Cell stress, sublethal damage, viable but non-culturable organisms

FSTC/DASC606

Definitions

• Stress
  — Departure from optimal conditions with the potential to decrease bacterial growth

• Sublethal injury or metabolic injury
  — The consequence of exposure to a chemical or physical process that damages but does not kill a microorganism (result of stress).

  • Wesche et al. JFP 72:1121, 2009

Stressors

• Physical
  — Temperature
    • Heat
    • Cold
  — Radiation
    • UV
    • Light
    • Ionizing radiation
  — Drying
  — Osmotic pressure

• Chemical
  — Acid
  — Alkaline
  — Antimicrobials

• Nutritional
  — Starvation

• Another definition of stress
  — Abiotic effectors that reduce the growth rate of a microorganism
Stress effects

- Sublethal injury in cells
  - Important in microbiological testing
- Stress response by cells
  - Important in food processing

Sublethal injury

- Stress results in metabolic injury
  - Expressed by the inability to grow in selective media, which non-injured cells can tolerate

Acid injury in *E. coli* O157:H7 by exposure to 0.5% lactic acid (pH 2.8)
Heat injury in *Salmonella Typhimurium* and *Escherichia coli* O157:H7 by heating at 54.5°C for 15 min

![Graph showing log CFU/ml vs. time for S. Typhimurium and E. coli O157:H7 under different conditions](image)

**Injury repair (resuscitation)**

- Exposing injured microorganisms to a growth medium without selective agents and/or repair compounds (pyruvate), and under optimal growth conditions, results in repair of injury.

**Repair methods**

- **Pre-enrichment**
  - Mix sample with a non-selective fluid medium
  - Used for qualitative testing
    - *Salmonella*
- **Dilutions in tryptic soy broth**
  - Incubate for 1.5 h then plate or follow more dilutions
  - Used for plate counts
- **Agar overlay**
  - Plate on non-selective medium, incubate for 1-2 h then overlay with selective medium
  - Used for plate counts
Stress response and its effects

- Bacteria use diverse strategies for protecting against stress damage
  - Stress response
- These strategies can be specific for stress agents
  - Reactive oxygen species
  - DNA radiation damage
- In addition, general stress response protects against various stress agents
  - Cross protection

General stress response

- During exponential phase, bacteria accumulate RNA polymerases (σ factors)
- At the end of the exponential phase the σ factors will express genes that enable continued growth with less nutrients
Stress response

• Stress response can be triggered under various life-threatening stress conditions
  – Acid, heat, etc.
• Metabolic shortcuts are followed for specific σ factors
  – Cross protection

Viable but non-culturable bacteria

• Bacteria are viable but do not grow in culture media
• VBNC is not stress
  – Stressed cells will grow after incubation in non-selective media
  – VBNC bacteria will not grow in selective or non-selective media
• Many bacteria enter VBNC state under stressful environmental conditions
  – Starvation, toxic metal ions, cold temperatures etc.
• In many cases, pathogens in VBNC state are still infective
  – Become restored upon colonization

Incidence of *V. vulnificus* in coastal waters by sampling month

![Incidence graph](image.png)

Oliver, 1987
Antagonistic and predatory activity in microorganisms

Bacterial antagonism

- Antagonism
  - Bacterial inhibition resulting from the activity of other microorganisms in the same environment
- Mechanisms for antagonism
  - Acid compounds
  - $\text{H}_2\text{O}_2$
  - Antibiotics
  - Volatile fatty acids
  - Bacteriocins
  - Competition

Examples of antagonistic bacteria

- Lactic acid bacteria (LAB)
- 10 strains of each of Streptococcus, Lactobacillus and Leuconostoc isolated from Mexican cheese made from non-pasteurized milk

<table>
<thead>
<tr>
<th></th>
<th>Ratio LAB: pathogen</th>
<th>% LAB strains that produced total inhibition in 100% of 13 strains of:</th>
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<tbody>
<tr>
<td></td>
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<td>Salmonella</td>
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<tr>
<td>Streptococcus</td>
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<tr>
<td>Lactobacillus</td>
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<td>Leuconostoc</td>
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<td>30:1</td>
<td>60</td>
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Predatory microorganisms

- *Bdellovibrio* and Like organisms (BALO)
  - *Bdellovibrio*
  - *Bacteriovorax*
  - *Vampirovibrio*
  - *Micavibrio*
  - *Vampirococcus (anaerobe)*
  - *Daptobacter (anaerobe)*
  - Invade the bacterial prey cell and feed on it

- Other predators
  - *Myxobacteria* (gliding bacteria)
    - Use exoenzymes to produce lysis of bacteria and feed on the released compounds
  - *Ensifer adhaerens*
    - Attaches to the prey and releases lysis factors
Possible applications

• Both LAB and BALO have been used as biocontrol agents against human or plant pathogens
• Bacteriophages also have been proposed as biocontrol agents.