thuringiensis strain BL40 (Hq180398)*
IMF4	*Bacillus sp. JM4 (Ef062991)*
IMF5	*Bacillus anthracis strain (HM854238)*
IMF6	*Bacillus sp. ITO01(Fr667164)*
IMF7	*Uncultured Bacillus sp. clone Filt.93 (Hm152681)*
IMF8	*Lysinibacillus fusiformis strain CBII-2 (Hm068891)*
IMF9	*Brevibacillus parabrevis strain M3 (Ab215101)*
IMF10	*Brevibacillus brevis strain P-30 (Hm185814)*
IMF11	*Paenibacillus sp. JAM-FM32 (Ab526335)*
IMF12	*Aeromicrobium erthreum (NR_024846)*
IMF13	*Staphyloccus hominis (Fj380978)*
IMF14	*Enterococcus faecium (Ab481104)*
IMF15	*Enterococcus durans (Eu419600)*
IMF16	*Cronobacter sakazakii ATCC BAA-894*
IMF17	*Enterobacter cloacae (Fp929040)*
IMF18	*Citrobacter koseri (CP000822)*
IMF19	*Escherichia vulneris (Af530476)*
IMF20	*Uncultured bacterium (Hm309271)*

culture independent

Strain number	Closest homologue (accession number)
PIF1	*Uncultured firmicutes.*
PIF2	*Streptococcus thermophiles strain T1*
PIF3	*Anoxybacillus flavithermus clone LK4*
PIF4	*Streptococcus thermophiles strain T1 1*
PIF5	*Flavobacterium frigidarium strain A2i*
PIF6	*Uncultured bacterium clone gir_aah96b03 16S*
PIF7	*Enterococcus faecium strain: ML4*
PIF8	*Uncultured bacterium clone 3-1B-G12*
PIF9	*Leuconostoc lactis YKLAB10*
PIF10	*Streptococcus uberis 0140J*
PIF11	*Lactococcus piscium strain: NJ119*
PIF12	*Lactococcus lactis subsp. Cremoris strain: YIT 2007*
PIF13	*Uncultured Streptococcus sp.clone ChR-II-cI12*
PIF14	*Lactococcus sp.YM05004*
PIF15	*Cronobacter sakazakii strain 05CHPL95*
PIF16	*Uncultured bacterium W05*
PIF17	*Uncultured bacterium W05*
PIF18	*Uncultured Trichococcus sp. TCE-134*
PIF19	*Uncultured firmicutes bacterium clone PLF34 16S*
PIF20	*Streptococcus thermophilus strain T1*

Conclusions (1)

To predict the outcome of pathogen host interactions (or to derive safe exposure levels), (do) we need to know?

- Host factors (age, health, immune status)
- Bacterial numbers
  - Genetic complement of strain
    - Growth potential, survival potential, virulence potential
  - History (exposure to stress)
    - Impact gastric transit, virulence potential
  - Food constituents
    - What about co-consumed foods?

Conclusions (2)

- Should inform policy, but how?
  - Specific regulations for specific strains (EHEC v *E. coli*)?
  - Specific regulations for specific foods?
    - Zero tolerance in high glutamate foods?
  - Specific advice for high-risk groups?
    - Certain combinations of foods to be avoided (e.g. intensive care)

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