7th International Food Safety Congress
3-4 November 2022 Istanbul-TURKEY
Grand Cevahir Hotel & Convention Center
"Safe food for now and future"

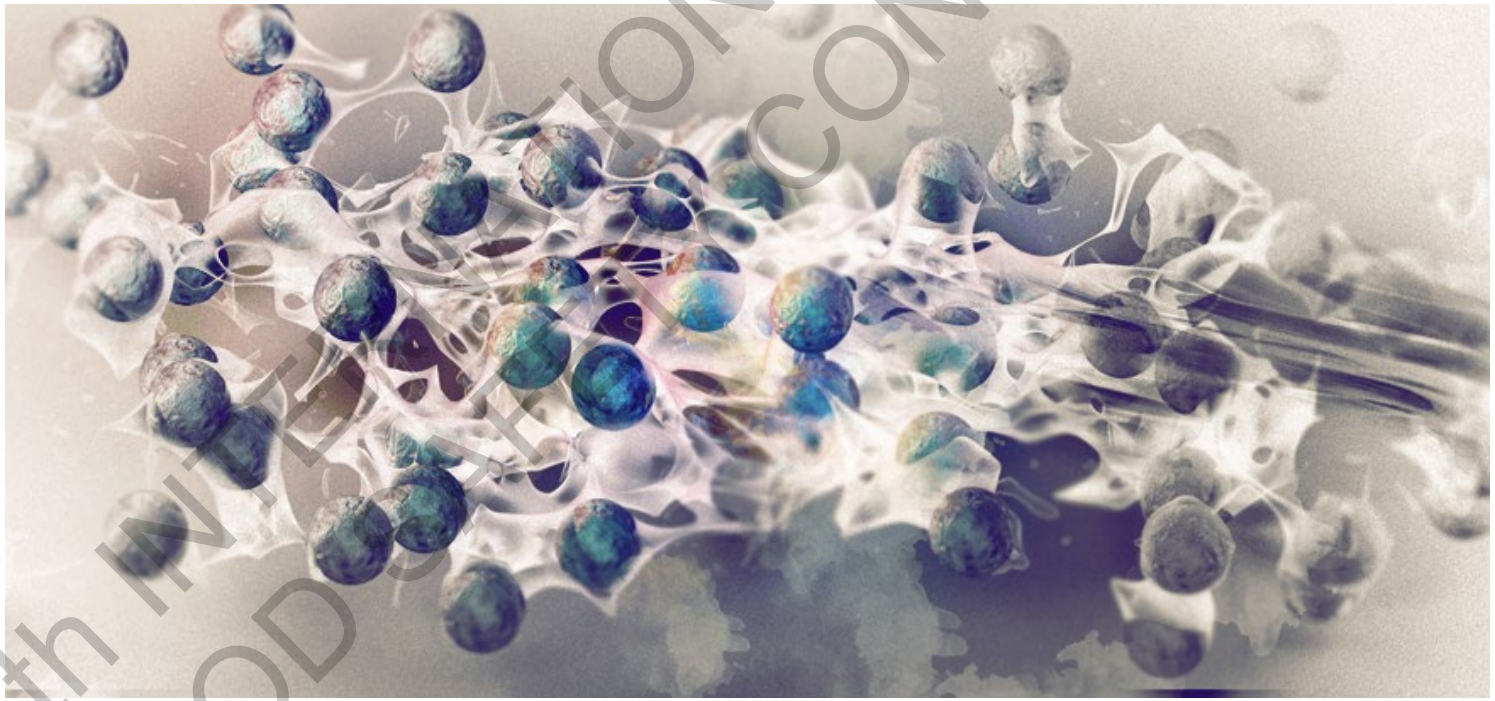
Biofilm Forming Bacteria in Meat Processing Facilities

dr. Ivana Čabarkapa

University of Novi Sad,
Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

What is a biofilm??

A highly organized community of microorganisms attached to a surface or interface and immersed in a self-produced extracellular matrix (ECM).

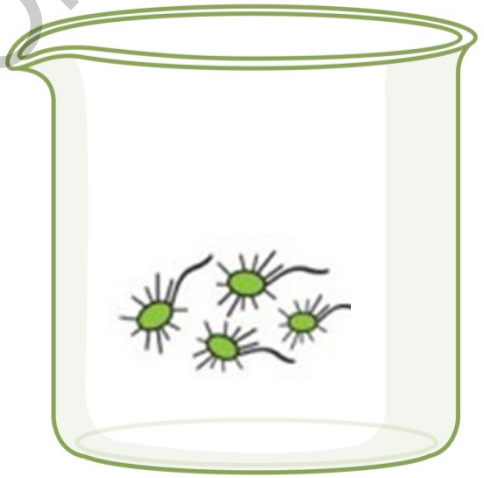


What is a biofilm??

- Biofilm represents a dominant lifestyle of bacteria in all environments.
- Approximately 90% of bacteria exist in the form of biofilms.

90%

Bacteria in biofilm

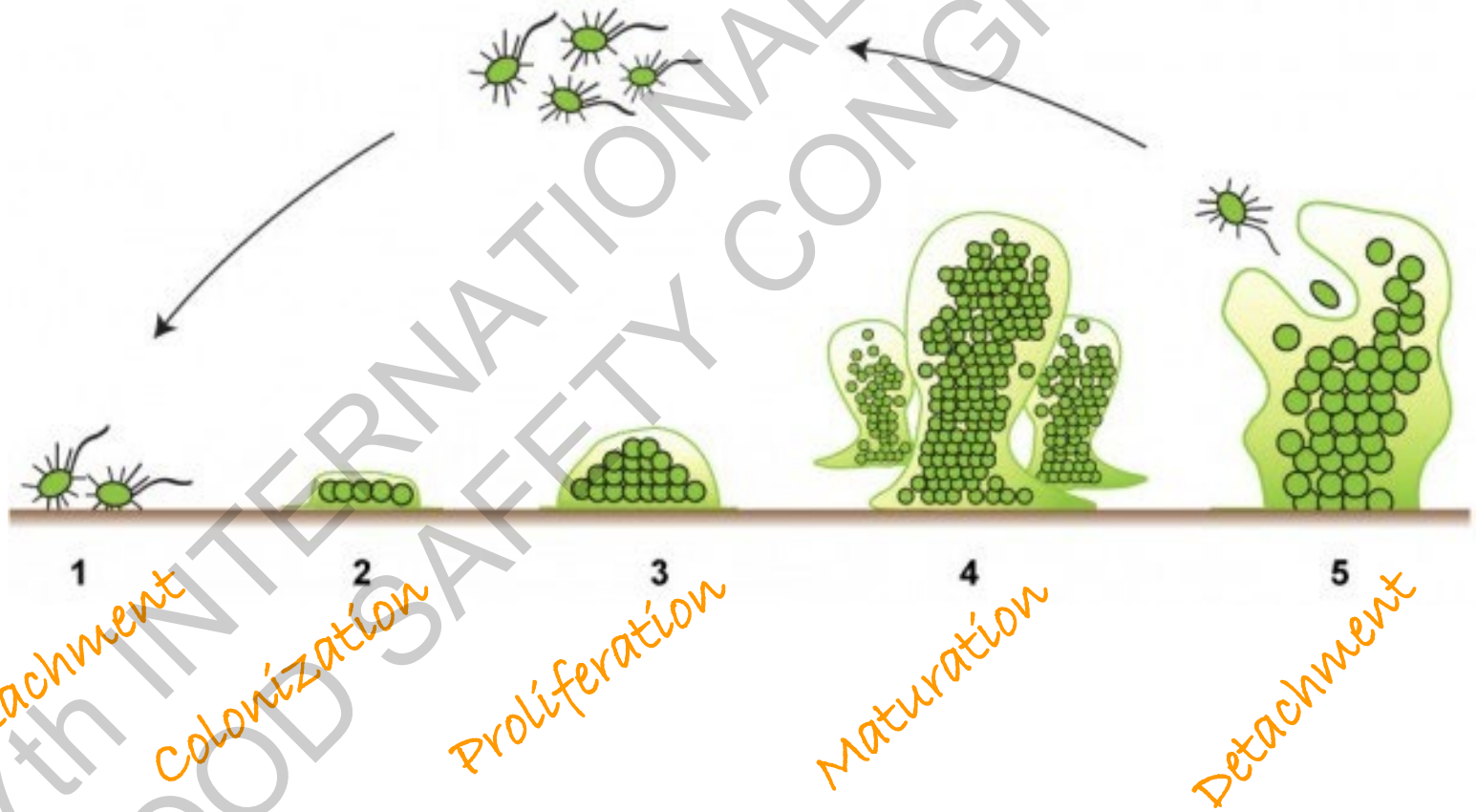


10%

Planktonic (free-floating) bacteria

Stages of biofilm formation

Biofilm formation is a complex process that involves five major stages.



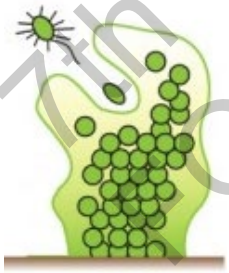
Biofilm formation

1. **Attachment:** Attachment of planktonic (free-floating) bacteria to surface;

2. **Colonization:** Transition of reversible to irreversible attachment followed by cell aggregation and production of extracellular polymers (EPS);

3. **Proliferation:** Development of microcolonies and early development of biofilm architecture;

4. **Maturation:** Growth of the attached multi-layered bacterial cells into the matured biofilm with the typical 3D biofilm structure;



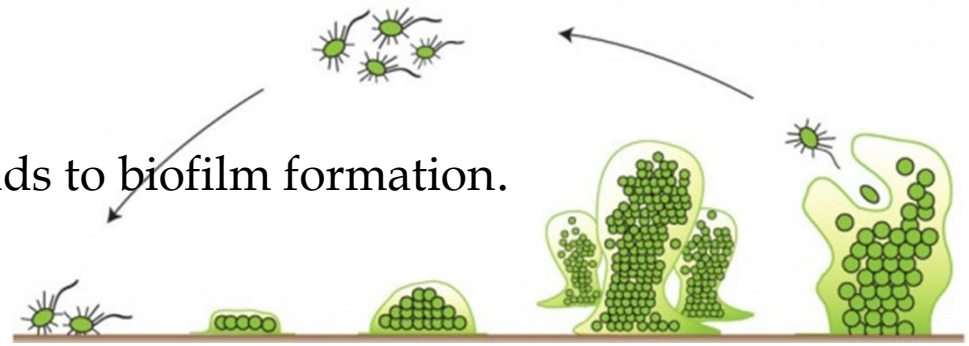
5. **Dispersal and/or detachment:** of cells from biofilm into the surrounding environment.

Why bacteria form biofilm?

- In response to environmental conditions, bacteria have developed a variety of strategies to adapt and survive.

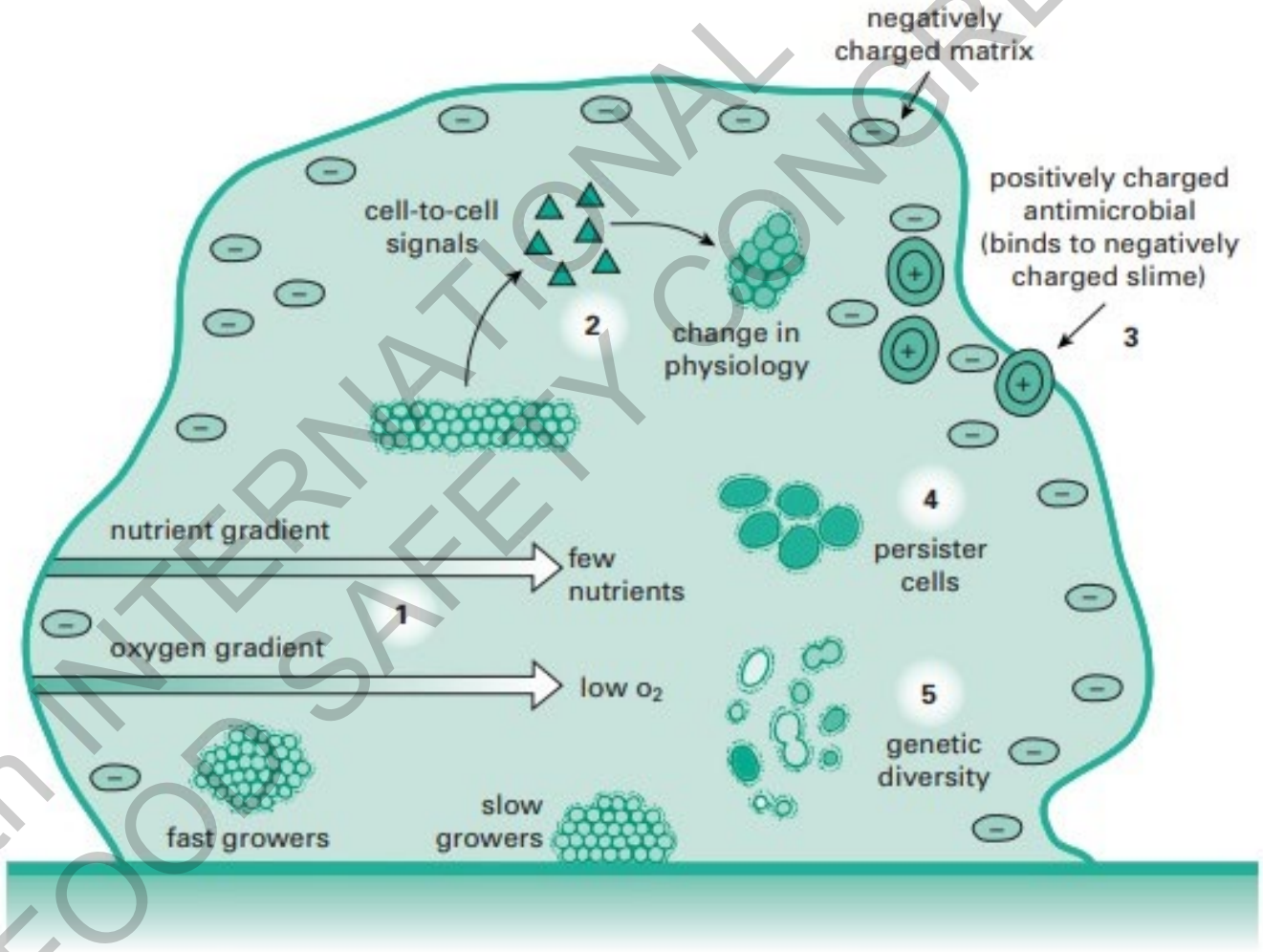
- The formation of multicellular communities known as biofilms is one such strategy, which is generally associated with the persistence and survival under different environmental conditions.
- Exposure of bacteria to a variety of stresses such as:

✓ alternations in moisture content,
✓ UV radiation,
✓ limited nutrients,
✓ extreme pH,
✓ extreme temperature,
✓ high salt concentrations,
✓ high pressure,
✓ antimicrobial agents→→leads to biofilm formation.



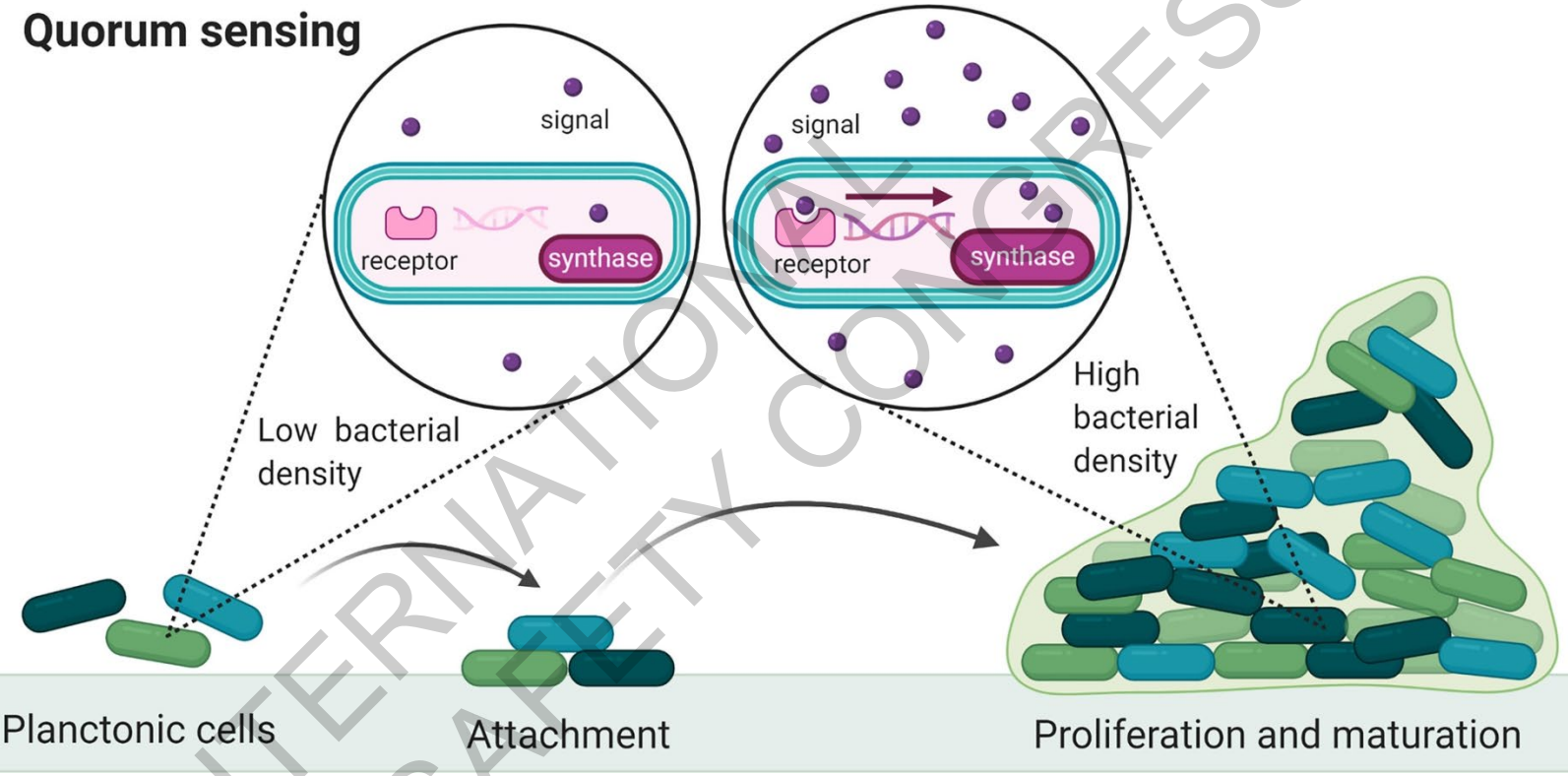
The biofilm as a fortress

Biofilm is a useful adaptation of micro-organisms, enabling them to survive in certain environments.



Quorum sensing

Bacteria can talk to each other!



QS is a bacterial cell–cell communication that regulates the expression of genes involved in virulence, competition, pathogenicity, and resistance.

QS involves the production, detection, and response to extracellular signaling molecules called autoinducers (AIs).

Biofilm in industry

- Biofilms formed in food processing environments represent a long-term source of food contamination, not only with food spoilage bacteria but also with food-borne pathogens such as *Salmonella* spp., *Campylobacter* spp., *Escherichia coli,* and *Listeria monocytogenes*.

- Adherence ability and biofilm formation of food-borne pathogens has been confirmed on different types of materials that are frequently used in the food industry as:

- glass,
- stainless steel,
- metal,
- teflon,
- marble,
- polystyrene, etc.






Biofilm in industry

- The existence of bacteria in biofilms in the food industry may cause **cross and post-process contamination** and **economic losses** by
- impairing heat transfer;
- increasing corrosion rate;
- reducing the shelf life of food products;
- increasing food spoilage;
- spreading of pathogenic bacteria and causing diseases related to foodborne pathogens.

- Some food-borne pathogens in food production plants may exist for several months or even years. These strains are known as “house strains”, and the assumption is that their existence is enabled due to their ability to form a biofilm.

Biofilm in industry

The places that are usually identified as the most common potential areas (hotspots) of biofilm development are:

- the receipt of raw materials,
- floors,
- walls,
- drains,
- pipes,
- ventilation systems,
- conveyor belts,
- knives or hatchets
- slicing machines,
- cutting boards,
- animal hide,
- intestinal tract,
- employees (hands, clothing, health),
- seasonings,
- packaging, and
- storage areas.

Biofilm in industry

- Biofilms are extremely difficult to eradicate.
- Biofilms can tolerate antimicrobial agents at concentrations of 10-1000 times, which are needed to inactivate genetically equivalent planktonic bacteria.
- Reasons for biofilm formation within industrial plants are commonly referred to improperly cleaned and sanitized equipment.

For planktonic bacteria

For bacteria enclosed into biofilm

Aim of study

Industry environments could be a carrier of a wide range of microbial contaminants, which can cause an adverse effect on food deterioration as well as compromise the safety of food products.

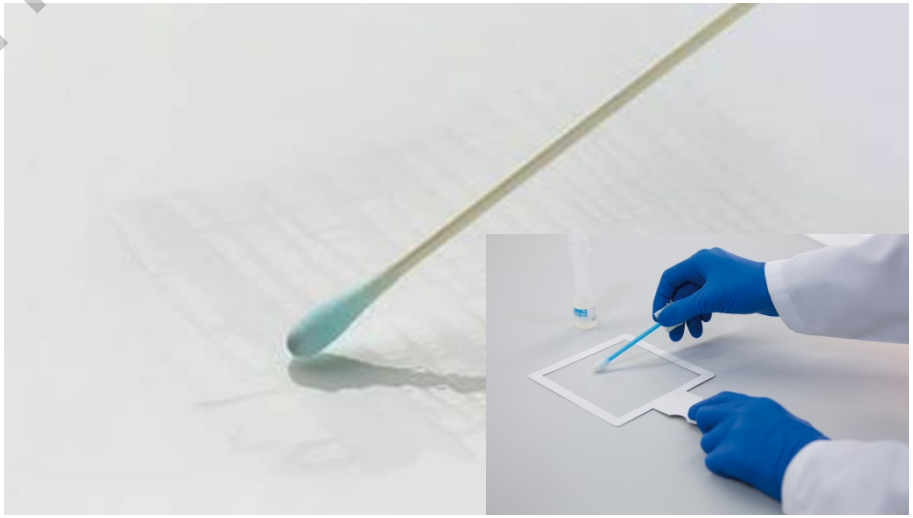
Meat processing facilities are particularly important as a potential source of contamination, not only with food spoilage bacteria but also with food-borne pathogens.

Detection presence of residual bacteria within meat processing facilities after cleaning and disinfection process.

Material and Methods

Sampling was conducted after cleaning and disinfection to increase the likelihood of targeting residual bacteria.

Samples were collected from 60 surfaces in meat processing facilities (slicing machines, cutting boards, knives, or hatchets) using a swab sampler, with neutralizing buffer.

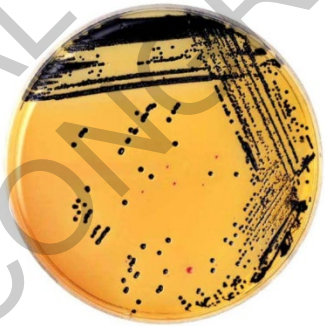


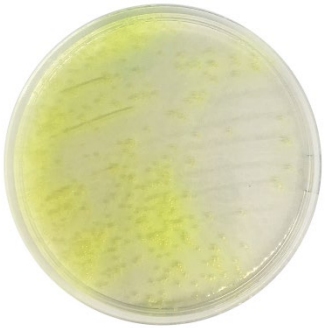
Material and Methods

Performed microbiological analyses:

- ✓ Total aerobic plate count,
- ✓ Total *Enterobacteriaceae* count,
- ✓ *Staphylococcus* spp.,
- ✓ *Listeria monocytogenes*,
- ✓ *Pseudomonas* spp. and
- ✓ *Salmonella* spp.

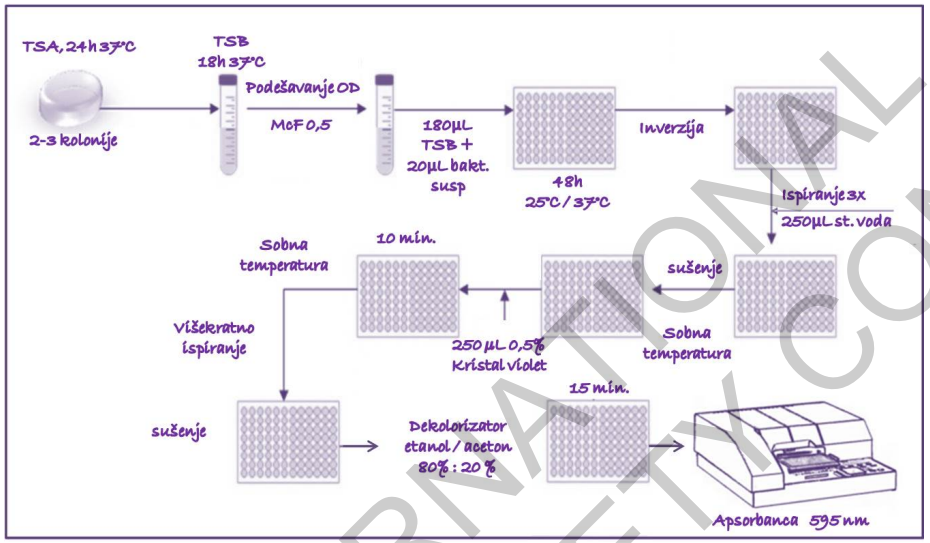
EN ISO methods

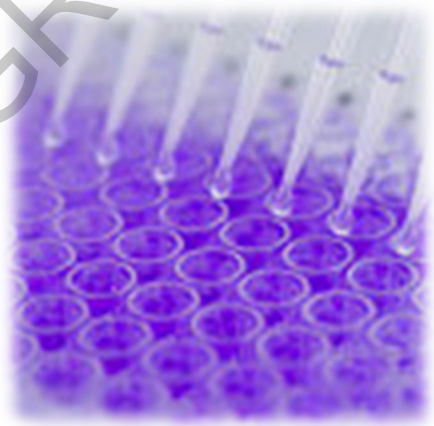




Material and Methods

Biofilm forming ability





$OD \leq ODc$ no biofilm

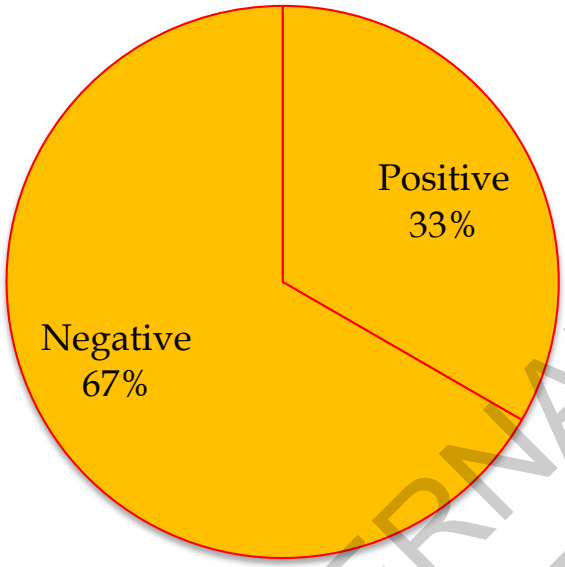
$ODc \leq OD \leq (2 \times ODc)$ weak

$(2 \times ODc) < OD \leq (4 \times ODc)$ moderate

$(4 \times ODc) < OD$ strong

Results

Out of 60 examined swabs, 20 (33.3%) were positive for the presence of microorganisms.



20 hotspots identified

All 20 hotspots were detected on food contact surfaces

(**cutters and associated equipment**).

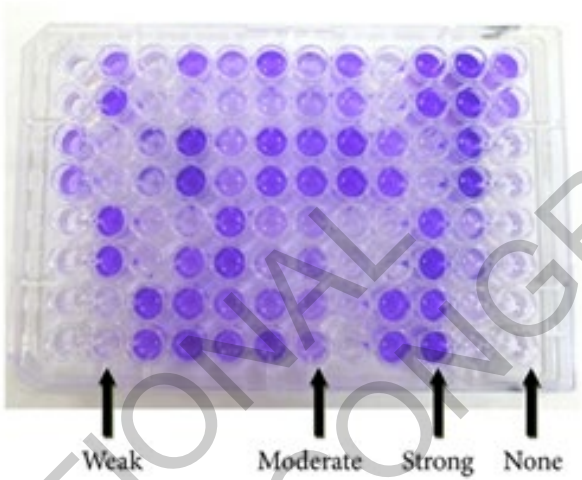
From these hotspots, we isolated bacteria of three different genera.
Enterobacteriaceae (*E. coli*),

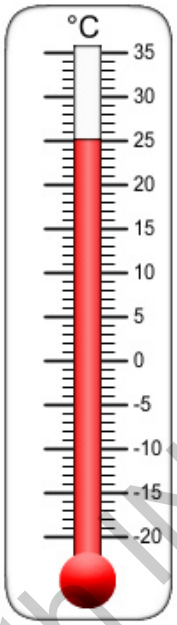
Pseudomonadaceae (*Pseudomonas*), and

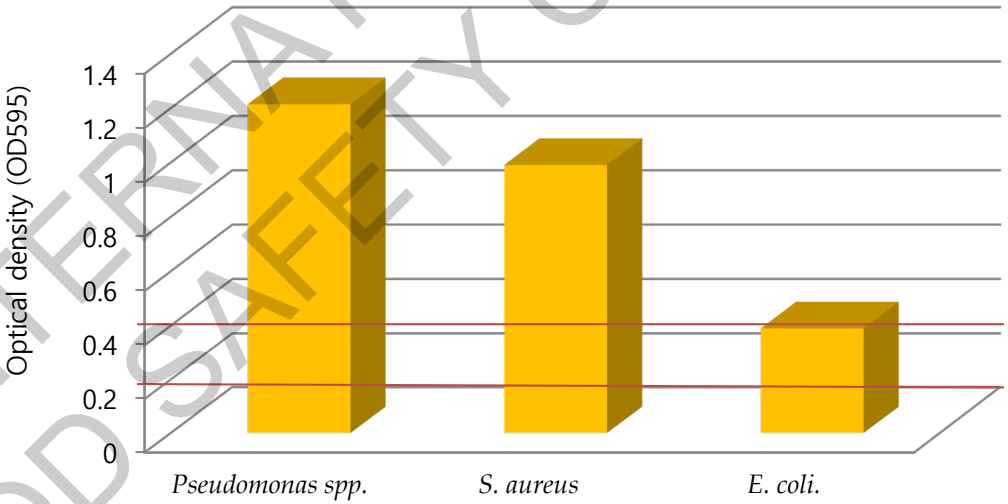
Staphylococcaceae (*S. aureus*)
indicating the presence of multi-species biofilms.

Results

48h







Strong

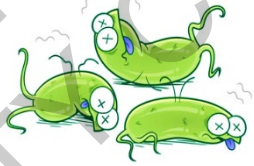
Moderate

Weak

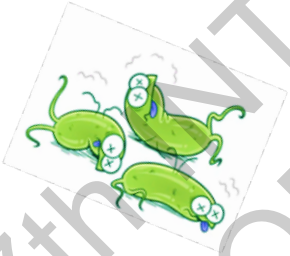
Conclusion remarks

Obtained results highlight the importance of properly sanitation and disinfection food processing equipment.

Food industry traditionally rely on cost-effective **chemical methods**, such as sodium hydroxide or sodium hypochlorite solutions, as well as **physical methods**, such as hot water steam, ozone or **mechanical removal** techniques.



Biofilm development on some food industry facilities **cannot be controlled** with these cost-effective methods.

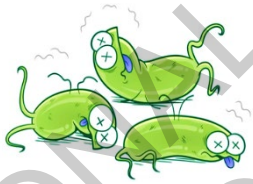


Conclusion remarks

SOLUTION - supporting existing techniques

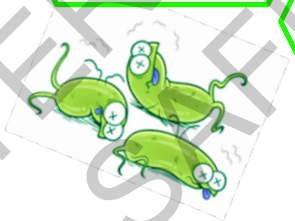
Enzymes

cellulases, proteases, glycosidases, DNAses, etc.



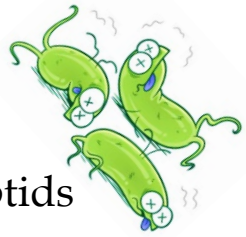
Bacteriophages and bacteriocins

QS inhibitors



Natural compouns

EOs
Antimicrobial peptids



Physical strategies

GRAS (Generally Recognized as Safe)

New technologies have been developed in recent years, but require approval from health authorities to be implemented at the industrial level.