Chapter 3: Cell damage and repair

Introduction

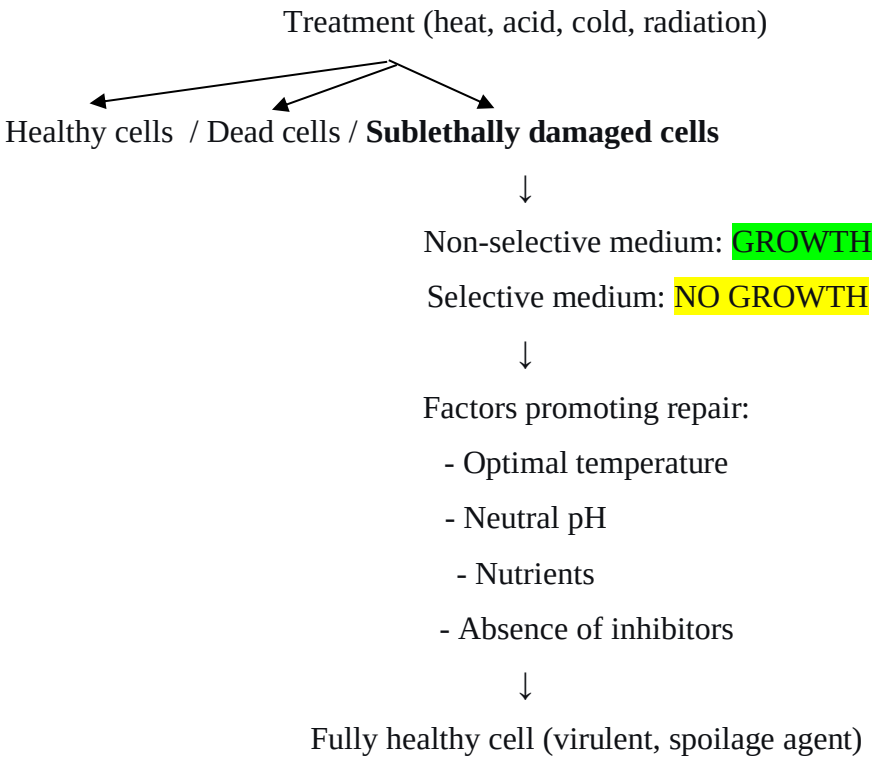
In food processing (heat treatment, salting, acidification, etc.), microorganisms do not die instantly. Some undergo damage that does not kill them immediately: these are **sublethally damaged cells**. These cells pose a major problem in food safety because they can repair themselves and become pathogenic or spoilage agents again.

1. Concept of the sublethally damaged cell

1.1. Definition

A **sublethally damaged cell** is a living cell that has suffered structural or metabolic alterations that prevent it from multiplying under selective culture conditions, whereas it can multiply under non-selective conditions (rich media with protective agents).

Simple example: A bacterium can grow on a non-selective agar medium (nutrient agar), but not on a medium containing salt (NaCl). After repair, it becomes capable of growing on the salty medium again.



1.2. Importance in the food industry

- ✓ **Positive aspects (benefits)**

- **Process control**: The presence of sublethally damaged cells after treatment indicates that the treatment was partially effective. Processing parameters can be optimized to eliminate them completely.
- **Food stabilization**: By preventing repair (cold storage, pH reduction), the microbiological quality of the food is ensured.

- ✓ **Negative aspects (risks)**
  - **Major health risk**: Sublethally damaged cells are not detected by classical methods (selective media). A food declared "pathogen-free" may still contain them, capable of repairing themselves in the digestive tract.
  - **False interpretations**: A standard microbiological analysis can dangerously underestimate the viable flora.
  - **Delayed spoilage**: Sublethally damaged spoilage microorganisms can repair during storage and deteriorate the product.

2. Types of sublethal damage

Type of damage	Cellular target	Example of cause
Membrane damage	Cytoplasmic membrane (loss of selective permeability)	Heating, freezing
DNA damage	Single or double strand breaks	UV radiation, oxidizing agents
RNA damage	Ribosomal or messenger RNA	Moderate heat
Enzymatic damage	Denaturation of key enzymes	Pressure, acids
Ribosomal damage	Ribosomal subunits	Heating

3. Detection techniques for sublethal damage

It is indispensable to develop detection methods since the great potential threat of sublethally injured microbes to food safety and public health. Due to the fact that injured cells can grow on the nonselective media, whereas not on selective media, culture-based methods are typically employed to analysis the sublethal injury. In addition, fluorescent staining, molecular biological and infrared spectroscopy methods have also been established, which mainly based on the changes in membrane

3.1. General principle

Compare growth on:

- **Non-selective medium** (nutrient agar, tryptone soya agar = TSA): counts all viable cells, including damaged ones.
- **Selective medium** (containing salt, bile salts, an antibiotic, etc.): counts only healthy cells.

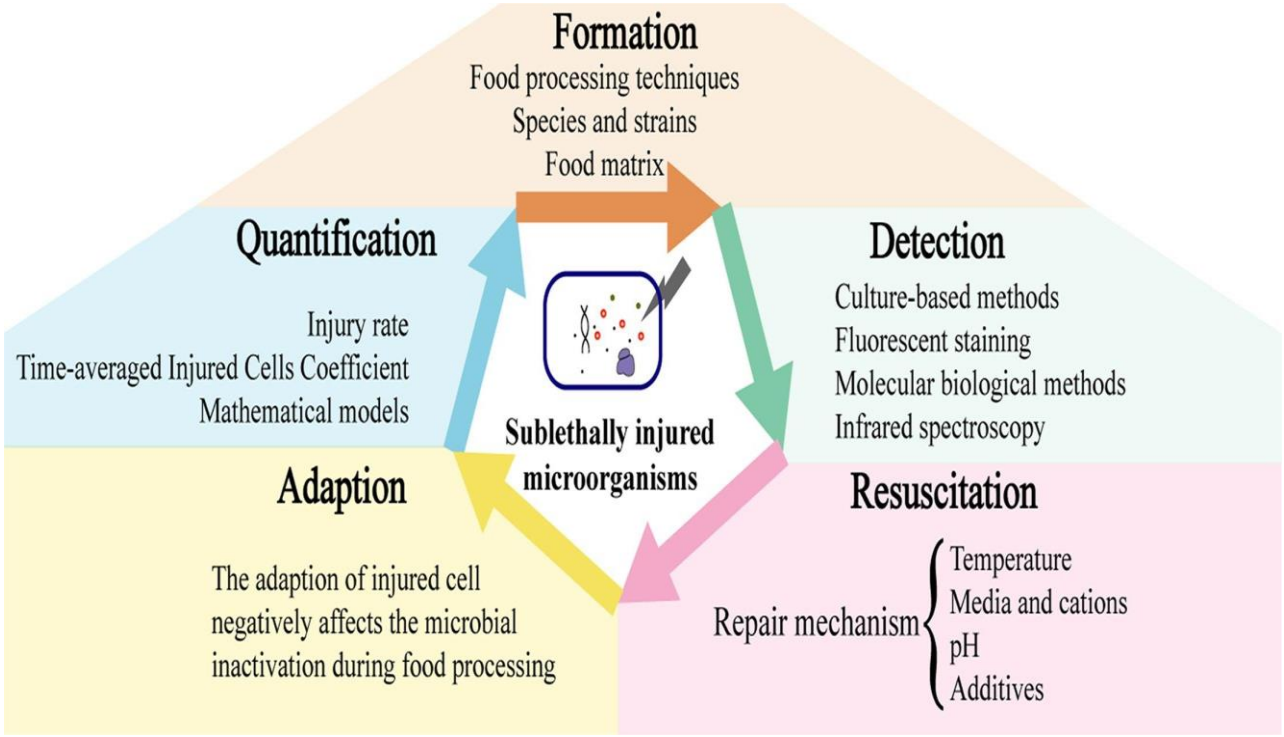
The difference between the two counts gives the number of sublethally damaged cells.

3.2. Culture media used

Type of Medium	Example	Use
Non-selective	Nutrient agar, TSA, PCA (for total flora)	Total viable count
Selective for membrane damage	TSA + NaCl (6-10%)	Detection of cells that have lost membrane integrity
Selective for metabolic damage	Minimal medium without amino acids	Detection of cells with increased nutritional requirements
Selective for pathogens (with prior repair)	Baird-Parker agar (*S. aureus*) + egg yolk	After repair in non-selective broth

❖ **Advantages and disadvantages of techniques**

Technique	Advantages	Disadvantages
Double counting (non-selective vs selective)	Simple, low cost, standardized	Does not indicate the type of damage; underestimates if repair is rapid
Fluorescence microscopy (LIVE/DEAD stains)	Rapid, direct visualization	Expensive equipment; does not detect all types of damage
Flow cytometry	Precise quantification, multiparametric	Very expensive, requires trained operator
Membrane potential measurement (dyes)	Specific for membrane damage	Does not detect internal damage (DNA, RNA)



❖ **Concrete examples of application**

- **Pasteurized milk**: Count on nutrient agar (37°C) vs nutrient agar + NaCl (10%). A difference >1 log means pasteurization generated sublethally damaged cells.
- **Acidified fruit juice**: Comparison on non-selective agar vs low-pH agar.
- **Irradiated foods**: Comparison with and without an antioxidant agent in the medium.

4. Factors determining the presence of sublethal damage and its repair

4.1 Microorganism-related factors

- **Species and strain**: *Listeria monocytogenes* repair its membranes better than *Salmonella*.
- **Growth phase**: Cells in stationary phase are more resistant but repair more slowly.
- **Previous physiological state**: Pre-adaptation to stress induces stress proteins (HSPs, chaperones).

4.2 Treatment-related factors

- **Stress intensity**: The more severe the treatment (close to lethal), the more extensive the damage and the longer the repair.
- **Nature of the stress**: Heat → mainly ribosomal and membrane damage; Acid → enzyme damage; Cold → membrane damage.
- **Exposure time**: Prolonged exposure increases the number of sublethally damaged cells up to a plateau.

4.3 Environmental factors after treatment (repair)

Factor	Effect on repair
Temperature	Optimal repair around growth temperature (20-37°C for pathogens). Too cold: blocks repair. Too hot: kills or prevents repair.
pH	Near-neutral pH promotes repair. Acidic pH (foods) inhibits it.
Oxygen availability	Aerobes: need O_2. Anaerobes: O_2 blocks repair.
Nutrient availability	Lack of amino acids, growth factors → no repair.
Presence of inhibitory substances	Salt, nitrites, preservatives → prevent repair (good for industry).

4.4 Food matrix-related factors

- **Water activity (aw)** : Low aw prevents repair (e.g., cheese, dry ham).
- **Structure**: Biofilms repair faster than free cells.
- **Interaction with background flora**: Competitive flora can inhibit repair (barrier effect).

5. Practical applications in quality control

In routine industrial practice

1. **Pathogen detection**: Use enrichment in non-selective medium before culture on selective medium (ISO 11290 standard for *Listeria*). This allows repair of sublethally damaged cells.
2. **Process validation**: Compare counts on non-selective and selective media to assess real effectiveness.
3. **Choice of indicators**: Do not rely solely on selective media; systematically use a repair medium.

6. Role of NER in managing sublethal cells

Nucleotide Excision Repair (NER) is a crucial mechanism that enables bacteria to detect and repair such lesions, thereby preserving genomic integrity and cell viability. The NER system is the main actor that prevents a sublethal cell from shifting into a lethal state. It allows the cell to:

1. **Survive** after an aggression
2. **Regain its genomic integrity** without mutation
3. **Resume its normal functions**

Without NER, a sublethal cell is ultimately doomed: it accumulates damage, eventually loses vital functions, or dies. With NER, it becomes a healthy and competitive cell again.

6.1. General mechanism of NER

NER involves a coordinated series of enzymatic reactions:

1. **Damage detection** – UvrA and UvrB proteins form a complex to locate DNA lesions.
2. **Incision** – UvrC makes cuts on both sides of the damage.
3. **Excision** – The damaged fragment is removed.
4. **Repair synthesis** – New DNA is synthesized using the complementary strand as a template.
5. **Ligation** – The new segment is sealed into place.

Key proteins involved: **UvrA, UvrB, UvrC, UvrD** (Uvr stands for "Ultraviolet resistance").

Sublethal cell (damaged DNA)

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Detection by UvrA/UvrB

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Incision / Excision of damaged fragment

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Synthesis using the intact complementary strand as template

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Ligation

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Healthy cell (DNA restored)

- NER excises **only the damaged fragment** (not the entire DNA)
- Synthesis uses the **intact complementary strand** as a template → no errors
- The cell retains all its functions after repair

6.2. Two types of NER in bacteria

- **Global Genome NER (GG-NER)**: Repairs damage across the entire genome, including non-transcribed regions. Essential for overall genomic stability.
- **Transcription-Coupled NER (TC-NER)**: Repairs lesions on transcribed strands of active genes. When RNA polymerase is blocked by a lesion, the **Mfd protein** (TRCF) removes it and recruits NER components for rapid repair.