Listeria monocytogenes

Factors contributing to survival in foods and food production environments

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The Issue

- *L. monocytogenes* (*Lm*) is a frequent contaminant of many foods
  - Dairy, meat products, produce, seafood
- Consequences of *Lm* contamination
  - Recalls
  - Outbreaks
- These issues are nothing new
- Why can't we keep *Lm* out of our food?

What do we know?

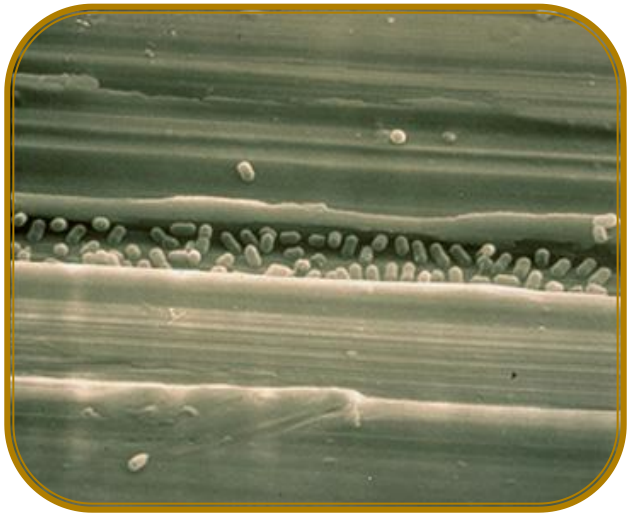
- *Lm* is ubiquitous
  - Unique
    - Saprophytic organism
      - Soil, water, animals, humans
- Psychrotrophic
  - Grows at temperatures of -0.4°C
    - Reliance of refrigeration
      - Positive selection for *Lm*

What do we know?

- pH tolerant
  - Grow over pH 4.3-11.0!
    - Survive at pH 2.5
- Halotolerant (osmotolerant)
  - Grow in 2 M NaCl, tolerate 3 M NaCl!
  - Unique
    - Similar to *Staphylococcus aureus*

What do we know?

- Biofilms
  - Well-established
    - Seed environment and product
      - Source of post-processing contamination
- So…
  - That's what we know…



www.microbewiki.kenyon.edu

Does this info help reduce *Lm*??

- General microbiological knowledge
  - Modelling, risk assessments, interventions
- Known this information for a long time
  - Has it helped?
- Let's look at listeriosis trends
  - FoodNet 2009 data

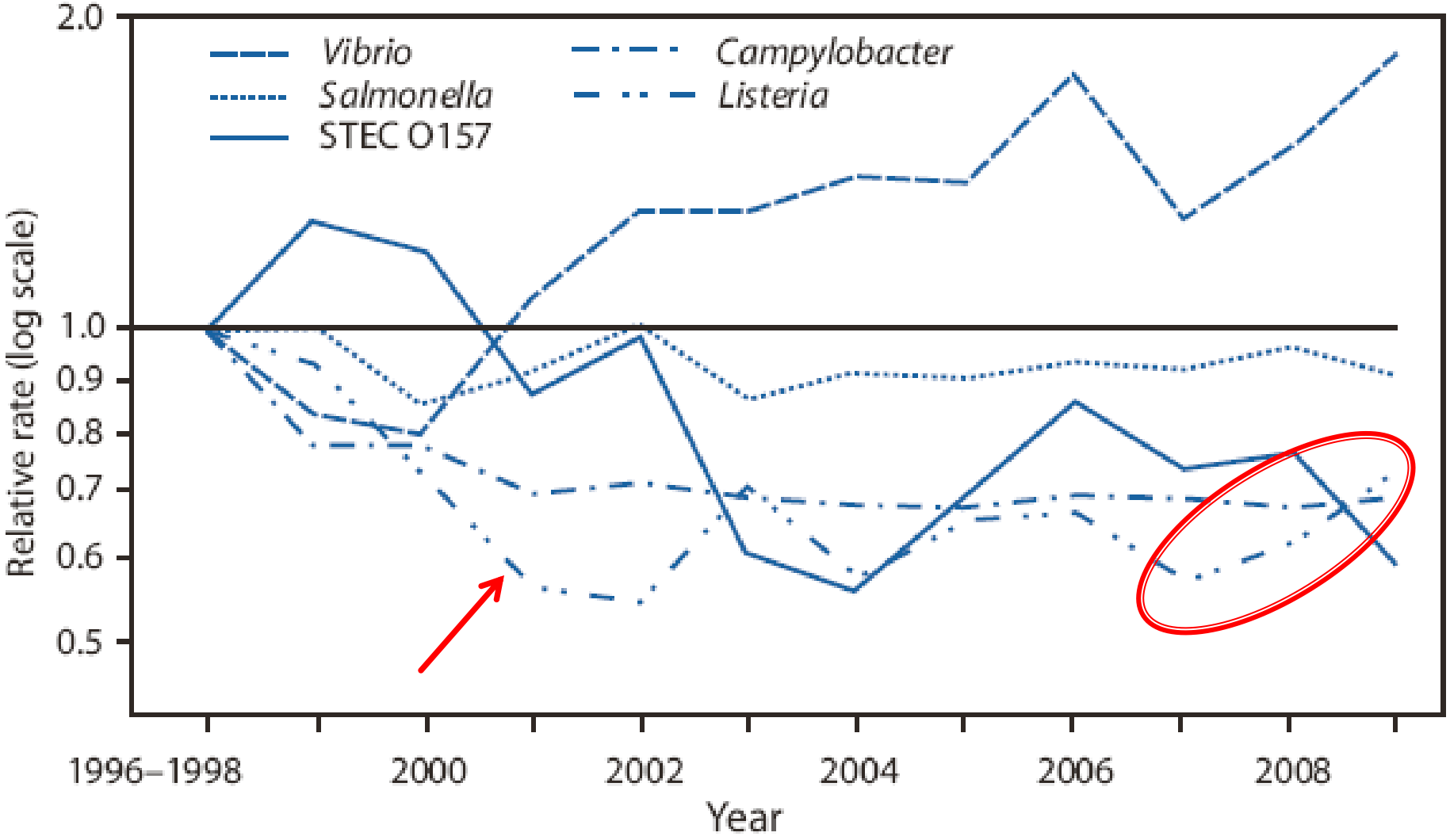
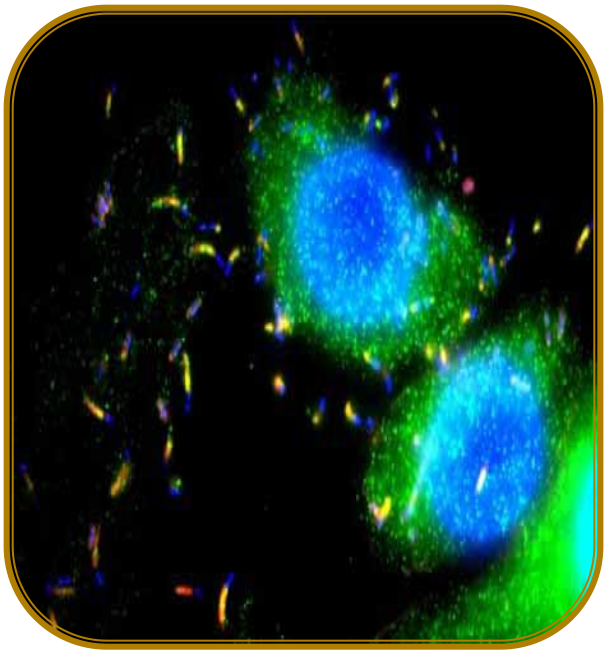


Figure 1. Relative rates of laboratory-confirmed infections compared with rates observed in 1996-1998 (FoodNet, 2010).

In Canada

- Outbreaks/recalls since 2008:
  - Ivanhoe Cheese (2008)
  - Maple Leaf Outbreak (2008)
  - Fromagerie Medard (2008)
  - Sienna Foods (2010)
  - Moonstruck Organic Cheese (2010)
  - Silani Sweet Cheese (2010)



U. California - Berkely

- What's this say about our interventions? Do we have effective control?

Does basic info help us control *Lm*?

- Well…
  - Yes, but we need to do better
- What can we do differently?

Understand *Lm* Physiology

- Need to understand how *Lm* behaves physiologically
  - Adapt our interventions accordingly

- *e.g.* Why can *Lm* grow at 0°C? Why is it resistant to acid, osmolarity and oxidative stress?
  - Are these observations independent, or are they linked?
    - Consequence?

How does *Lm* grow at <4°C?

- Refrigeration back-bone of our food chain
- Physiological adaptation
  - Modifies membrane lipids
  - Induces cold shock proteins
  - Accumulates cryo-protectants
    - L-carnitine
      - Found in meat and dairy products (Mitchell, 1978)
  - Induction of sigma B (σ^B)
    - Stimulates L-carnitine uptake pump

How does *Lm* survive acid stress?

- Commonly employed hurdle strategy
  - How do *Lm* cells survive?
- Physiological adaptation
  - Acid shock proteins
    - Mild acid exposure prepares leads to increased acid resistance
  - Glutamate decarboxylase system (GAD)
    - Responsible for survival at pH 2.5
  - Induction of sigma B (σ^B)
    - Turns on GAD

How does *Lm* survive osmotic stress?

- Commonly employed hurdle strategy (i.e. salt to reduce water activity [a_w])
- Physiological adaptation
  - Salt shock protein (Ssp) induction
  - Actively imports osmoprotectants
    - L-carnitine, betaine
  - Induction of σ^B
    - Stimulates L-carnitine uptake pump

Link between stress responses

- σ^B
  - General stress response regulator
    - Coordinates all stress responses
  - Elevated expression in stationary phase
    - Cells in food production environment are in a stationary-like survival state
  - Induced by stress

- What's the consequence of σ^B induction?

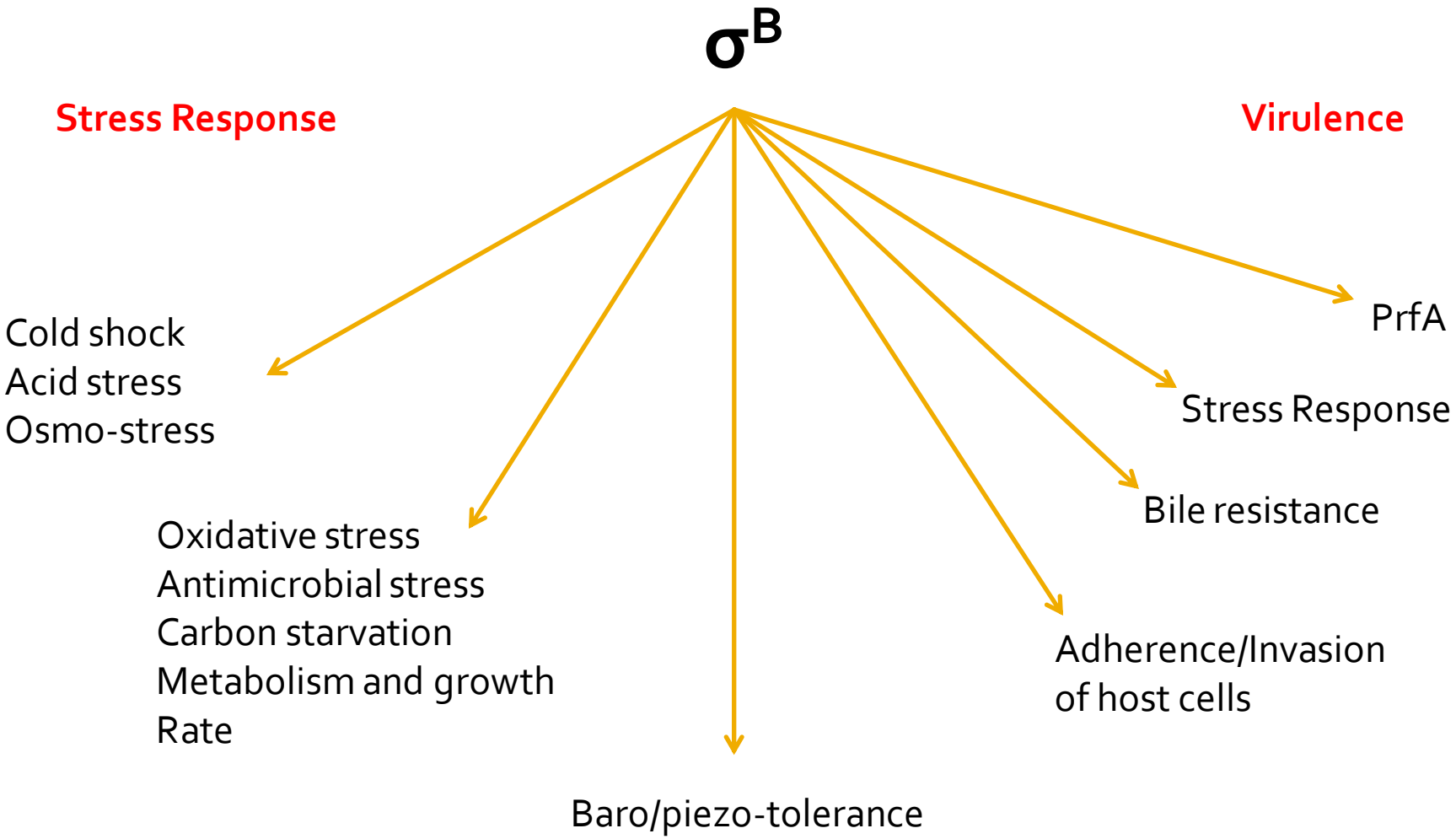
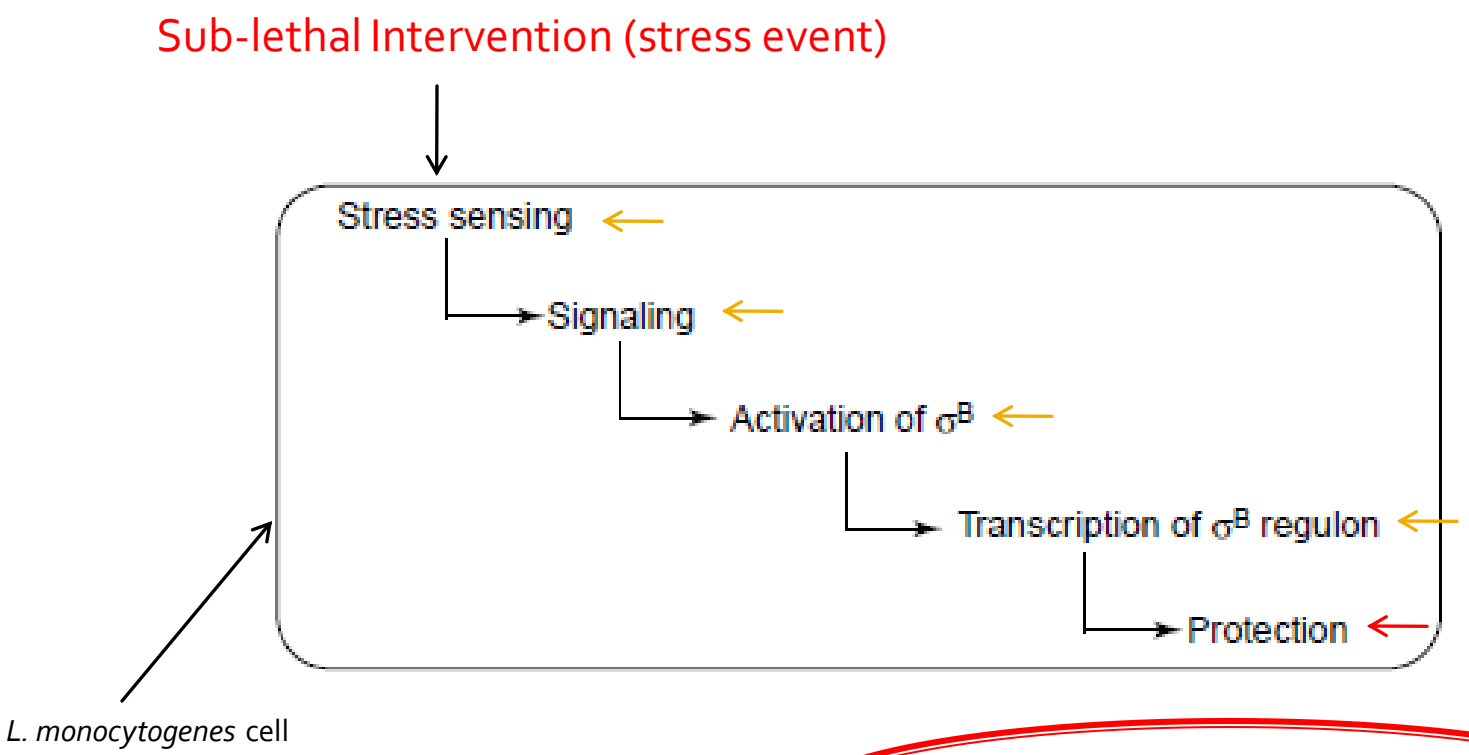


Figure 3. Known links between σ^B, stress response and virulence in *Lm*.

Consequence of σ^B induction?



Protection = Cross-protection to diverse stress types!

(Adapted from van Schaik and Abee, 2005)

Example of Cross-Protection - Cheese

Production Environment Factors

- Starvation
- Antimicrobials
- Cold temperature

Product Factors

- L-carnitine
- Reduced water activity (a_w)

σ^B induction

- Increased resistance to reduced a_w
- Improved survival and growth
- Increased virulence?

Example of Cross-Protection – Meat Products

Production Environment Factors

- Starvation
- Antimicrobials
- Cold temperature

Product Factors

- NaCl
- Organic acids
- L-carnitine

σ^B induction

- Increased resistance to reduced a_w
- Increased resistance to high acid
- Improved survival and growth
- Increased virulence?

How does this information help?

- Helps us understand why *Lm* remains a significant foodborne pathogen
  - Physiologically geared for food chain survival

- Interesting biological attributes
  - *i.e.* cold adaptation, resistance to acid, osmolarity *etc*.
    - Not just abstract facts
  - Physiologically coordinated events (via σ^B) maximizing survival
    - On-going evolution

σ^B Physiology and Tailoring Interventions

- Need to consider physiological state of *Lm*
  - Intervention efficacy assessments
    - Consequences?
- *e.g.* High hydrostatic pressure (HHP) processing
  - Meats/meat products

σ^B Physiology and Tailoring Interventions

- HHP (cont)
  - Environment/Product factors
    - $\uparrow \sigma^B$
      - Uptake of L-carnitine (cryoprotectant)
  - Cryoprotectants = Baroprotectants!

- How are efficacy assessments of HHP performed?

Determination of HHP Efficacy

- *Lm* grown in lab, product inoculated, assessed
  - Issue?
    - Physiologically sensitive state!!
- How would you do this properly?
  - Inoculate product, place at 4°C
    - Product/temperature → ↑ σ^B and barotolerance
      - More accurate efficacy assessment
- Strategy applicable to other interventions

Tailoring Interventions

- Ideal intervention?
  - No induction/repression of σ^B
    - Possible?
- What do we do?

- Consider the integrated/related stress response physiology of *Lm*
  - Cells in food production environments are geared to survive

Tailoring Interventions

- Need to increase *Lm* cellular damage
  - Over-whelm stress response
  - More effective hurdles
    - Bigger hurdles ↓ *Lm*
      - Balancing game with quality
    - More hurdles
- Goal
  - Reduce *Lm* population
  - More effectively suppress proliferation

Source: Gabriel Moisa

Consequences of Failed Environmental Interventions

- Example – Production facility
  - Unable to successfully eliminate *Lm*
    - Recurring positives over the course of a year
      - Issue?
        - Contaminated product/recall/outbreak
  - Fundamental issue?
    - Ineffective elimination equates to positive selection!!
      - Selecting strains:
        - Biofilms
        - Resistant to "your" interventions
          - *Lm* adapts!

www.directindustry.com/prod

Summary

- *Lm* isn't your average pathogen
  - Exceptional stress response
    - Ideally suited to make your life miserable
- Interventions
  - Consider the physiology
    - Reflect resistant state
- Hurdle strategy
  - More aggressive
    - More and bigger hurdles

Are we doing enough?

- Most of the time…
  - *Lm* can evolve
    - Is that good enough?

Questions and Contact Info

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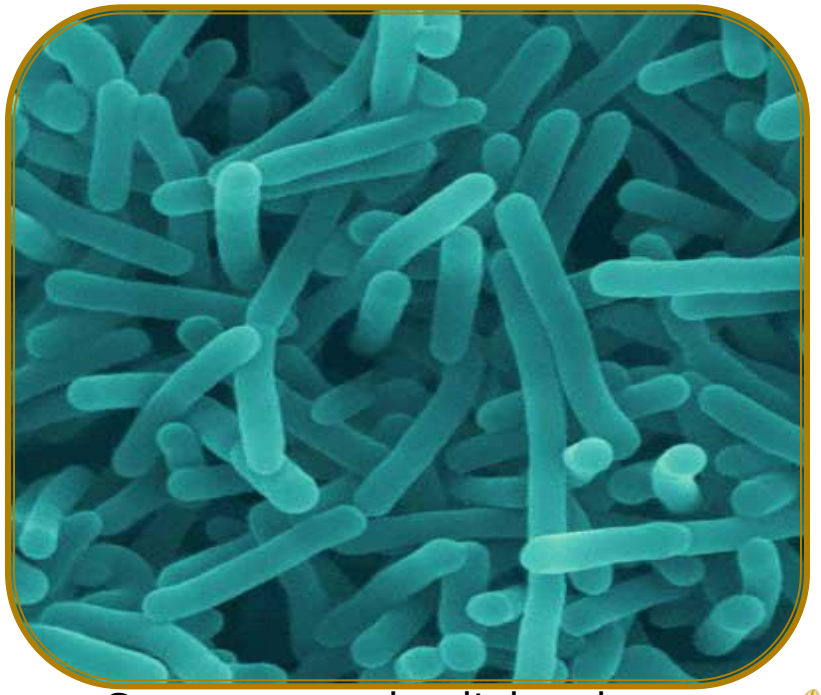

Source: www. koolielu.edu.ee