Gram+ Foodborne Pathogens and Toxins
Food Biology Fundamentals

Listeria monocytogenes,
Staphylococcus aureus
and
Bacillus cereus

Main Textbook:

"Food Microbiology: Fundamentals and Frontiers", 4th edition, Chapter III

Top Five Pathogens Contributing to Domestically Acquired Foodborne Illnesses Resulting in Death (CDC 2011)

Pathogen	Estimated number of deaths	90% Credible Interval	%
***Salmonella*, nontyphoidal**	378	0–1,011	28
Toxoplasma gondii	327	200–482	24
Listeria monocytogenes	255	0–733	19
Norovirus	149	84–237	11
Campylobacter spp.	76	0–332	6
Subtotal			88

The Gram Stain

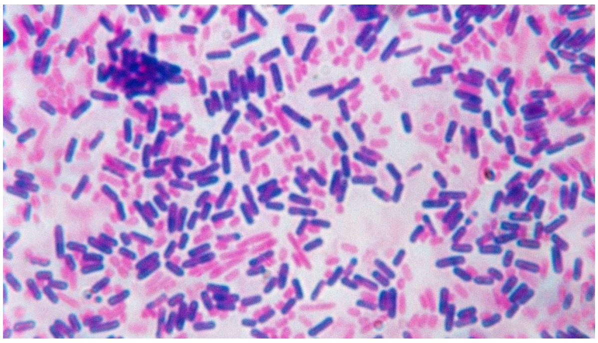
≠

Hans Christian Joachim Gram
(13 September 1853 – 14 November 1938)
was a Danish bacteriologist noted for his development of the Gram stain, still a standard technique to classify bacteria and make them more visible under a microscope.

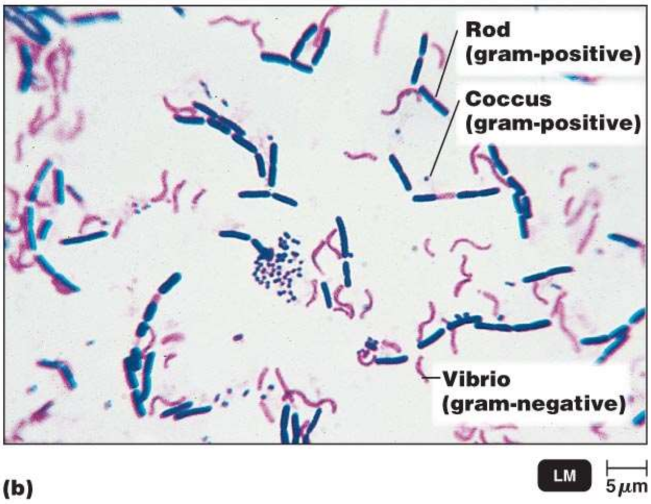
"The Differential Staining of Schizomycetes in Sections and in Smear Preparations" Fortschr. Med. 2:185–89, 1884

Gram Stain

- Classifies bacteria into Gram-positive or Gram-negative
  - Gram-positive bacteria tend to be killed by penicillin and detergents
  - Gram-negative bacteria are more resistant to antibiotics



Micrograph of Gram-Stained Bacteria

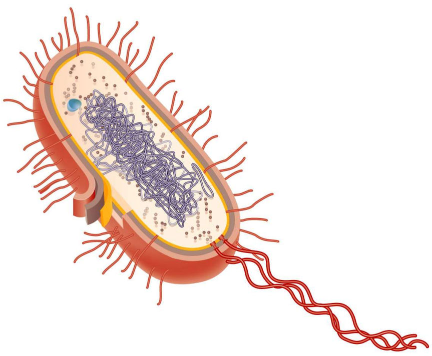


(b)

Figure 3.12b

Cell Wall

- Prevents osmotic lysis
- Made of peptidoglycan (in bacteria)



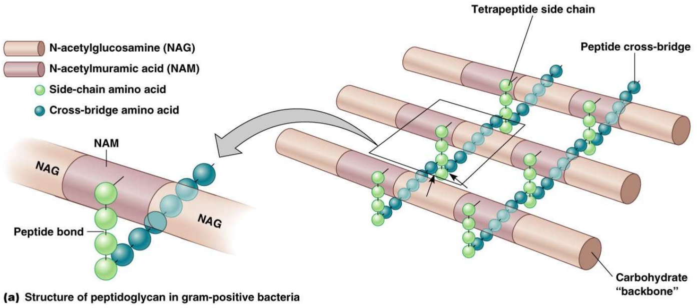
(a)

(b)

Figure 4.6a–b

Peptidoglycan

- Polymer of disaccharide
  N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM)
- Linked by polypeptides



(a) Structure of peptidoglycan in gram-positive bacteria

Figure 4.13a

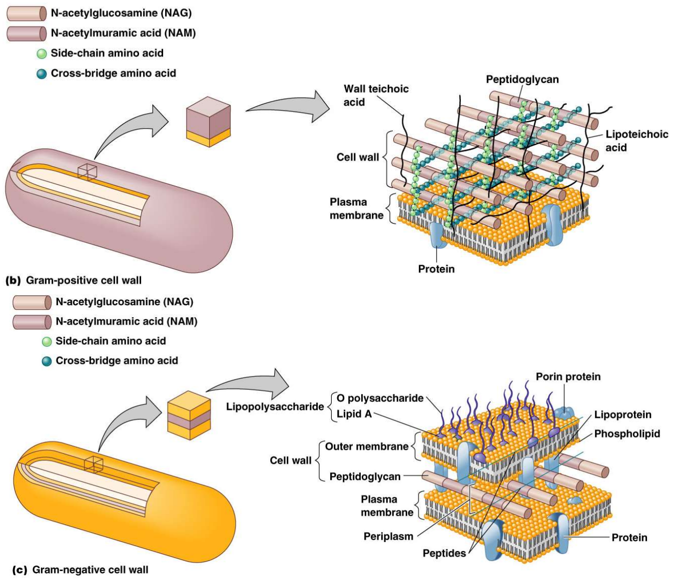
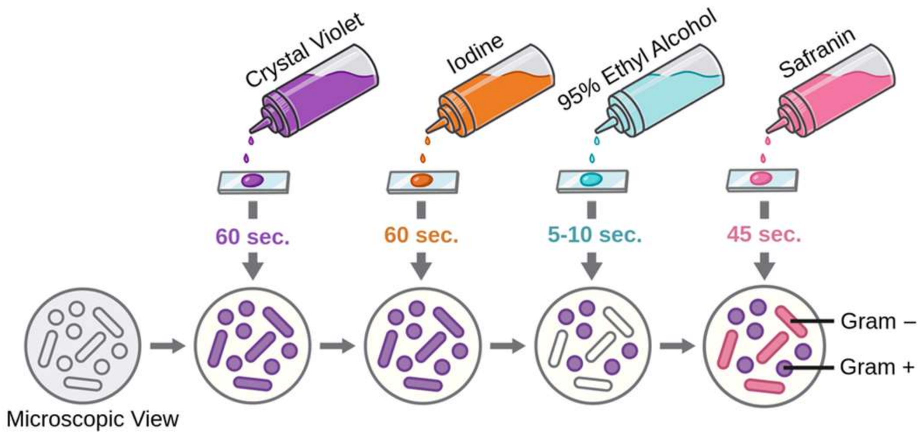


Figure 4.13b–c



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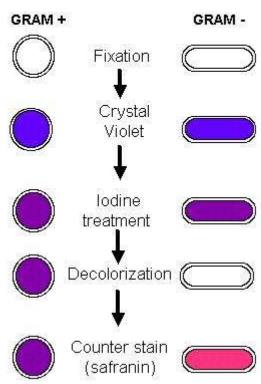
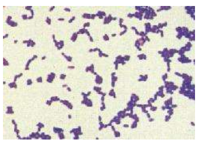
The Gram Stain

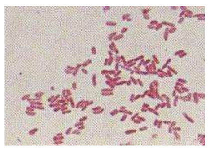
make smear of culture on slide

- stain with:
  - crystal violet
  - iodine
- distain with:
  - alcohol
- counter stain:
  - safranin
- examine microscopically

Blue = Gram^{+} Pink = Gram^{-}







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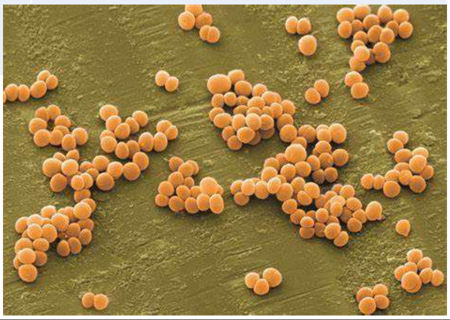
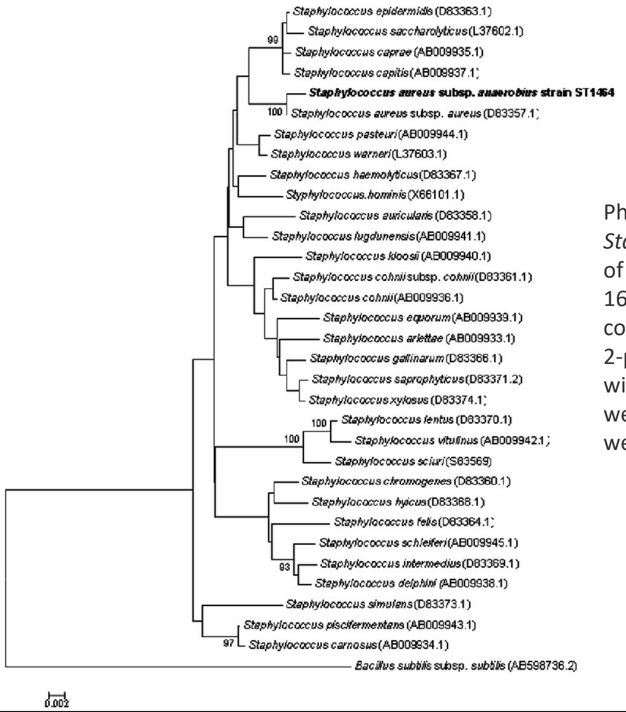
Differences Associated with Gram Stain

	Gram+	Gram-
• heat resistance	more	less
• toxins	exo-	endo
• nutrients requirements	complex	simple
• detergents	very sensitive	less sensitive
• penicillin	susceptible	resistant
• outer membrane	no	yes
• peptidoglycan	thick	thin

Staphylococcus aureus	Gram-positive 19 species in Staphylococcus genera Coagulase and termonuclease positive messofiles	Toxins production A, B, C1, C2, C3, D, E
Growth temperatures: from 7ºC up to 47.8ºC Enetrotoxins productin 10ºC-46ºC, with optimum at 40ºC-45ºC	pH: from 4.0 up to 9.8 With optimum at 6.0-7.0	Water activity: Resistance up to 8.3 Resistance to 10-20% NaCl
Caused by bacteria, toxins. Incubation period: 0.5-8h. Gastro-intestinal desorders, perifer neuro system, blod presure. 0.5-10 ug/100g toxin/food	*Stephylococcus aures* is present in nature, animals. In humans, *S. aureus* is part of the normal microbiota present in the upper respiratory tract, and on skin and in the gut mucosa.	



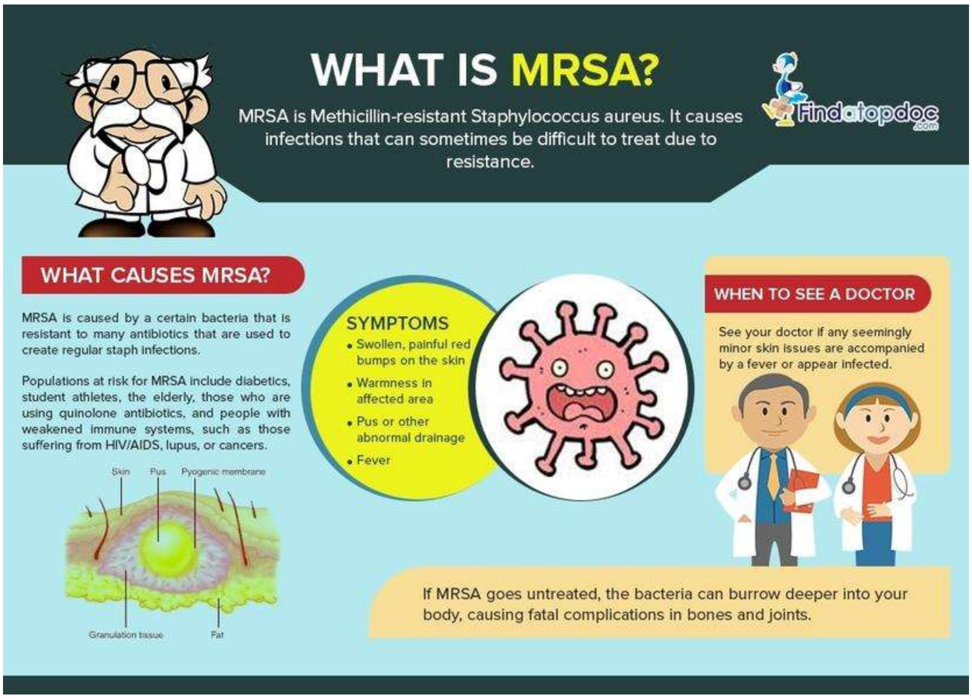


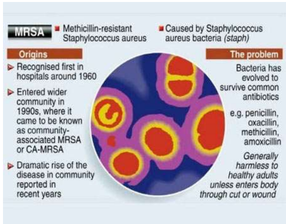
Phylogenetic tree depicting the relationship between *Staphylococcus aureus* subsp. *anaerobius* and other members of the genus *Staphylococcus* based on 1,311 base pairs of the 16S rRNA gene sequence aligned in Muscle. The tree was constructed by using the Neighbor-Joining method and Kimura 2-parameter model using MEGA5 software [26] and rooted with *Bacillus subtilis* subsp. subtilis. Bootstrap consensus trees were inferred from 100 replicates, only bootstrap values > 90% were indicated.

DOI: 10.4056/sigs.3748294

Of significance to humans are various strains of the species *Staphylococcus aureus* and *S. epidermidis*. While *S. epidermidis* is a mild pathogen, opportunistic only in people with lowered resistance, strains of *S. aureus* are major agents of wound infections, boils, and other human skin infections and are one of the most common causes of food poisoning. *S. aureus* also causes meningitis, pneumonia, urinary tract infections, and mastitis, an infection of the breast in women or of the udder in domestic animals. In addition, local staphylococcal infections can lead to toxic shock syndrome, a disease associated with the liberation of a toxin into the bloodstream from the site of infection.

One strain that is of great concern to humans is methicillin-resistant *S. aureus* (MRSA), which is characterized by the presence of a mutation that renders it resistant to methicillin, a semisynthetic penicillin used to treat staphylococcus infections that are resistant to mold-derived penicillin. This strain of *S. aureus* was first isolated in the early 1960s, shortly after methicillin came into wide use as an antibiotic. Today methicillin is no longer used, but the strain of MRSA to which it gave rise is commonly found on the skin, in the nose, and in the blood and urine of humans.


WHAT IS MRSA?
MRSA is Methicillin-resistant Staphylococcus aureus. It causes infections that can sometimes be difficult to treat due to resistance.
Findatopdoc.com
WHAT CAUSES MRSA?
MRSA is caused by a certain bacteria that is resistant to many antibiotics that are used to create regular staph infections.
Populations at risk for MRSA include diabetics, student athletes, the elderly, those who are using quinolone antibiotics, and people with weakened immune systems, such as those suffering from HIV/AIDS, lupus, or cancers.
Skin
Pus
Pyogenic membrane
Granulation tissue
Fat
SYMPTOMS
• Swollen, painful red bumps on the skin
• Warmness in affected area
• Pus or other abnormal drainage
• Fever
WHEN TO SEE A DOCTOR
See your doctor if any seemingly minor skin issues are accompanied by a fever or appear infected.
If MRSA goes untreated, the bacteria can burrow deeper into your body, causing fatal complications in bones and joints.



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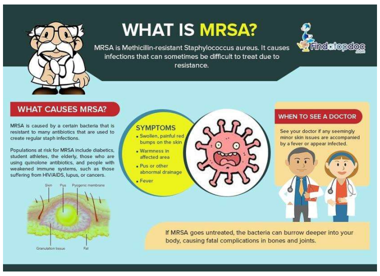

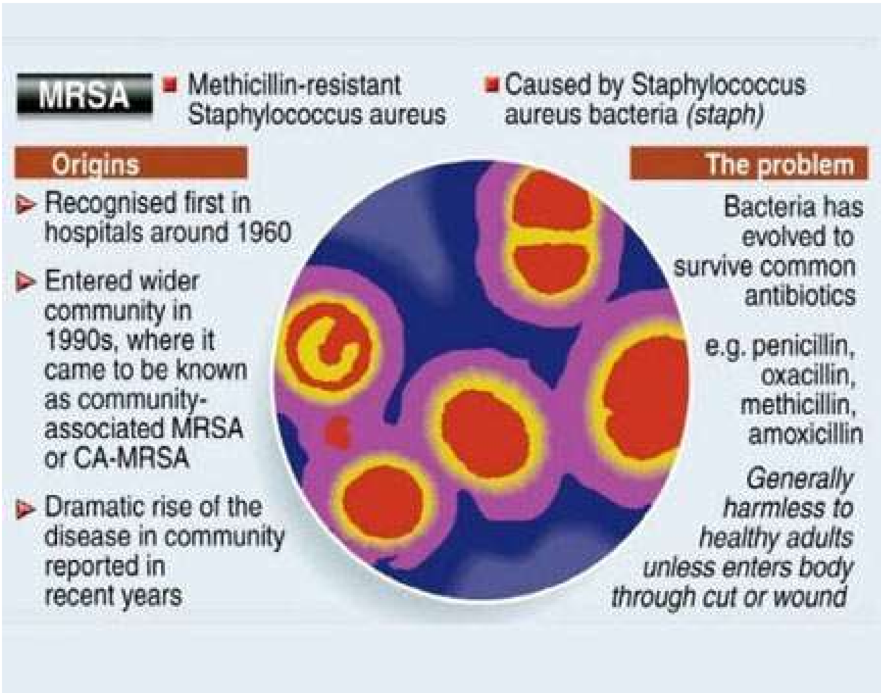
MRSA
Methicillin-resistant Staphylococcus aureus
Caused by Staphylococcus aureus bacteria (staph)
Origins
Recognised first in hospitals around 1960
Entered wider community in 1990s, where it came to be known as community-associated MRSA or CA-MRSA
Dramatic rise of the disease in community reported in recent years
The problem
Bacteria has evolved to survive common antibiotics
e.g. penicillin, oxacillin, methicillin, amoxicillin
Generally harmless to healthy adults unless enters body through cut or wound

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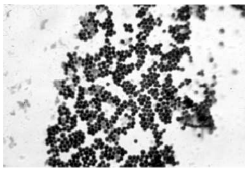
Staphylococcus aureus



- Gr^+, non-motile, asporogenous cocci,
- "grape-like" clusters

limiting factors	aerobic	anaerobic
pH	4.8	5.5
doubling time	25 min	56 min
A_w	0.86	0.92

241,148 illnesses in 2011 (3% of total foodborne infections) - CDC

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Staphylococcal Food Poisoning

- Staphylococcal food poisoning is caused by the ingestion of an enterotoxin produced in improperly stored foods.
- *S. aureus* is inoculated into foods during preparation.
- The bacteria grow and produce enterotoxin in food stored at room temperature.
- The exotoxin is not denatured by boiling for 30 minutes.
- Foods with high osmotic pressure and those not cooked immediately before consumption are most often the source of staphylococcal enterotoxicosis.
- Diagnosis is based on symptoms. Nausea, vomiting, and diarrhea begin 1~6 hours after eating and last about 24 hours.
- Laboratory identification of *S. aureus* isolated from foods is used to trace the source of contamination.
- Serological tests are available to detect toxins in foods.

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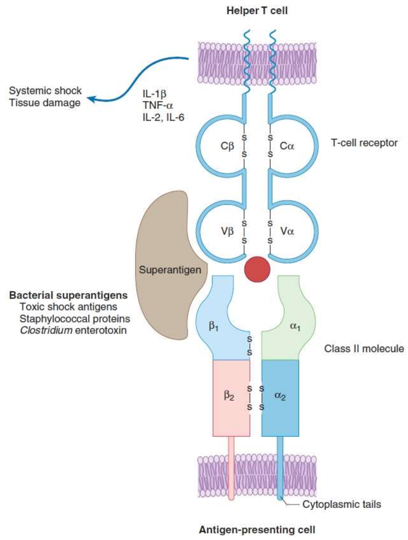
Staphylococcus aureus

- enterotoxin
- effective at 1 µg/kg
- protein of 239 amino acids
- serological types: A, B, C, D, F
- very compact
- heat resistance:
  - cells: $D_{55°C}$ = 0.95 to 8.0 min
  - toxin: $D_{121°C}$ = 9.9 to 34 min
  - z= 30°C

Staphylococcal (and clostridial) Toxins: Introduction

- Staphylococcal enterotoxins (Ses) are **superantigens** and belong to the family of **pyrogenic toxins**
- **Superantigens** are defined by their ability to stimulate a large fraction of T-cells via interaction with the T-cells receptor (TCR) Vβ domain
- Superantigens include staphylococcal enterotoxin A (SEA) and toxic shock syndrome toxin (TSST)
- The superantigen by-passes normal processing and directly bridges the TCR with the MHC class II molecule*, causing cells to divide and differentiate into effector cells and release T-cell factors (IL-2, TNF-α, and IL-1β)
- Because the number of T-cells that share Vβ domains is high (up to 10% of all T-cells), large numbers of T-cells (regardless of antigen specificity) may be activated by superantigens
- This can lead to massive systemic disruption with symptoms similar to septic shock including occurrence of severe tissue injury and possible multiple organ failure



*MHC class II molecules: major histocompatibility complex (MHC) molecules normally found only on professional antigen-presenting cells such as dendritic cells, mononuclear phagocytes, some endothelial cells, thymic epithelial cells, and B cells.
IL, interleukin; *TNF*, tumor necrosis factor.

https://doi.org/10.1016/B978-0-323-07447-6.00005-3
Elsevier's integrated review immunology and microbiology / Jeffrey K. Actor. – 2nd ed.

Table 21.3 Prevalence of *S. aureus* in several foods

Product	No. of samples tested	% Positive for *S. aureus*	No. of *S. aureus* CFU/g[a]	Reference
Ground beef	74	57	≥100	135
	1,830	8	≥1,000	24
	1,090	9	≥100	107
Big-game meat	112	46	≥10	126
Pork sausage	67	25	100	135
Ground turkey	50	6	≥10	47
	75	80	>3.4	48
Salmon steaks	86	2	>3.6	36
Oysters	59	10	>3.6	36
Blue crabmeat	896	52	≥3	148
Peeled shrimp	1,468	27	≥3	137
Lobster tail	1,315	24	≥3	137
Assorted cream pies	465	1	≥25	141
Tuna pot pie	1,290	2	≥10	149
Delicatessen salads	517	12	≥3	104

[a]Determined by either direct plate count or most-probable-number techniques.

doi:10.1128/9781555818463.ch21

Staphylococcus aureus

Food Poisoning Syndrome:

- onset: 0.5 to 6 hours
- recovery: 24 to 72 hours
- major symptoms: vomiting, diarrhea
- other symptoms: nausea, salivation, cramps, retching, prostration

Staphylococcal Toxic Doses

- According to FDA, doses of SE* causing illness result when populations of greater than 10^5 *S. aureus* cells per gram of contaminated food are present
  - When grown in milk, detectable levels of SEA* and SED* were found with **10^4 and 10^7** CFU/ml of the strains-producers, respectively
- Approx. 1 ng of SE per gram of contaminated food is sufficient to cause symptoms associated with staphylococcal food poisoning (SFP)
  - Typically, 1 to 5 μg of ingested toxin is associated with many outbreaks
  - The minimum dose of SEA required to cause SFP in school children was found to be 144 ± 50 ng
  - Low-fat milk outbreak of SFP (Japan): total intake of SEA per capita 20 to 100 ng

  - *SE: staphylococcal enterotoxin
  - SEA: staphylococcal enterotoxin A
  - SED: staphylococcal enterotoxin D

doi:10.1128/9781555818463.ch21

Staphylococcus aureus Case Study

- Flight from Tokyo to Paris via Anchorage and Copenhagen
- 196 of 343 passengers, 1 of 20 crew developed symptoms
- food eaten:
  - 1 h post Anchorage, snack, served to all
  - 5.5 h post Anchorage, steak dinner, crew
  - 5.6 h post Anchorage, cheese omelet w/ ham, passengers only
- onset:
  - 0.5 to 5.5 h after breakfast, average 2.5 h
- symptoms:
  - diarrhea (88%), vomiting (82%)
  - cramps (74%), nausea (68%)

doi: 10.1016/s0140-6736(75)90183-x

Staphylococcus aureus Investigation

- Isolated SED-producing *S. aureus* from fecal swabs of 5 patients and from left over omelet and ham
- Isolated SED from omelet and ham
- Breakfast prepared day before flight, held at room temperature for 6 h during preparation
- Held 14.5 h at 10°C prior to the flight
- Held at room temperature during the flight, then heated
- Isolated SED-producing *S. aureus* from inflamed lesion on the hand of the cook who had made the breakfast

doi: 10.1016/s0140-6736(75)90183-x

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Staphylococcal Food Poisoning (1989)

- Staphylococcal enterotoxin found in canned mushrooms from China
- 4 outbreaks, > 100 cases

English, et al., Dec. 1990, Food Technol. p. 74

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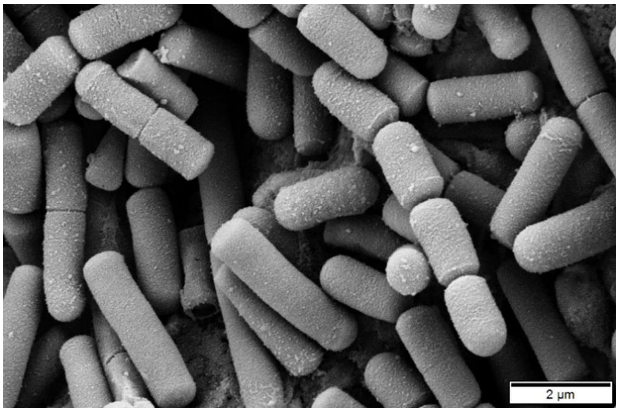
Would a *Staphylococcus* by Any Other Name be So Toxic?

- *Staphylococcus intermedius* producing SEA
- Implicated in outbreak involving 263 cases
- linked with consumption of butter and margarine in October, 1991

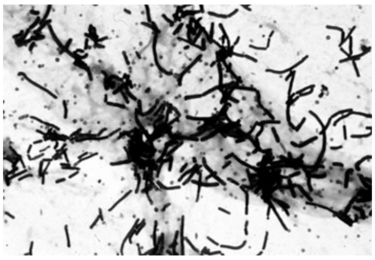
Bennet, et al., Abs. 81st Ann Mtg. IAMFES, 1994

Bacillus cereus	Gram-positive, spore formation,mesofil 48 species of *Bacillus* Catalase positive, oxidase variable	NB: identification is important tool. 16s rRNA can be not suficient
Growth temperatures: from 10ºC up to 48ºC Optimal 28ºC-35ºC	pH: from 4.9 up to 9.3	Water activity: 0.95 7.5% NaCl – inhibition
Caused by bacteria, toxins Gastroenterite Incubation time 12-24h Presence of 10^7-10^9 CFU Toxin is destroyed after 55ºC for 20 min; below pH 4.0	*Bacillus cereus* is present in nature, soil, water, respiratory tract, vegetables. Can be find in 40% of rice samples	

Bacillus cereus



- aerobic
- Gram$^+$, motile rods
- protoplasm is granular
- acid, no gas from glucose
- acid from sucrose, glycerol
- no acid from mannitol, arrabinose, xylose
- metabolizes citrate

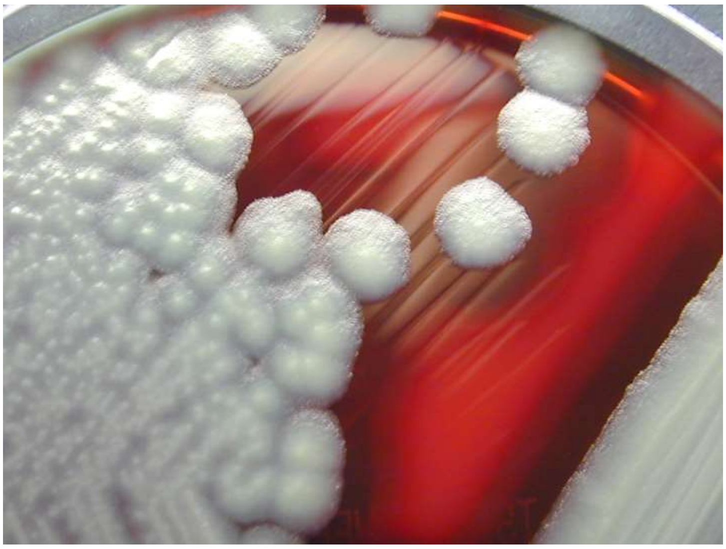


http://vm.cfsan.fda.gov/~mow/chap12.html

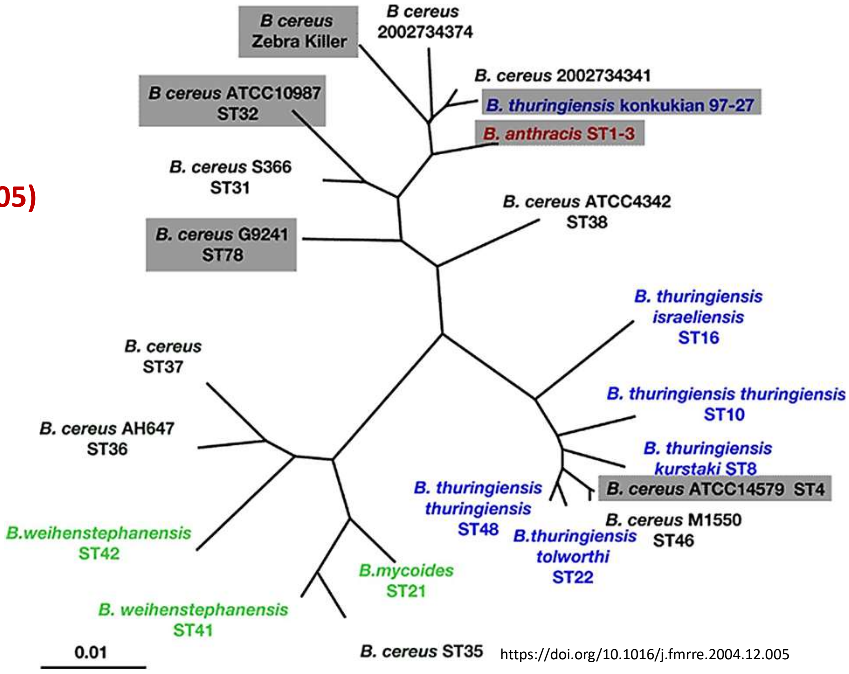
Colonies of *B. cereus* were originally isolated from a gelatine plate left exposed to the air in a cow shed in 1887.

The specific name, cereus, meaning "waxy" in Latin, refers to the appearance of colonies grown on blood agar.

In the 2010s, examination of warning letters issued by the US Food and Drug Administration issued to pharmaceutical manufacturing facilities addressing facility microbial contamination revealed that the most common contaminant was *B. cereus*.



Multi-locus sequence typing (MLST) neighbor-joining phylogenetic tree of representatives of the *Bacillus cereus* group of organisms (2005)



https://doi.org/10.1016/j.fmrre.2004.12.005

Because of *B. cereus*' ability to **<u>produce lecithinase</u>** and its **<u>inability to ferment mannitol</u>**, there are some proper selective media for its isolation and identification such as mannitol-egg yolk-polymyxin (MYP) and polymyxin-pyruvate-egg yolk-mannitol-bromothymol blue agar (PEMBA).
B. cereus colonies on MYP have a violet-red background and are surrounded by a zone of egg-yolk precipitate.

List of differential techniques and results that can help to identify *B. cereus* from other bacteria and *Bacillus* species.

- ✓ Anaerobic growth: Positive
- ✓ Voges Proskauer test: Positive (test used to detect acetoin)
- ✓ Acid produced from
- ✓ d-glucose: Positive
- ✓ l-arabinose: Negative
- ✓ d-xylose: Negative
- ✓ d-mannitol: Negative
- ✓ Starch hydrolysis: Positive
- ✓ Nitrate reduction: Positive
- ✓ Degradation of tyrosine: Positive
- ✓ Growth at above 50 °C: Negative
- ✓ Use of citrate: Positive

The *B. cereus* groups

- *Bacillus cereus sensu stricto* (strictly speaking)- an opportunistic pathogen
  - *Bacillus cereus* – foodborne pathogens
  - *Bacillus anthracis* - the etiological agent of anthrax
  - *Bacillus thuringiensis* – entomopathogen

- *Bacillus cereus sensu lato* (in the broad sense)
  - *Bacillus mycoides*
  - *Bacillus pseudomycoides*
  - *Bacillus weihenstephanensis*
  - *Bacillus toyonensis*
  - *Bacillus cytotoxicus*
  - *Bacillus manliponensis*
  - *Bacillus gaemokensis*
  - *Bacillus bombysepticus*
  - *Bacillus bingmayongensis*
  - *Bacillus* sp. 7_6_55CFAA_CT2
  - *Bacillus wiedmannii*

doi: 10.1038/srep46430
https://doi.org/10.1016/j.fmrre.2004.12.005

Table 19.2 Examples of foods involved in *B. cereus* food poisoning events

Type of food	Country	No. of people involved	Type(s) of syndrome[a]
Barbecued chicken	Many countries	—[b]	E, D
Cooked noodles	Spain	13	D
Cream cake	Norway	5	D
Eclair (pastry)	Thailand	>400	E (D)
Fish soup	Norway	20	D
Hibachi steak	United States	11	E, D
Lobster pâté	United Kingdom	—	D
Meat loaf	United States	—	D
Meat with rice	Denmark	>200	D
Milk	Many countries	—	E, D
Milkshake	United States	36	?
Pea soup	The Netherlands	—	D
Sausages	Ireland, China	—	D
School lunch	Japan	1,877	E
Scrambled egg	Norway	12	D
Several rice dishes	Many countries	—	E, D
Stew	Norway	152	D
Turkey	United Kingdom, United States	—	D
Vanilla sauce	Norway (many countries)	>200	D
Vegetable sprouts	United States	3	E, D
Wheat flour dessert	Bulgaria	—	D

[a] E, emetic syndrome; D, diarrheal syndrome.
[b] —, not available.

The emetic syndrome is an **intoxication that is caused by ingestion of a cyclic peptide toxin called cereulide that is pre-formed in the food during growth by *B. cereus***.

doi:10.1128/9781555818463

Table 19.3 Characteristics of the two types of illness caused by *B. cereus*[a]

Characteristic	Diarrheal syndrome **generally, no fever**	Emetic syndrome
Dose causing illness	10^5–10^7 cells (total)	10^5–10^8 cells (per g in foods)
Toxin produced	In the small intestine of the host	Preformed in foods
Type of toxin	Protein; enterotoxin(s)	Cyclic peptide; emetic toxin
Incubation period	8–16 h (occasionally >24 h)	0–5 h
Duration of illness	12–24 h (occasionally several days)	6–24 h
Symptoms	Abdominal pain, watery diarrhea occasionally with nausea	Nausea, vomiting, and malaise (sometimes followed by diarrhea, due to production of enterotoxin)
Foods most frequently implicated	Meat products, soups, vegetables, puddings/sauces, and milk/milk products	Fried and cooked rice, pasta, pastry, and noodles

[a]Based on references 35, 53, and 74.

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doi:10.1128/9781555818463

Table 19.5 Toxins produced by *B. cereus*

Toxin	Type/size	Food poisoning
Hemolysin BL (Hbl)	Protein, 3 components	Probably
Nonhemolytic enterotoxin (Nhe)	Protein, 3 components	Yes
Cytotoxin K (CytK)	Protein, 1 component, 34 kDa	Yes, 3 deaths
Emetic toxin (cereulide)	Cyclic peptide, 1.2 kDa	Yes, several deaths

doi:10.1128/9781555818463

- Haemolysin IV, or CytK, is a 34 kDa cytotoxic necrotic and haemolytic protein.
- The deduced amino-acid sequence is 37% identical to that of *B. cereus* haemolysin II and 30% identical to that of the α-hemolysin from *S. aureus*.
- From a structural point of view, CytK (like HlyII) belongs to the family of oligomeric β-barrel pore-forming toxins.
- Secreted in a soluble form, converted into a transmembrane pore by the assembly of an oligomeric β-barrel, with the hydrophobic residues facing the lipids and the hydrophilic residues facing the lumen of the channel.
- CytK can spontaneously form oligomers that are resistant to SDS but not boiling (similar to other β-barrel pore-forming toxins).

doi:10.3390/toxins5061119

Table 19.4 Properties of the *B. cereus* emetic toxin cereulide[a]

Trait	Property/activity
Molecular mass	1.2 kDa
Structure	Ring-shaped peptide
Isoelectric point	Uncharged
Antigenic	No
Biological activity in living primates and Asian house shrew	Vomiting; Inhibition of mitochondrial activity (fatty acid oxidation)
Receptor	5-HT_3 (stimulation of the vagus afferent)
Ileal loop tests (rabbit, mouse)	None
Cytotoxic	No
HEp-2 cells	Vacuolation activity
Stability to heat	90 min at 121°C
Stability to pH	Stable at pH 2–11
Effect of proteolysis (trypsin, pepsin)	None
Conditions under which toxin is produced	In food (rice and milk at 12–32°C)
Mechanisms of production	Produced by a nonribosomal peptide synthetase

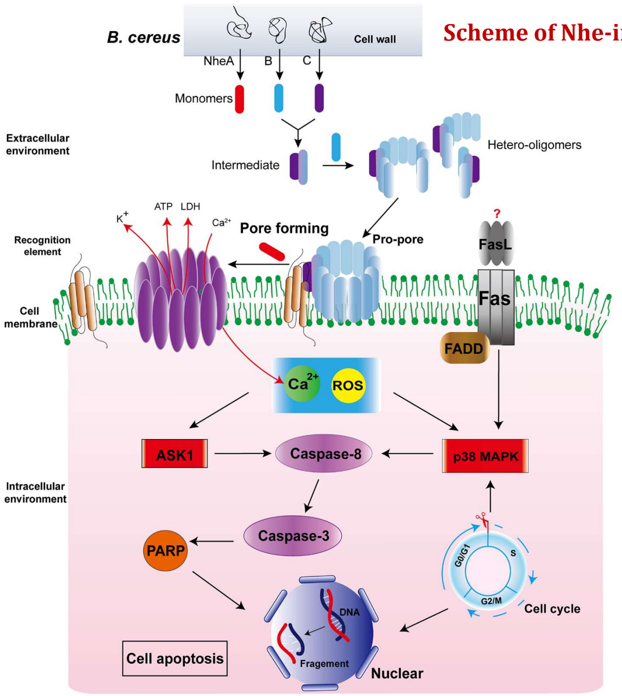
[a]Based on references 2, 3, 27, 53, 74, 75, and 82.

doi:10.1128/9781555818463

- Dodecadepsipeptide with a structure similar to the potassium ionophore valinomycin.
- Cyclic molecule, 36-membered ring with alternating ester and amide bonds and the structure (d-*O*-Leu-d-Ala-l-*O*-Val-l-Val)$_3$.
- Molecular weight is 1165 Da and predicted pI is 5.52.
- Very hydrophobic, essentially insoluble in aqueous solution.
- Seven cereulide synthesis genes comprise the *ces* operon.
- Cereulide transcription depends on Spo0A and AbrB (direct repressor of the main σ^A-dependent promoter of the *ces* operon) regulators.
- Cereulide production appeared to be independent of late stages of sporulation.

https://doi.org/10.1016/C2010-1-67744-9

Non-ribosomal peptide synthetases are modular enzymes that catalyze synthesis of important **peptide** products from a variety of standard and **non**-proteinogenic amino acid substrates.



Scheme of Nhe-induced apoptosis cascade

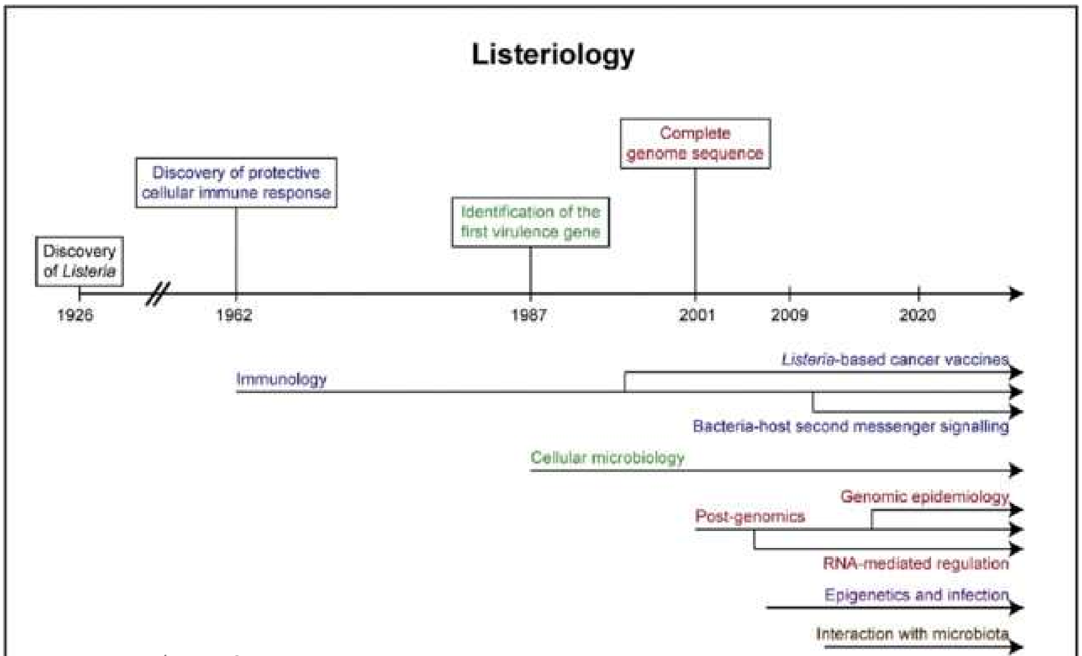
- Pore formation by the Nhe complex results in the accumulation of Ca^{2+} and ROS in the cytoplasm and activation of Fas.
- That leads to cell cycle arrest at G_0/G_1 phase accompanied by activation of p38 MAPK.
- Then ASK1 and p38 MAPK induce cleavage of caspase-8, which activates its downstream proteins to mediate apoptosis.
- Nhe activates the death receptor pathway by a still unknown (2018) mechanism.

ASK1, apoptosis signal-regulating kinase 1; **Caspase**, cysteinyl aspartate specific proteinase; **FADD**, Fas-associated death domain; **Fas**, tumor necrosis factor superfamily receptor member 6; **FasL**, Fas ligand; **MAPK**, mitogen-activated protein kinase; **Nhe**, non-hemolytic enterotoxin; **PARP**, poly ADP-ribose polymerase; **ROS**, reactive oxygen species

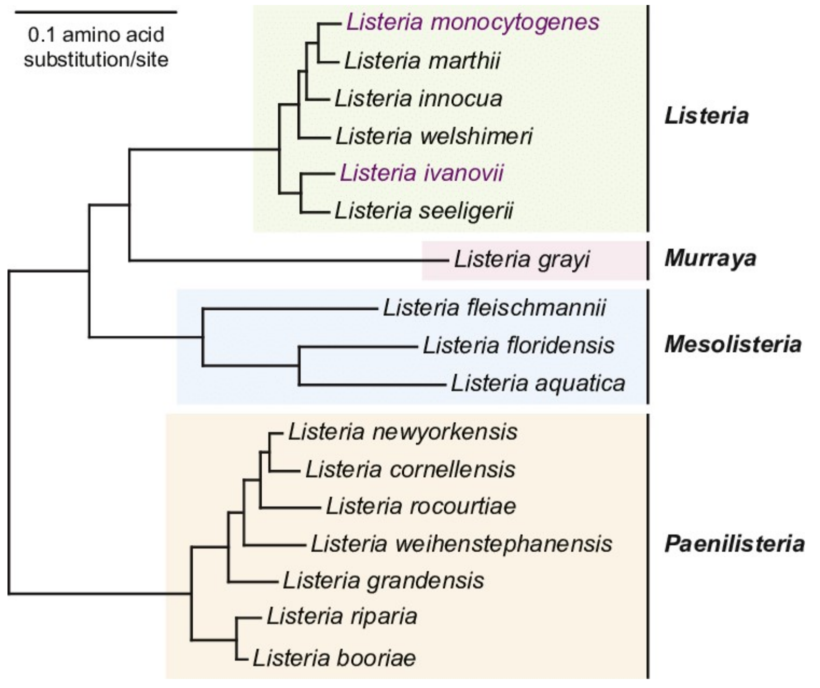
https://doi.org/10.1111/cmi.12684

Foods associated with *Bacillus cereus* outbreaks

- Meats and gravies
- Sauces and puddings
- Miscellaneous vegetable dishes
- Fried rice and other rice products



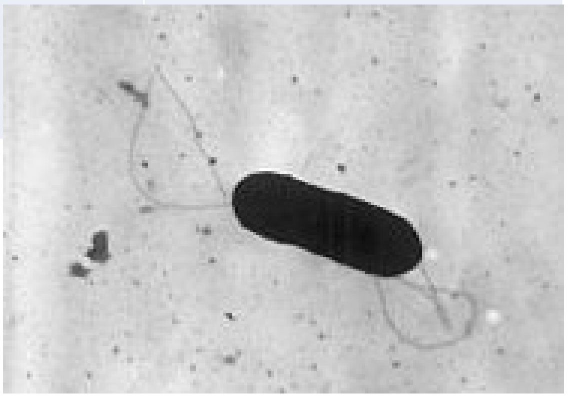
•DOI: 10.1016/j.micinf.2016.10.009



DOI: 10.1016/j.micinf.2016.10.009

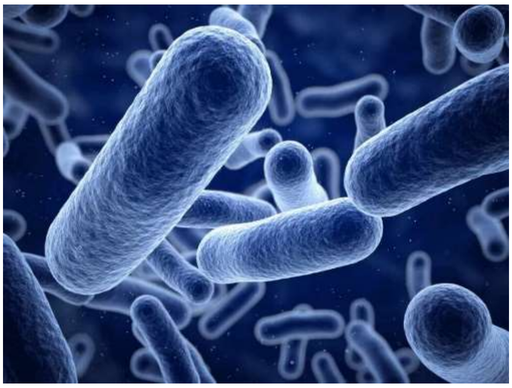
Phylogenomic tree of Listeria species. The two pathogenic species Listeria monocytogenes and Listeria ivanovii are highlighted in purple letters. The proposed novel genera names are displayed on the right. This tree does not include yet Listeria goaensis, the newcomer to be proposed by T. Chakraborty and S. Barbuddhe.

Listeria monocytogenes	Gram-positive, non spore formation, facultative anaerobe	
Growth temperatures: from 2.5ºC up to 44ºC	pH: from 4.5 up to 9.5	Water activity: Optimal 0.97 Resistance up to 30% NaCl
Pregnant Elderly, Children, Imunocompromised, transplantant Multiplication inside the cells Septimia, entiridic, bacterimia, febre, diarrea GIT disorders	Humans and other animals are reservoir for Listeria. Warm blood animals, birds, fish, larvas, insects	



Listeria monocytogenes

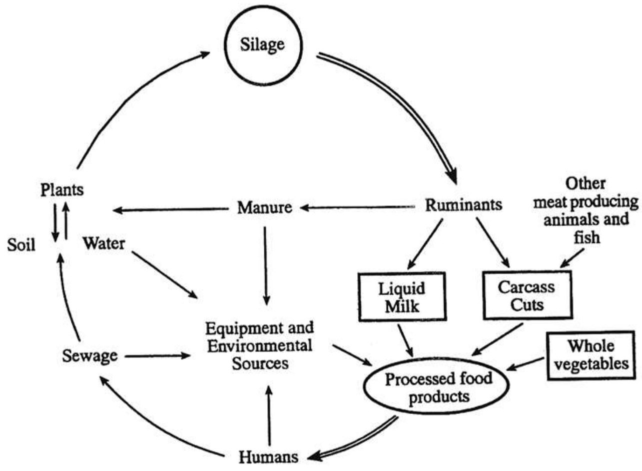
- **Bactéria ubiquitária**
- **Não esporulada**
- **Termosensível**
- **Formadora de biofilmes em equipamentos e utensílios**
- **Recontaminação após o cozimento**
- **Durante o fatiamento**
- **Durante o manuseio para o consumo**



Listeria monocytogenes

- Gram^{+}, small, non-sporeforming rod
- 1-2 X 0.5 µM Long
- Can Have "Y", "V", or even streptococcal forms
- Motile at 20-25°C, but not at 37°C
- Serotype Based on O & H Antigens
  - there are 13 serotypes that can cause disease, but more than 90% of human isolates belong to 1/2a, 1/2b, and 4b
- Produces haemolysin
- Infective dose may be as low as 8 CFU/G

Figure 20.2 Potential routes of transmission of *L. monocytogenes*



Circles or ovals indicate areas of greatest risk of *L. monocytogenes* multiplication. Boxes indicate where direct consumption of minimally processed products (e.g., whole fresh vegetables, cooked carcass cuts of meat and fish, and effectively pasteurized milk) presents a low risk. Double arrows indicate consumer at risk.

doi:10.1128/9781555818463.ch20f2

L. monocytogenes - environmental factors

- Temperature
- range: 0 - 45° C
- optimum: 30-37°C

- Cells more resistant to acid and salt at 5°C than 30°C

- pH
- range: 5.0-9.6
- optimum: 6.5

- Cells gradually die off at pH <4.8

Table 20.1 Relative risk ranking and predicted number of cases of listeriosis for the total U.S. population on a per serving and per annum basis[a]

Relative risk ranking	Predicted median no. of cases of listeriosis for 23 food categories					
	Per serving basis[b]			Per annum basis[c]		
		Food	Cases		Food	Cases
1	High risk	Deli meats	7.7×10^{-8}	Very high risk	Deli meats	1,598.7
2		Frankfurters, not reheated	6.5×10^{-8}	High risk	Pasteurized fluid milk	90.8
3		Pâté and meat spreads	3.2×10^{-8}		High fat and other dairy products	56.4
4		Unpasteurized fluid milk	7.1×10^{-9}		Frankfurters, not reheated	30.5
5		Smoked seafood	6.2×10^{-9}	Moderate risk	Soft unripened cheese	7.7
6		Cooked ready-to-eat crustaceans	5.1×10^{-9}		Pâté and meat spreads	3.8
7	Moderate risk	High fat and other dairy products	2.7×10^{-9}		Unpasteurized fluid milk	3.1
8		Soft unripened cheese	1.8×10^{-9}		Cooked ready-to-eat crustaceans	2.8
9		Pasteurized fluid milk	1.0×10^{-9}		Smoked seafood	1.3
10	Low risk	Fresh soft cheese	1.7×10^{-10}	Low risk	Fruits	0.9
11		Frankfurters, reheated	6.3×10^{-11}		Frankfurters, reheated	0.4
12		Preserved fish	2.3×10^{-11}		Vegetables	0.2
13		Raw seafood	2.0×10^{-11}		Dry/semidry fermented sausages	<0.1
14		Fruits	1.9×10^{-11}		Fresh soft cheese	<0.1
15		Dry/semidry fermented sausages	1.7×10^{-11}		Semisoft cheese	<0.1
16		Semisoft cheese	6.5×10^{-12}		Soft ripened cheese	<0.1
17		Soft ripened cheese	5.1×10^{-12}		Deli-type salads	<0.1
18		Vegetables	2.8×10^{-12}		Raw seafood	<0.1
19		Deli-type salads	$5.6 \times 10^{-1}3$		Preserved fish	<0.1
20		Ice cream and other frozen dairy products	4.9×10^{-14}		Ice cream and other frozen dairy products	<0.1
21		Processed cheese	4.2×10^{-14}		Processed cheese	<0.1
22		Cultured milk products	3.2×10^{-14}		Cultured milk products	<0.1
23		Hard cheese	4.5×10^{-15}		Hard cheese	<0.1

[a] Table adapted from reference 6.
[b] Food categories were classified as high risk (>5 cases per billion servings), moderate risk (≤5 but ≥1 case per billion servings), and low risk (<1 case per billion servings).
[c] Food categories were classified as very high risk (>100 cases per annum), high risk (>10 to 100 cases per annum), moderate risk (>1 to 10 cases per annum), and low risk (<1 case per annum).

... and the take-home message is?...

doi:10.1128/9781555818463

Bad News on Refrigeration

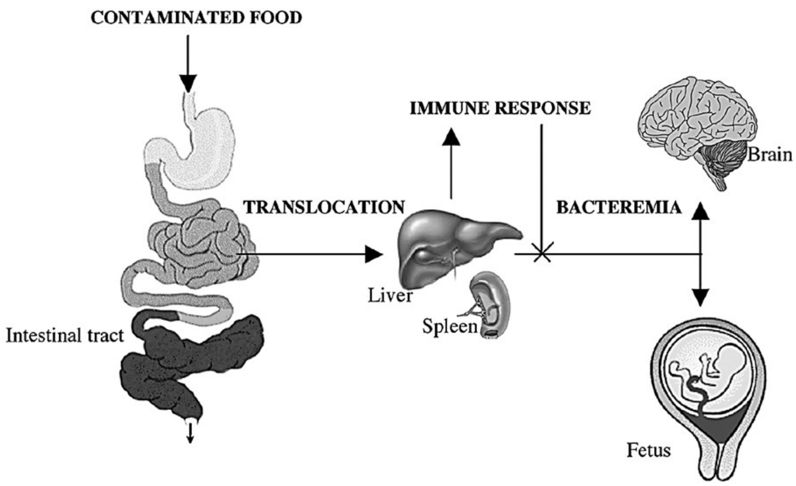
Hutton, et al., 1991, J Food Safety 11:255-267

- 15 CITY - 100 SUPERMARKET STUDY OF REFRIGERATOR CASES
- 90% OVER 4.5 °C
- 20% OVER 10°C

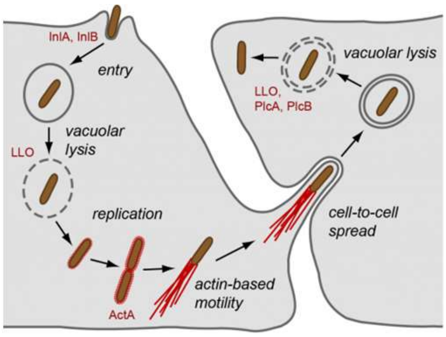
Woodburn, J. Am. Diet. Assoc. 87:322-326

- 21% OF HOME REFRIGERATORS OVER 10°C

Figure 20.3 Schematic representation of the pathophysiology of *Listeria* infection.
doi:10.1128/9781555818463.ch20f3 ("borrowed" without referencing from: DOI: 10.1128/CMR.14.3.584–640.2001)



Listeria: a Brief Story of Invasion



- Intenalin A and B (InlA/B) assist *Listeria* in phagocytosis
- Once inside a phagocyte, it can lyse the phagosome using Listeriolisin O (LLO)
- After being released from the phagosome, listeria hijacks the cell cytoskeleton, inducing actin polymerization to propel itself inside cytoplasm, using ActA
- Once listreria reaches the cell membrane, it can rupture that cell and spread to a neighboring one directly, without an extracellular phase

https://www.biolegend.com/newsdetail/2260/

Listeria outbreaks case: Los Angeles Mexican Soft Cheese

- 93 Perinatal cases:
  - 19 Still births
  - 10 Postnatal deaths
- 49 Adult cases:
  - 18 Deaths
- Linked with Mexican soft cheese
- Same phage type isolated from cases and processing plant
- Plant's milk throughput exceeded capacity of pasteurizer
- Cheese was alkaline phosphate positive

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Listeria outbreaks case: Canadian Maritime Provinces (1979-1981)

- Peaked in summer of each year
  - 34 Perinatal cases:
    - 5 Abortions
    - 4 Stillborn
    - 23 Live birth but critically ill, 27% mortality
    - 2 “well babies"
  - 33 % Fatality in adult cases
  - Probable cause: coleslaw
  - Cabbage was “organically” grown on farm with active cases of ovine enteritis

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L. monocytogenes - thermal resistance

L. monocytogenes **strain Scott A, FREE CELLS**

T (°C)	D (SEC)
57.8	290
66.1	7.3
68.9	3.0
71.7 (161°F)	0.9

COMPARE WITH	**D (SEC) AT 71.7°C**
Pseudomonas fraggi	1.17
E. coli	1.17
Yersinia	1.17
Staphylococcus aureus	1.20

71.1 °C FOR 15 SEC PRODUCES 15 LOG KILL

CURRENT PASTEURIZATION (72°C OR 161°F - 15 SEC) APPEARS ADEQUATE

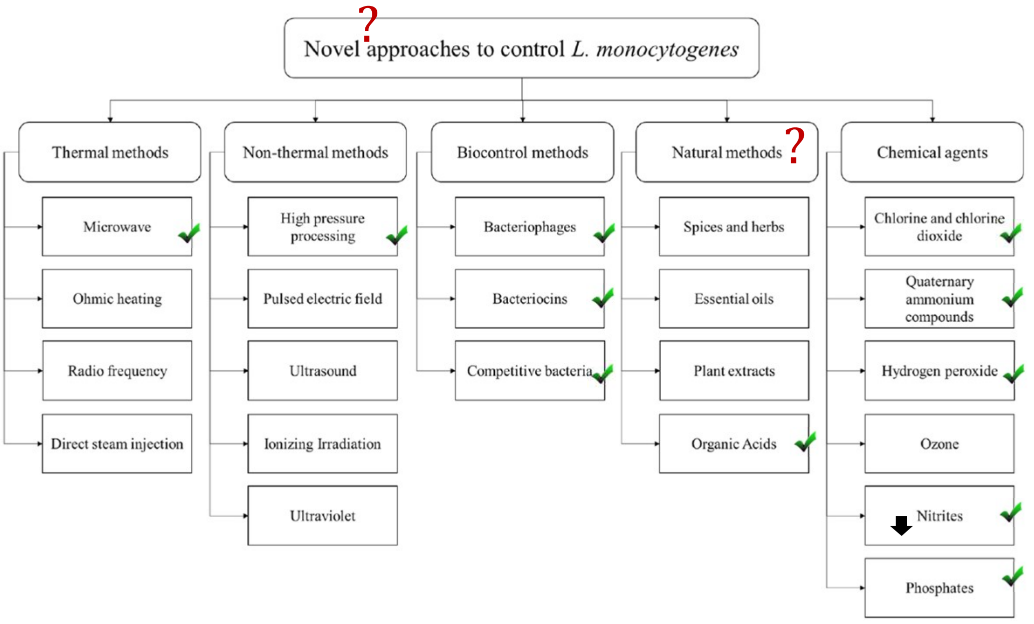
Listeria monocytogenes Recommendations

National Advisory Committee on Microbial Criteria For Foods

Int. J. Food Microbiol. 14:185-246.

STATUS:
- Organism widespread in environment
- Cases are relatively rare
- Organisms cannot be eliminated from environment
- Educational efforts are incomplete

RECOMMENDATIONS :
- Develop effective disease surveillance
- Target specific foods
- Minimize presence, survival, growth in foods
- Keep temperature <40°F (4°C)
- Educate (everybody)



doi:10.3390/foods11121760

Rapid Tests

- Rapid tests are widely used to detect specific antibodies or pathogens and are characterized by the availability of results in a few minutes (5 to 30 min)
- A rapid test that can diagnose a disease or detect pathogens in places with limited resources or when no pre-treatment or complex processing of suspect samples is required, is classified as a point-of-care test (POCT)
- Rapid tests have been applied in several areas such as:
  - diagnostic medicine,
  - environmental,
  - veterinary medicine,
  - quality control in industries,
  - food safety through the detection of toxins, allergens , and pathogens such as *L. monocytogenes*

doi:10.1080/1040841X.2021.1911930

Methods for detection of listeria

Method	Working Principles	Advantages	Disadvantages
Molecular Methods			
Multiplex PCR	Simultaneously amplifies multiple target DNA sequences and quantifies by detecting fluorescent probes attached to the DNA fragments	Rapid and high-throughput analysis	High cost, complex, and difficult in optimization
Real-time nucleic acid sequence-based amplification (NASBA)	Amplifies nucleic acid (generally by converting single-stranded RNA into cDNA) under isothermal condition and detects fluorescent probes attached to the target fragment	Operates without thermal cycling equipment and can detect viable microbial cells	Complexity in handling RNA
Loop-mediated isothermal amplification (LAMP)	Six primers target eight specific regions of target DNA, producing cauliflower-like structure of DNA bearing multiple loops. Assay performed under isothermal conditions, amplification products detected by agarose gel electrophoresis or fluorescent dye	Greater yield, lower detection limit, operates without thermal cycling equipment	Requires complex primer designing system, which can limit specificity
Oligonucleotide-based microarray	A glass slide coated with chemically synthesized oligonucleotide probes detects target DNA or RNA labeled with fluorescent dye.	Simultaneous identification and typing of microbial strain	Require high amount of target DNA or RNA

doi:10.3390/foods11121760

Methods for detection of listeria

Method	Working Principles	Advantages	Disadvantages
Microfluidic Systems			
Microfluidics lab-on-a-chip	Microchip with integrated microprocessor, pumps, valves, thermocycler, fluorescence detection module, to purify *L. monocytogenes* cells, and detect using real time-PCR	Fully automated purification and detection method	Lower sensitivity
Phage-Based Methods			
Phage protein	Listeria cells incubated with GFP-tagged phage protein and fluorescence measured after removal of unbound protein	Rapid and precise glycotype determination	Requires validation and further development
Phage amplification	Phages replicate inside viable target cells and lyse the cells to release progeny cells along with host DNA and intracellular components which can be detected using qPCR, ELISA, or enzyme assays	Rapid and detects viable cells	Complex and low throughput

doi:10.3390/foods11121760

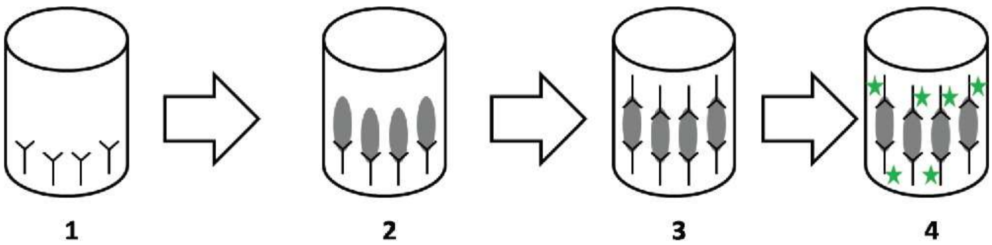
Methods for detection of listeria

Method	Working Principles	Advantages	Disadvantages
	Immunological Methods		
Immunomagnetic capture	Labelled Immunoglobulin G and aptamer-conjugated magnetic nanoparticles form sandwich-type immuno-complex in the presence of *L. monocytogenes*, detects fluorescence	Can detect *L. monocytogenes* without pre-enrichment	Requires validation and further development
Lateral flow immunoassay	Sample flows through four sections of immunoassay strip: sample pad, conjugate pad (target binds with antibody labeled by color particles), nitrocellulose pad (captures target and conjugate), and absorbent pad; detects target as presence or absence of line colors	Low cost, rapid, and easy to operate	Low sensitivity and may require pre-treatment of samples; the potential for false positives due to the interference from sample matrix, these assays often need to be optimized for detection of bacteria from a specific food matrix

Spectroscopy-based and biosensors-based methods are also under development…

doi:10.1080/10408398.2013.775567
doi:10.3390/foods11121760

Simplified diagram of an immunologically based method, ELISA technology



(1) Antibodies specific for the target pathogen are fixed on the surface of plastic wells.

(2) Antibodies capture the target pathogen antigens if present, and all un-bound material in the sample is removed.

(3) Added enzyme-conjugated antibodies specific for the target pathogen completes the “sandwich.”

(4) A colorimetric or fluorescent substrate specific for the enzyme is added, and the amount of product formed over time can be used to quantify the amount of target bacteria present in the initial sample.

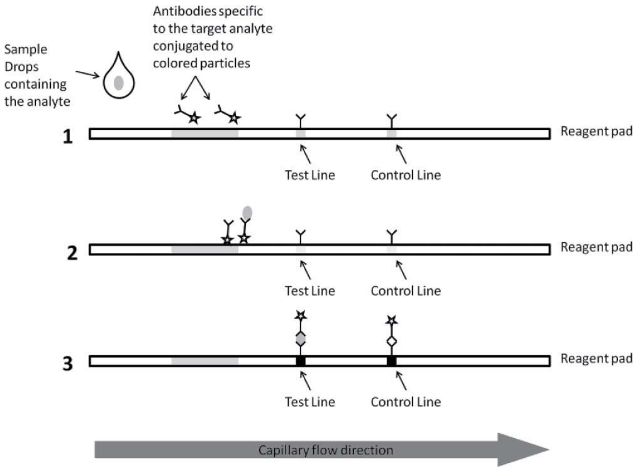
doi:10.1080/10408398.2013.775567

Lateral flow assay, aka lateral flow immunochromatographic assay (LFIA)

- The immunological technique most used as a rapid test or POCT (point-of-care test)
- The assay principle is based on the sample containing the analyte of interest introduction in the sample pad
- The sample moves laterally by the action of capillarity through different zones of a strip
- The first zone is the conjugated pad which contains molecules conjugated to colored or fluorescent labels
  - Colloidal gold nanoparticles conjugated to specific antigens or antibodies that act as detection agents are commonly used
- The detection zone is formed by porous membranes, such as the nitrocellulose (NC) membrane, containing specific biological reagents immobilised in test and control lines
  - Polyclonal antibodies are widely used in the test lines as a capture agent for the conjugate analyte
- The recognition of the analyte in the sample promotes staining in the test line and the result can be read with the naked eyes
- The test operation is certified by observing color in the control

doi:10.1080/1040841X.2021.1911930

Simplified diagram of the lateral flow assay



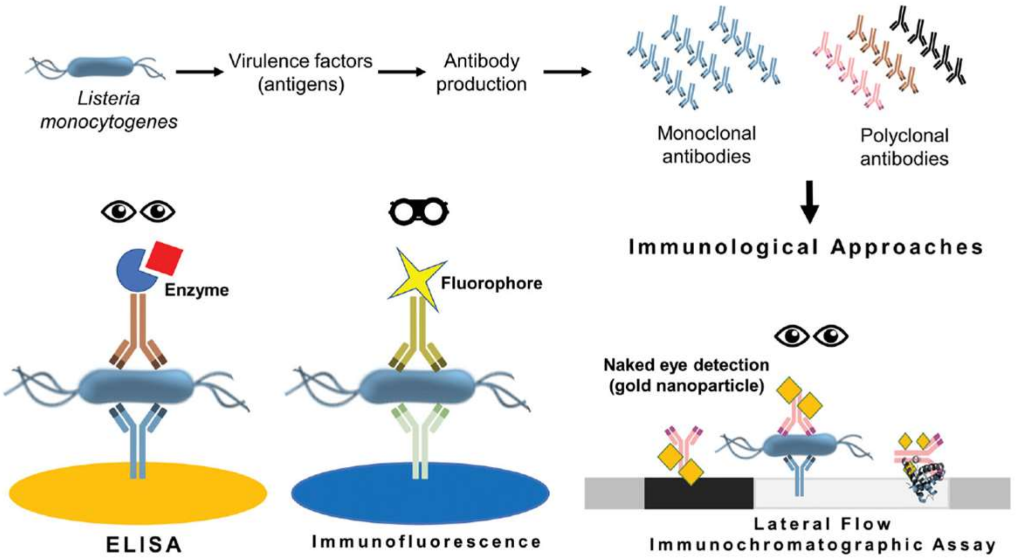
(1) Enriched sample is deposited in the reagent pad.

(2) Migration through the reagent pad and attachment to conjugated antibodies.

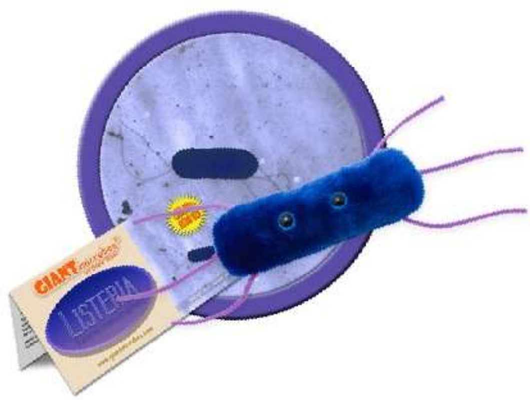
(3) Two visible (analyte and control) lines represent a positive result.

doi:10.1080/10408398.2013.775567

Immunoassay formats include ELISA (Enzyme- Linked Immunosorbent Assay), Immunofluorescence Assay, and Lateral Flow Immunochromatographic Assay



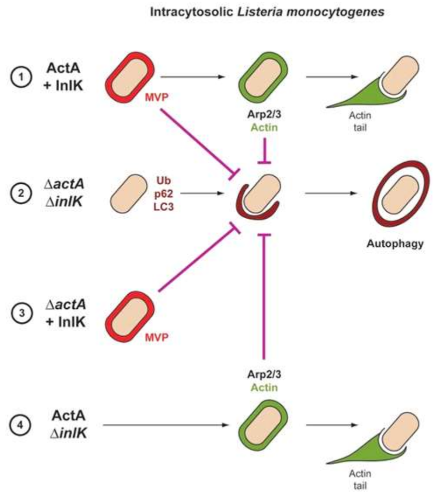
doi:10.1080/1040841X.2021.1911930



Acknowledgment to prof. Mike Chikindas, Rutgers University, NJ, USA
for collaborating in preparation of the material

https://www.giantmicrobes.com/us/products/listeria.html

Strategies Used by *L. monocytogenes* to Avoid Autophagic Recognition



(1) **ActA and InlK are coexpressed** by the bacterium: InlK recruits MVP (red) to the surface of the bacterium. ActA subsequently replaces InlK, and actin (green) replaces MVP to disguise the bacteria and prevent ubiquitinated protein (Ub) recruitment/formation, p62 recognition and LC3 recruitment;

(2) **Neither ActA nor InlK is expressed**: *Listeria* is surrounded by ubiquitinated proteins, p62 and LC3, leading to autophagy;

(3) **In the absence of ActA, InlK recruits MVP** and efficiently protects bacteria from ubiquitinated protein recruitment/formation, p62 recognition and LC3 recruitment;

(4) **ActA is expressed, but not InlK**: the recruitment of the Arp2/3 complex and actin is sufficient to prevent ubiquitinated protein recruitment/formation, p62 recognition and LC3 recruitment.

MVP = major vault protein, main component of cytoplasmic ribonucleoprotein particles named vaults, interacts with InlK ; **P62** = autophagy receptor; **ActA** = Actin assembly-inducing protein, triggers the recruitment of the Arp2/3 complex to mediate actin polymerization and to propel listeria through a mammalian host cell; **InlK** = internalin-like protein

doi: 10.4161/auto.8.1.18218

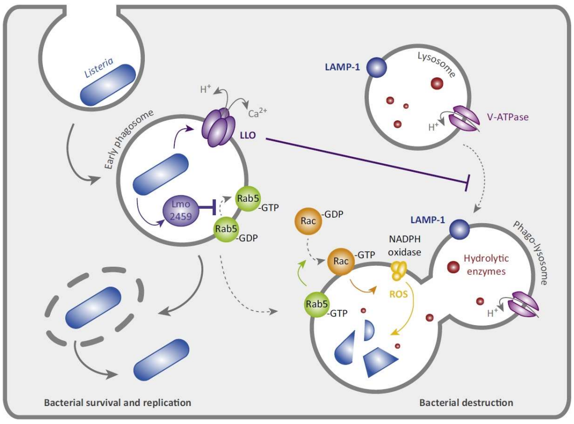
Listeria monocytogenes- disease

- Low grade "flu-like" infection - not serious, except in pregnant woman (who abort)
  - Up to 16% women carry *L. monocytogenes* without illness (Larmont and Postlethwaite, 1986, J. Infection 13:187-193)
- Listeric meningitis- headache, drowsiness, coma
  - 50% fatality rate; if very young and old are excluded, this drops to 30%
- Perinatal infection
  - 0.15% to 2.0% of all perinatal mortality
- Encephalitis
- Psychosis
- Infectious mononucleosis
- Septicemia

- Organelle manipulation by *Listeria* determines the outcome of infection
- Disruption of mitochondrial dynamics affects the efficiency of *Listeria* infection
- Bacteria secrete nucleomodulins to reprogram host cell transcription
- *Listeria*-induced perturbations in ion homeostasis impact on all organelles

DOI: https://doi.org/10.1016/j.tcb.2015.01.003

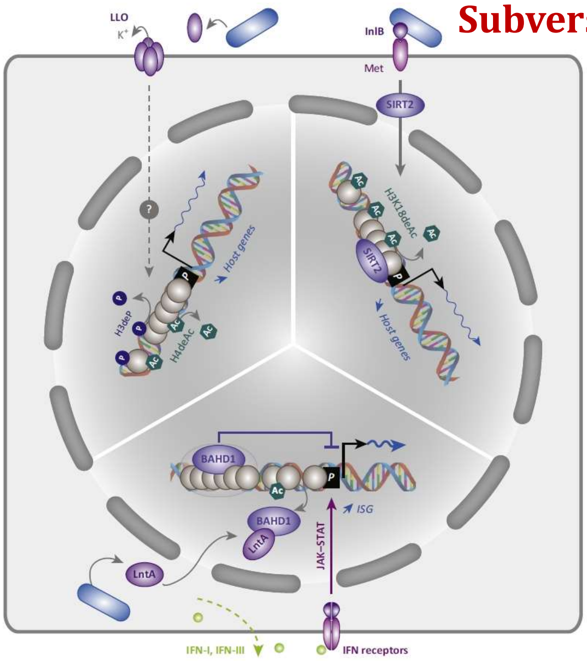
Inhibition of Phago-Lysosomal Maturation by Listeria



- After phagocytosis by macrophages, the bacteria-containing phagosome may fuse with LAMP-1 (lysosomal-associated membrane protein 1)-positive lysosomes to generate a phago-lysosome.
- Reactive oxygen species (ROS) produced by NADPH oxidase and the action of hydrolytic enzymes exert toxicity which is enhanced by the acidification of the organelle, resulting in bacterial killing and degradation.
- The secretion of listeriolysin (LLO) by *Listeria* decreases phagosomal calcium concentration and increases pH, which impedes phago-lysosomal fusion.
- Another secreted effector, Lmo2459, blocks the maturation of the phagosome via the inhibition of Rab5.

http://dx.doi.org/10.1016/j.tcb.2015.01.003

Subversion of Nuclear Functions by Listeria



- **(Left)** By secreting the pore-forming toxin listeriolysin (LLO), Listeria generates potassium efflux from the host cell, and this promotes global deacetylation of histone H4 and dephosphorylation of histone H3 on serine 10. This results in the downregulation of a subset of host genes.
- **(Right)** Binding of Listeria InlB to its receptor Met at the host cell membrane triggers, via a PI3K/Akt-dependent signaling cascade, the translocation of the lysine deacetylase SIRT2 to the nucleus. SIRT2 associates with chromatin at the promoter of various host genes, deacetylates lysine 18 on H3, and thereby represses these genes.
- **(Bottom)** In response to Listeria infection of epithelial cells, type I and III interferons (IFNs) are produced. However, the expression of IFN-stimulated genes (ISGs) is repressed downstream of JAK–STAT signaling as a result of condensation of a repressive complex at their promoter by the host chromatin component BAHD1. Under specific conditions, Listeria secretes the nucleomodulin LntA, which translocates to the nucleus, binds directly to BAHD1 and inhibits its function, resulting in enhanced expression of ISGs.

http://dx.doi.org/10.1016/j.tcb.2015.01.003