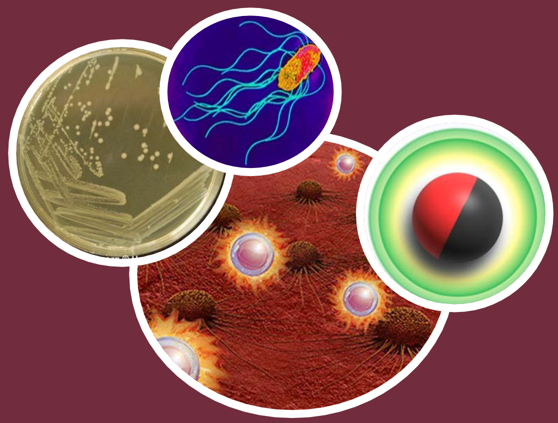
**NanoSpain 2017**
**San Sebastian, Spain**
**8 March 2017**



# **Hyperthermia** produced by magnetic nanoparticles as an alternative method to **control** a major **foodborne pathogen**

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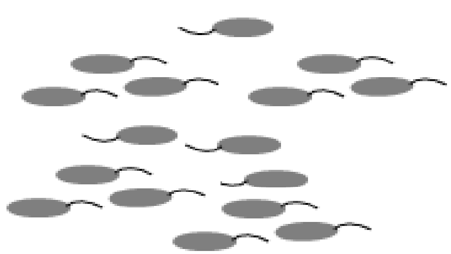
**Chemical disinfection**

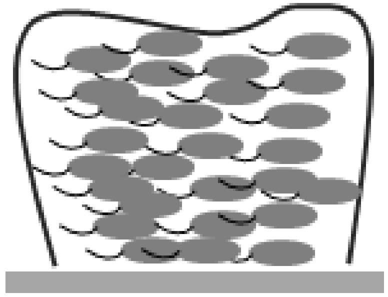
- Traditional approach.
- Widely applied in food industry.
- Many associated disadvantages.

**Exposure to sub-lethal concentrations ⇒ Microbial resistance**

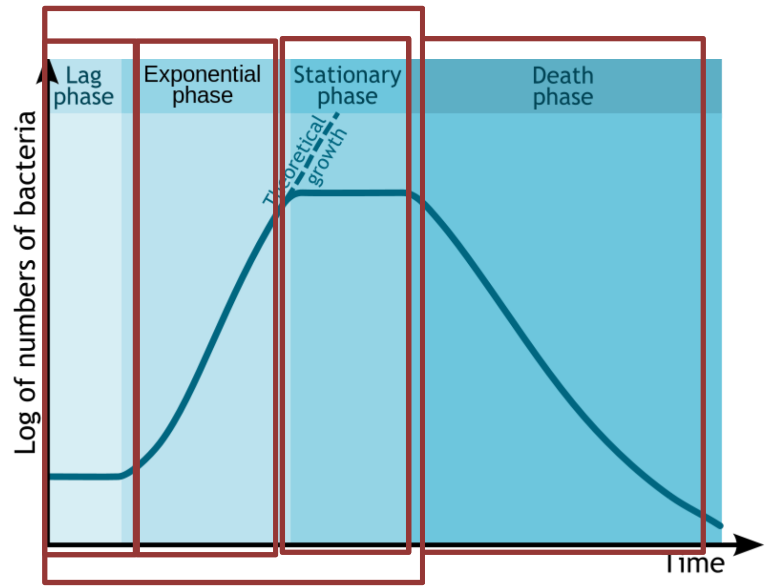
**Planktonic**



**Biofilm**



## Planktonic growth



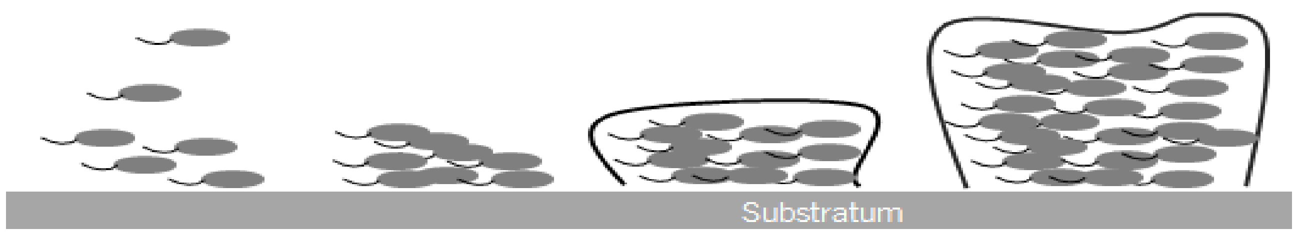

Lag phase – adaptation to new conditions.

Exponential phase – maximum growth rate.

Stationary phase – equal growth and death rates.

Death phase – bacteria die (injurious conditions).

## Biofilm formation




- Biological communities.
- Structure and polymeric matrix confer protection and resistance to the cells.
- Significantly more tolerant to disinfection.

# New disinfection methods

**Safer**

- Lower toxicity
- No by-products

**Chemical free**

- No enhancement of bacterial resistance

**More effective**

- Different metabolic states
- Different life forms

## SPM iron oxide nanoparticles

- Biocompatible and non-toxic.
- Relatively high Ms.
- No coercive forces or remanence.
- Versatility.

## Magnetic hyperthermia

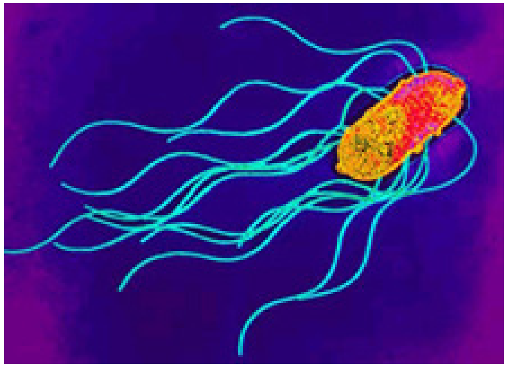
- ✓ Cancer therapy.
- ✓ Bacterial infections.
- ? Different bacterial populations.
- ? Foodborne pathogens.

# Aims of this study

Bactericidal action on different planktonic populations.

Bactericidal action on biofilms.

Effect on membrane integrity and biofilm structure.



***Salmonella enterica***

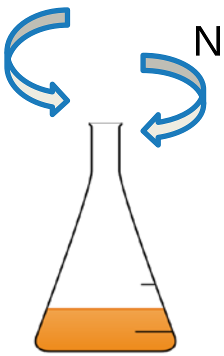
**Evaluate magnetic hyperthermia's potential against one of the most important foodborne pathogens.**

# Methodology

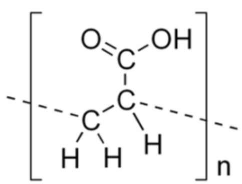
**Magnetic nanoparticles (MNPs): Poly-acrylic acid-coated magnetite (<d>10±4 nm; C=16 g/L)**

-$Fe^{2+}$ / $Fe^{3+}$ salts

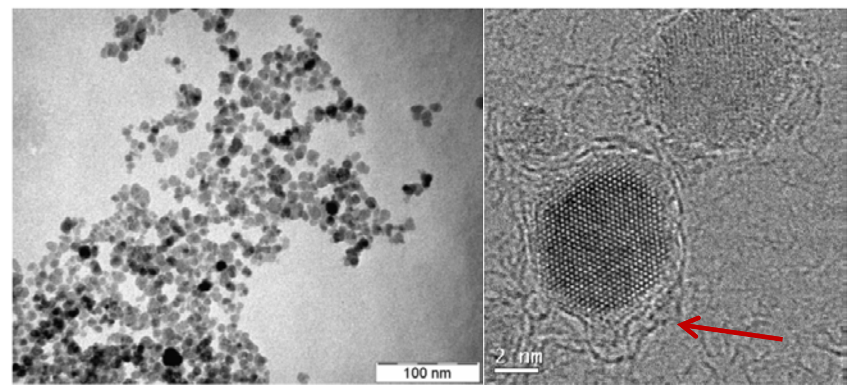
$NH_4OH$

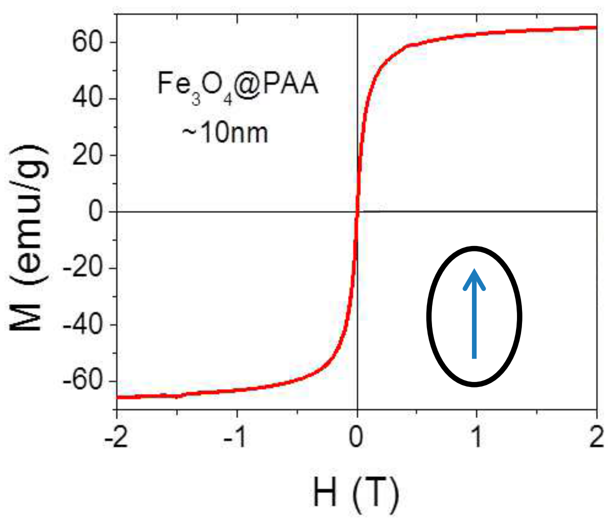


**co-precipitation method**




**+ PAA**



# Methodology

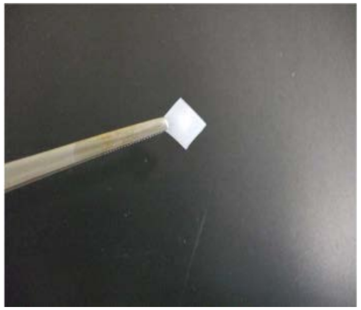
***S. enterica*** **strain: NCTC 13349**

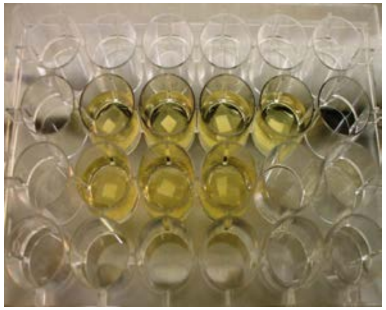
**Planktonic cells**
(saline; C ≈ 1x$10^8$ CFU/ml)

**Lag** (2h)

**Exp.** (4h)

**Stac.** (18h)





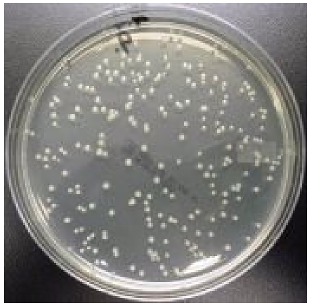
**Biofilms**
(3 days; RT; 120 rpm; TSB replaced each 24h )

**Magnetic Hyperthermia**
(873kHz ; 100 Oe)

500 µl MNPs + 500 µl bacteria

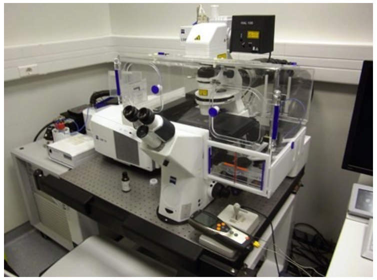
500 µl MNPs + 500 µl saline + biofilm

**CFU enumeration**



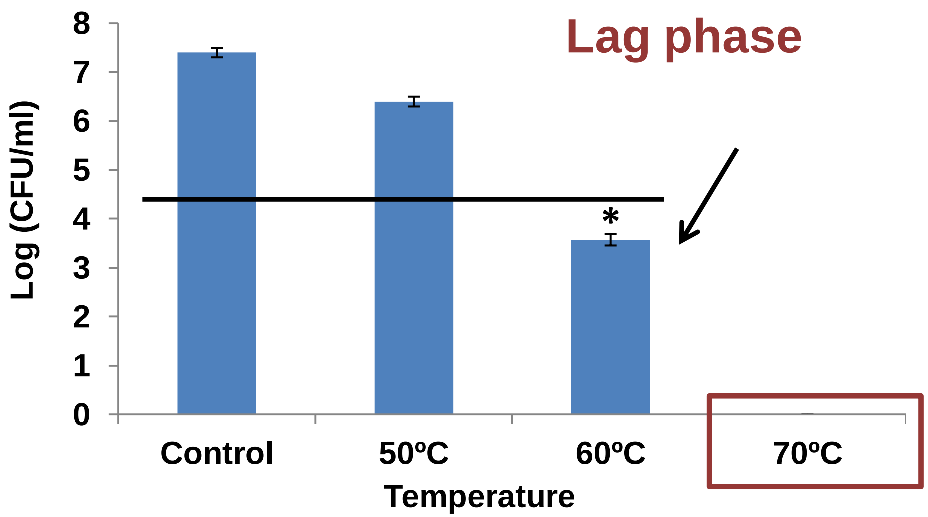
**Bacterial survival**

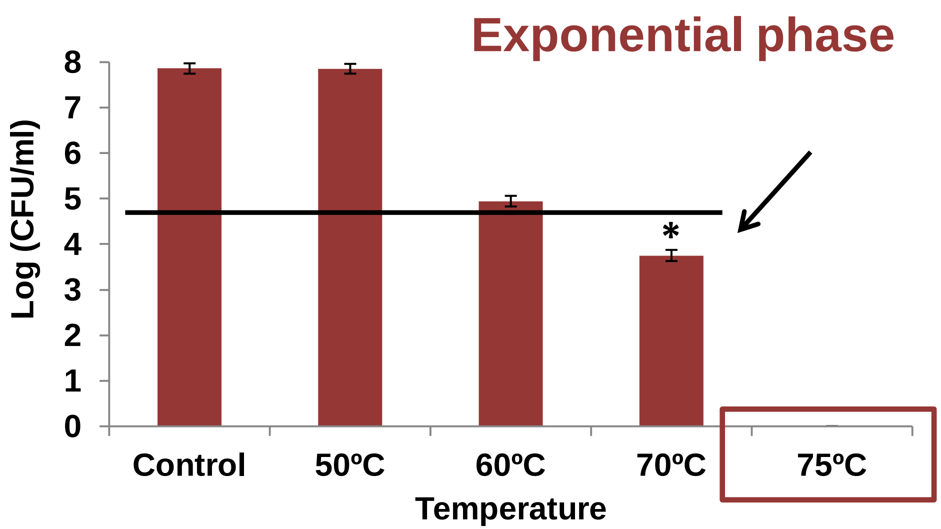
**CLSM**

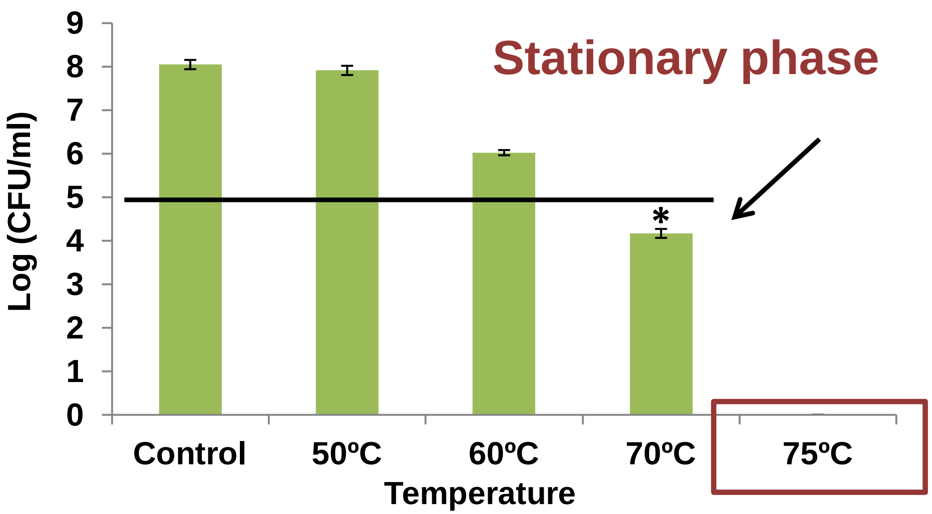


**Membrane integrity**
**Biofilm structure**

# Planktonic survival



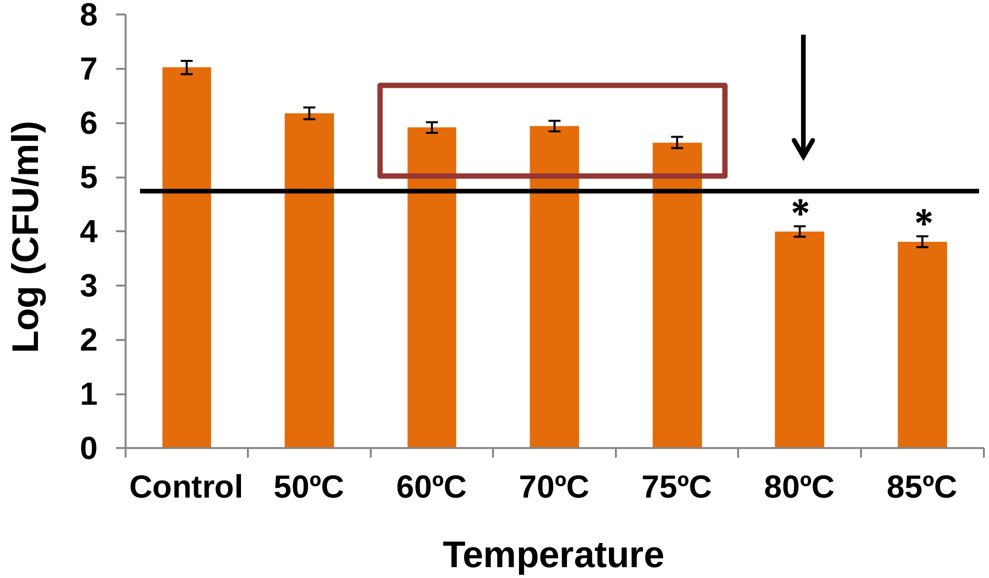




- Significant survival reduction on all planktonic populations.

- Lower temperature to reduce Lag phase survival.

- Eradication achieved for all phases (lower temperature on Lag phase).

# Biofilm survival



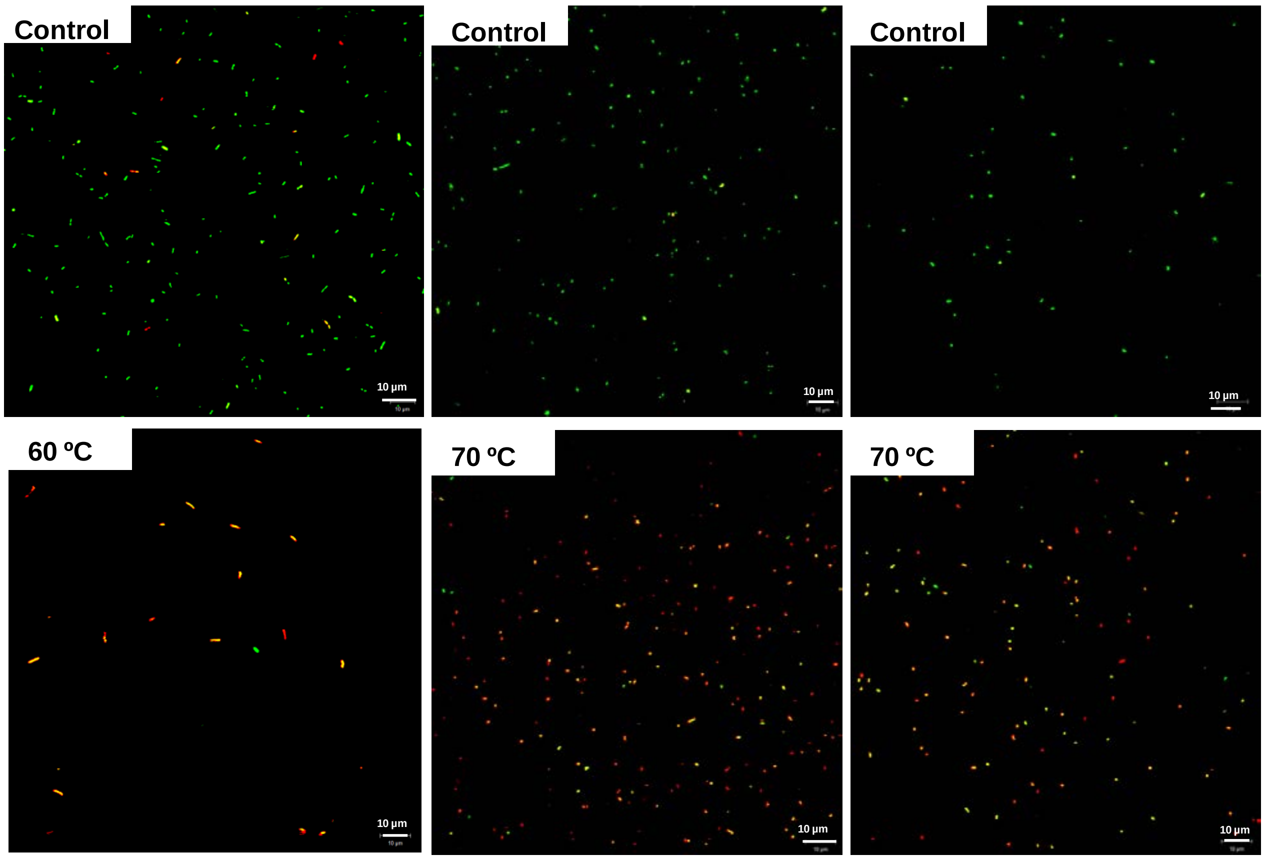

- Significant survival reduction (3 Log) achieved at 80 ºC.

- 6 Log remaining at the eradication temperatures for planktonic cells (70 ºC and 75 ºC).

- No eradication achieved.

# Membrane integrity

**Lag** | **Exponential** | **Stationary**

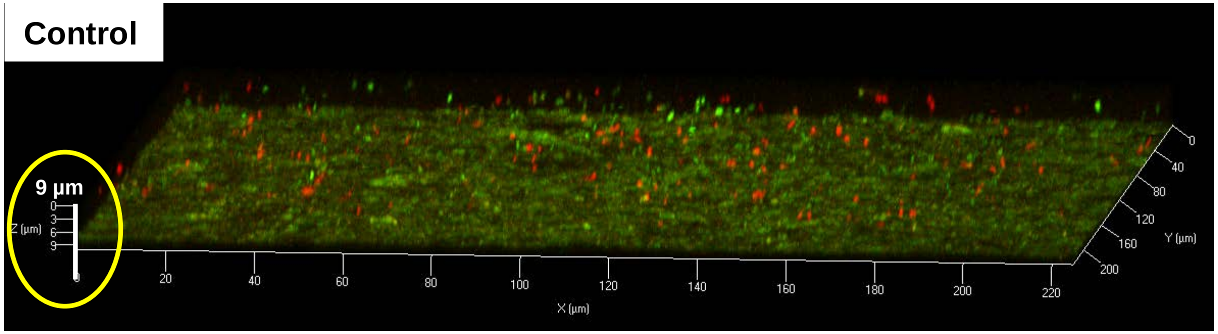


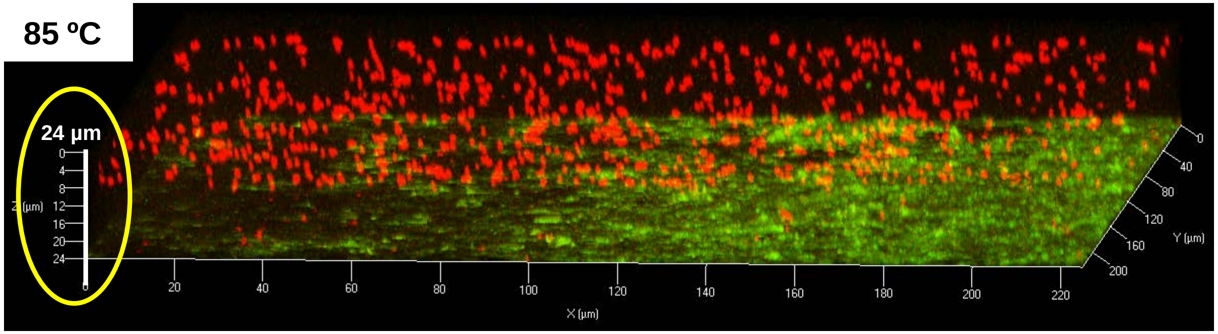

## Planktonic cells

- MH has promoted cell death (red-stained).

- Yellow/orange cells are not dead yet but have injured membranes.

# Biofilm structure



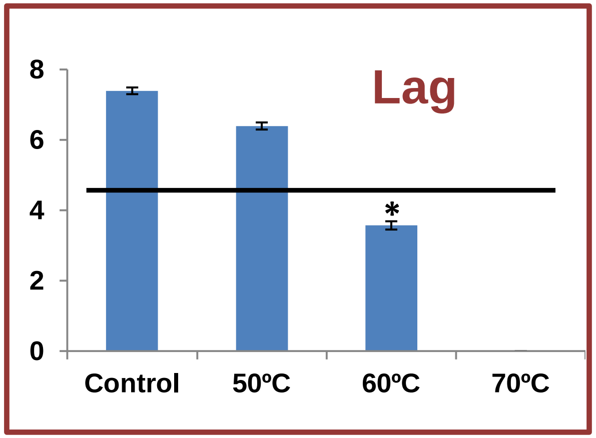



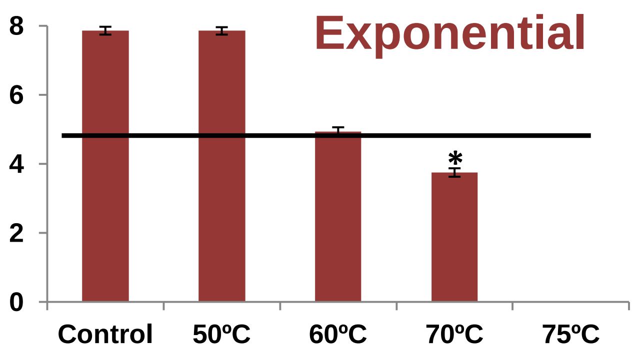

**- Dead cells (red-stained) were preferentially located in the upper layers.**

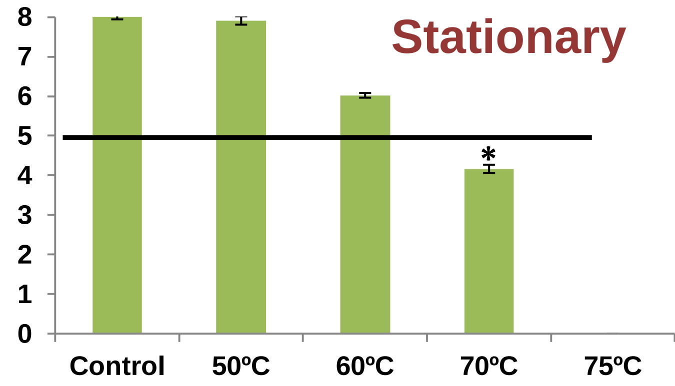
**- Biofilms exposed to MH were (apparently) thicker than controls.**

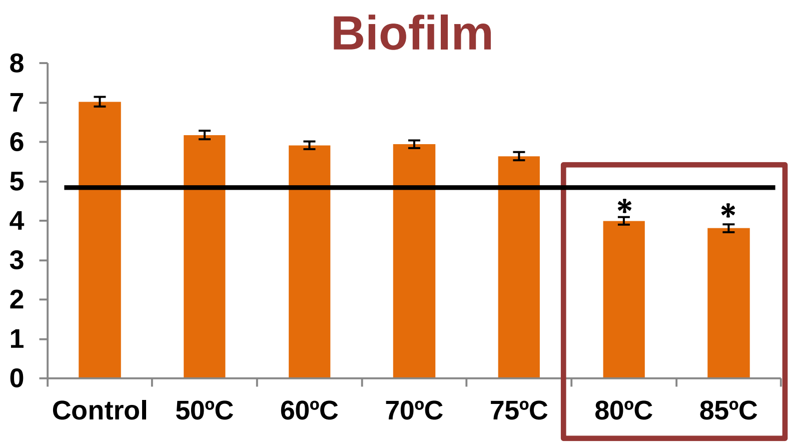
# Conclusions

**Bactericidal action**



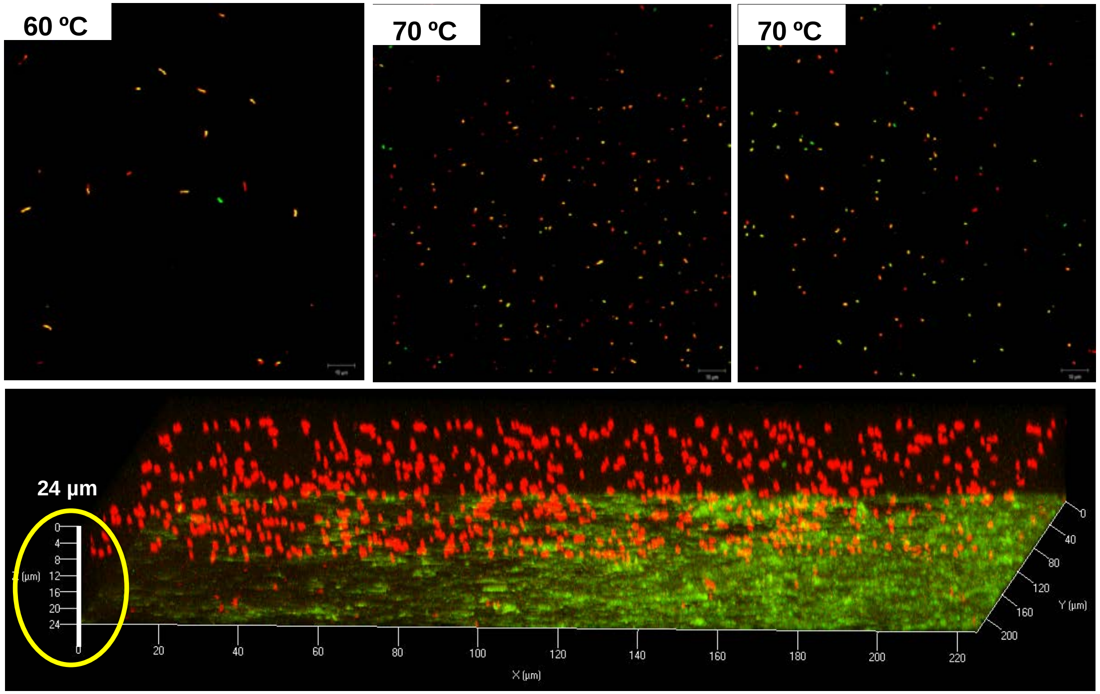

- MH was effective against all planktonic phases and biofilms (3 Log reduction).

- Lag phase was more susceptible to MH than the others planktonic phases (adaptation stage).

- MH was more effective against planktonic cells than biofilms

# Conclusions

## Membrane integrity & Biofilm structure




- MH affected membrane integrity of bacteria.

- MH affected biofilm structure.

- Higher thickness after MH can be due to detachment of dead cells.

# Final Conclusions

MNPs allowed an efficient heating of solutions containing an important foodborne pathogen – S. *enterica*.

MH lead to a significant viability reduction of planktonic cells concerning all growth phases.

Even though MH was more efficient against planktonic cells, the survival and the structure of biofilms were also affected by this treatment.

↓

**MH has high potential as an alternative method to control foodborne pathogens, presenting a promising bactericidal character against both planktonic cells and biofilms.**

# Acknowledgments

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