

## Chapter 25 Foodborne Listeriosis

Tsuei-Yun Fang

節錄自Modern Food Microbiology, Jay, J. M., 7th ed.



### TAXONOMY OF *LISTERIA*

- ◆ **Gram-positive, facultative anaerobic, non-sporeforming rods.**
- ◆ The primary pathogenic species, *L. monocytogenes* (李斯特菌; 單核细胞增生性李斯特菌), is represented by 13 serovars.



## GROWTH

- ◆ **B vitamins** and some **amino acids** are required.
- ◆ Glucose enhances growth of all species, and **L(+)-lactic acid** is produced.
- ◆ Grow in the presence of **10% or 40% (w/v) bile**, and about **10% NaCl**.
- ◆ Do not grow in the presence of 0.02% sodium azide.



## Effect of pH and NaCl

- ◆ Although the listeriae **grow best in the pH range 6-8**, In general, some species/strains will grow over the pH range of 4.1 to around 9.6 and over the **temperature range of 1°C to around 45°C**.
- ◆ Lower pH → time to visible growth increased.
- ◆ Increase salt concentration → time for visible growth increased



## Effect of Temperature and $a_w$

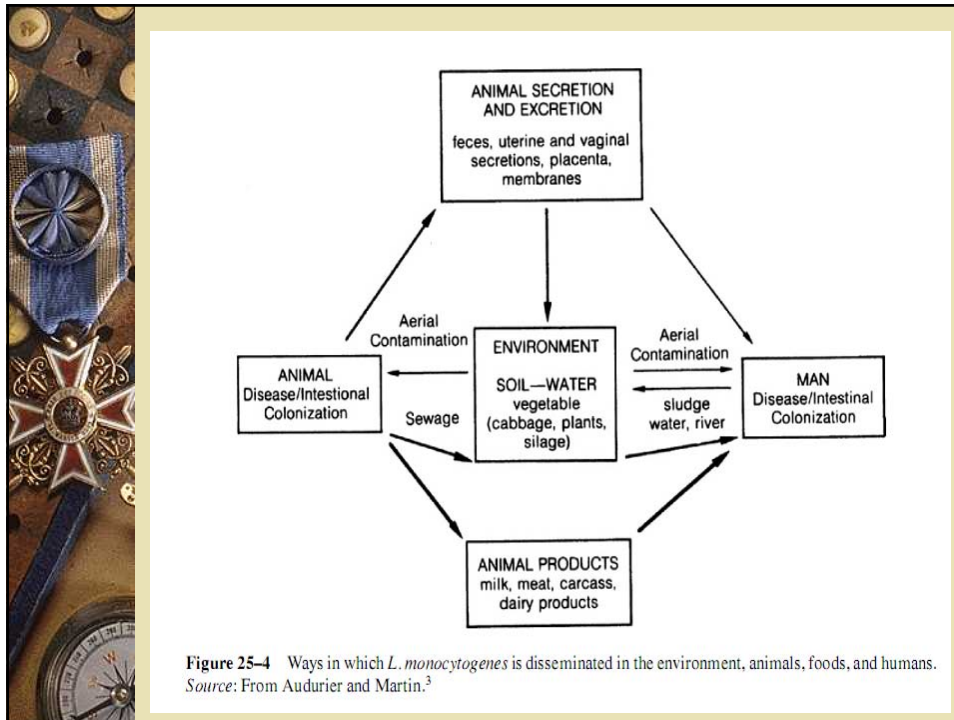
- ◆ The mean **minimum growth temperature** on trypticase soy agar of 78 strains of *L. monocytogenes* → a range of **0.5 - 3°C**.
- ◆ The **maximum growth temperature** for listeriae is around **45°C**.
- ◆ *L. monocytogenes* is second only to the staphylococci as a foodborne pathogen in **being able to growth at  $a_w$  values <0.93**.



## DISTRIBUTION

### ◆ The Environment

- The listeriae are widely distributed in nature → decaying vegetation and in soils, animal feces, sewage (污水), silage (青貯飼料), and water.
- Ways in which *L. monocytogenes* is disseminated throughout the environment → Figure 25-4.



## DISTRIBUTION

### ◆ Foods and Humans

- Any fresh food product of animal or plant origin may harbor varying numbers of *L. monocytogenes*.

### ◆ Prevalence

- Although the numbers of *L. monocytogenes* in foods tend to be so low that direct enumeration methods are without value, samples are sometimes found that contain numbers  $> 10^3/g$ .
- Table 25-3



**Table 25-3** High Numbers of *L. monocytogenes* per Gram or Milliliter Reported for Various Food Products

Chocolate milk (USA, 1994)	$\sim 10^9$
Goat's milk soft cheese (England, 1989)	$>10^7$
Cheese outbreak (Switzerland, 1983–1987)	$10^4$ – $10^6$
Temperature abused ricotta cheese	$3.6 \times 10^6$
Smoked mussels (Tasmania, 1991)	$>10^6$
Chicken roll (USA, 1990)	$1.9 \times 10^5$
Pâté (Great Britain, 1990)	$10^3$ – $10^6$
Raw pork skins (USA, 1991)	$4.3 \times 10^4$
Roast beef (USA, 1991)	$3.6 \times 10^4$
Vacuum-packaged corned beef, 1992	$3.3 \times 10^4$
Pâté (Australia, 1990), mean number	$8.8 \times 10^3$
Cabbage (USA, 1991)	$1.4 \times 10^3$

## THERMAL PROPERTIES

- ◆ Standard pasteurization protocols for milk are adequate for destroying *L. monocytogenes* at levels of  $10^5$ - $10^6$ /mL
  - **145°F (63°C) for 30 min (low temperature long time, LTLT)**
  - **161°F (72°C) for 15 sec (primary high temperature short time, HTST, method)**



## VIRULENCE PROPERTIES

- ◆ Of listerial species, *L. monocytogenes* is the pathogen of concern for humans.
- ◆ The most significant virulence factor associated with *L. monocytogenes* is **listeriolysin O**.



## Listeriolysin O and Ivanolysin O

- ◆ The pathogenic/virulent strains of *L. monocytogenes* produce  **$\beta$ -hemolysis** on blood agar. The responsible protein for hemolysis is **Listeriolysin O (LLO)**
- ◆ It is produced mainly **during the exponential growth phase** (對數生長期).





## Listeriolysin O and Ivanolysin O

- ◆ Sorbate at a level of 2% inhibited LLO synthesis at 35°C under aerobic or anaerobic conditions.
- ◆ **Thiol-dependent exotoxins** ← activated by SH-compounds such as cysteine

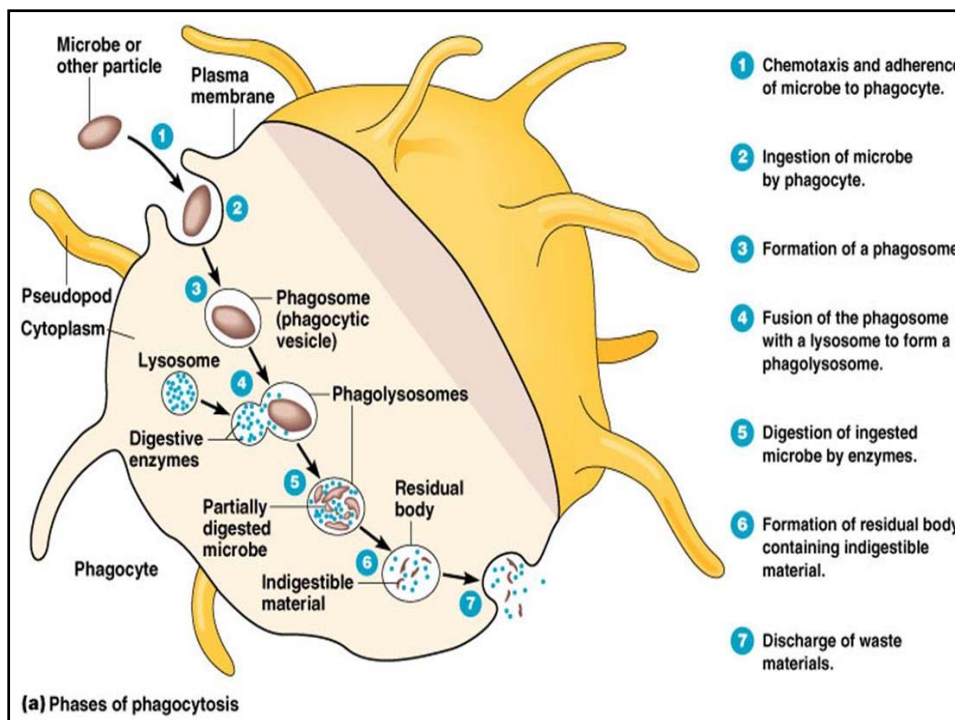


## Intracellular Invasion

- ◆ From the intestinal tract, the organism **invades tissues**, including the **placenta** in pregnant women, and **enters the blood stream**, from which it reaches other susceptible body cells.
- ◆ As an **intracellular pathogen**, it must **first enter susceptible** (易受影響的) **cells**, and then it must possess means of **replicating within these cells**.

## Intracellular Invasion

- ◆ In the case of phagocytes (吞噬細胞), entry occurs in two steps: **directly into phagosomes (吞噬小體) and from the phagosomes into the phagocyte's cytoplasm.**
- ◆ Phagocytosis (吞噬作用)在先天免疫殺菌作用中是個很重要的過程，當細菌和phagocyte上的receptor結合之後，細菌會在巨噬細胞裡形成phagosome，之後phagosome會和lysosome(溶酶體)結合，溶酶體會釋放出酵素，在酸性環境下，這些酵素能夠將細菌破壞。







## Intracellular Invasion

- ◆ *L. monocytogenes* survives inside macrophages by **escaping from phagolysosomal membranes into the cytoplasm** (cytosol), and this process is facilitated in part by **LLO**
- ◆ In nonphagocytic cell lines, entry requires **surface-bound proteins of the bacterium** designated InlA and InlB.



## Monocytosis-Producing (單核細胞增生) Activity

- ◆ *L. monocytogenes* cell has the **lipopolysaccharide (LPS)-like substance** on cell envelope (細胞外被膜).
- ◆ In gram-negative bacteria, LPS is located in the outer membrane, but **listeriae and other gram-positive bacteria do not possess outer membranes.**



## Monocytosis-Producing (單核細胞增生) Activity


- The LPS-like substance can has **monocytosis-producing activity** → induce **the production of monocytes** (單核細胞)
- It possesses low tissue toxicity and is serologically inactive, but it kills macrophages (巨噬細胞) *in vitro*.



## INCIDENCE AND NATURE OF THE LISTERIOSIS SYNDROMES

### Incidence (發生率)

- ◆ Table 25-5
- ◆ Listeriosis is a **rare disease in humans** despite frequent exposure to the causative (致病的) organism.



**Table 25-5** Some of the Suspected and Proven Foodborne Listeriosis Outbreaks and Cases

Year	Source	Cases/Deaths	Location
1953	Raw milk	2/1	Germany
1959	Fresh meat/poultry*	4/2	Sweden
1960–1961	Various/unknown	81/?	Germany
1966	Milk/products	279/109	Germany
1979	Vegetables/milk?†	23/3	Boston
1980	Shellfish	22/6	New Zealand
1981	Cole slaw	41/18	Canada
1983	Pasteurized milk†	49/14	Boston
1983–1987	Vacherin Mont D'Or	122/34	Switzerland
1985	Mexican-style cheese	142/48	California
1986–1987	Vegetables?†	36/16	Philadelphia
1987–1989	Pâté	366/63	United Kingdom
1987	Soft cheese	1	United Kingdom
1988	Goats' milk cheese	1	United Kingdom
1988	Cooked-child-chick.	1	United Kingdom
1988	Cooked-child-chick.	2	United Kingdom
1988	Turkey franks	1	Oklahoma
1989	Pork sausage	1	Italy
1989	Alfalfa tablets	1	Canada
1989	Salted mushrooms	1	Finland
1989	Shrimp	9/1	United States (Conn.)
1989	Pork sausage	1	Italy
1990	Raw milk	1	Vermont
1990	Pork sausage	1	Italy
1990	Pâté	11/6	Australia
1991	Smoked mussels	3/0	Australia
1992	Smoked mussels	4/2	New Zealand
1992	Goat meat (from Calif.)	1	Canada
1992	Pork tongue in jelly	279/85	France
1993	Pork rillettes	39/0	France
1994	Chocolate milk	52/0	USA
1994	Pickled olives	1	Italy
1995	Brie cheese	17/0	France
1998–1999	Wieners	ca. 101/ca. 21	United States
1999–2000	Pork tongue in jelly	26/7	France
2000–2001	Homemade Mexican-style cheese	12/0	United States
2002	Deli turkey meat	46/7	10 USA states

\*Suspected.  
†Epidemiologically linked; organisms not found.

## Source of Pathogens

- ◆ Although the organism is known to be fairly common in environmental specimens, it also exists in healthy humans at rates **from less than 1% to around 15%.**



## Syndromes

- ◆ symptoms depends on **the state of the host**.
- ◆ Nonpregnant healthy individuals are highly resistant to infection by *L. monocytogenes*.
- ◆ When susceptible adults contract the disease, **meningitis (腦膜炎)** and **sepsis (敗血症)** are the most commonly recognized symptoms.



## Syndromes

- ◆ Pregnant females who contract the disease may not present any symptoms, but when they do, they are **typically mild and influenzalike**. Abortion, premature birth, or stillbirth (死產) is often the consequence (不好的結果) of listeriosis in pregnant females.
- ◆ When a newborn is infected at the time of delivery, listeriosis symptoms typically are those of **meningitis (腦膜炎)**, and they typically begin 1-4 weeks after birth.



## RESISTANCE TO LISTERIOSIS

- ◆ **Resistance or immunity to intracellular pathogens** such as viruses, animal parasites, and *L. monocytogenes* is mediated by **T cells**, lymphocytes that arise from bone marrow and undergo maturation in the thymus (hence, T for thymus derived).



## RESISTANCE TO LISTERIOSIS

- ◆ Once a pathogen is inside a host cell, it cannot be reached by circulating antibody, but the presence of the pathogen is signaled by **structural changes in the parasitized cell**, and **T cells are involved in the destruction of this invaded host cell**, which is no longer recognized as "self".



## PERSISTENCE OF *L. MONOCYTOGENES* IN FOODS

- ◆ Because it can grow over the temperature range of about **1- 45°C** and the pH range of **4.1 to around 9.6**, *L. monocytogenes* may be expected to survive in foods for long periods of time.
- ◆ The overall resistance of *L. monocytogenes* in foods is consistent with its persistence in many nonfood environmental specimens.



## REGULATORY STATUS OF *L. MONOCYTOGENES* IN FOODS

- ◆ especially in **ready-to-eat products**
- ◆ Some countries have established **legal limits on the numbers of organisms that are permissible in foods, especially ready-to-eat products**, whereas others have suggested guidelines or criteria that do not have legal standing.



## REGULATORY STATUS OF *L. MONOCYTOGENES* IN FOODS

- ◆ The United States government has the most rigid policy whereby *L. monocytogenes* has been designated as an **“adulterant”** (攪雜物). This means that any ready-to-eat food that contains this organism can be considered adulterated and, thus, be subject to **recall** (收回), and/or **seizure** (查封).
- ◆ The U.S. requirement is **the absence of the organism in 50-g samples**. **Zero tolerance** generally means **the absence of the organism in 25-g samples**.