UNIVERSITÀ DEGLI STUDI DI TERAMO

FOODQA
TRAINING

DAY 3: 31 JANUARY 2018

DISINFECTION AND BIOPRESERVATION

PROF. ANTONELLO PAPARELLA

apaparella@unite.it

ANTIMICROBIAL AGENTS

❶ Physical agents: e.g. heat, radiations, etc.

❷ Chemical agents (disinfectants): gases, liquids, solids

THE IDEAL DISINFECTANT

Wide antimicrobial spectrum

High activity

Activity in presence of organic compounds and soaps

Rapid action

Long-lasting action

Chemical stability

Low toxicity in working conditions

THE IDEAL DISINFECTANT

No odour/taste given to foods

Noncorrosive for metals

Nonstaining for surfaces

High water solubility

Compatibility with sewage treatment plants

Easy application

Easy rinsing

Economical

FACTORS AFFECTING THE EFFECTIVENESS OF DISINFECTANTS

Concentration

Contact time

Temperature

pH

Formulation

Interfering substances

Type of microorganisms (species and strain)

MECHANISM OF ACTION

❶ Alteration of permeability of cytoplasmic membrane

❷ Damage to proteins or nucleic acids

Alteration of permeability of cytoplasmic membrane

Antimicrobials can damage membrane lipids and proteins, thus causing a loss of cytoplasmic material.

Damage to proteins

The functional properties of proteins depend on their 3-dimensional structure. Antimicrobials can break hydrogen bonds, covalent bonds, and SS-bonds.

Damage to nucleic acids

Heat, radiations and disinfectants can damage nucleic acids and hamper cell growth or modify physiological metabolic functions (e.g. enzyme synthesis).

ANTIMICROBIAL RESISTANCE OF MICROORGANISMS

MAX RESISTANCE Bacterial endospores

Mycobacteria

Protozoan cysts

Protozoan vegetative forms

Gram-negative bacteria

Fungi

Nonenveloped viruses

Gram-positive bacteria

MIN RESISTANCE Enveloped viruses

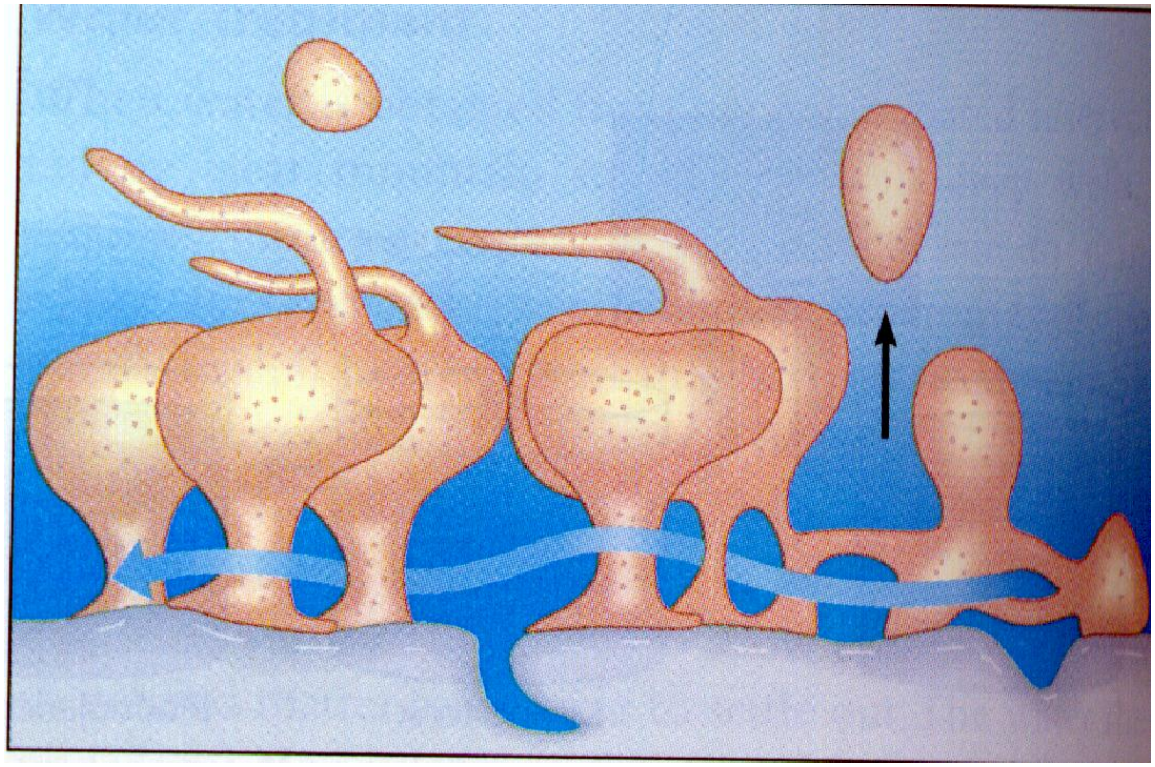
ANTIMICROBIAL RESISTANCE OF MICROORGANISMS

GRAM-NEGATIVES: due to outer membrane and in particular to porins (e.g. *Pseudomonas* spp.)

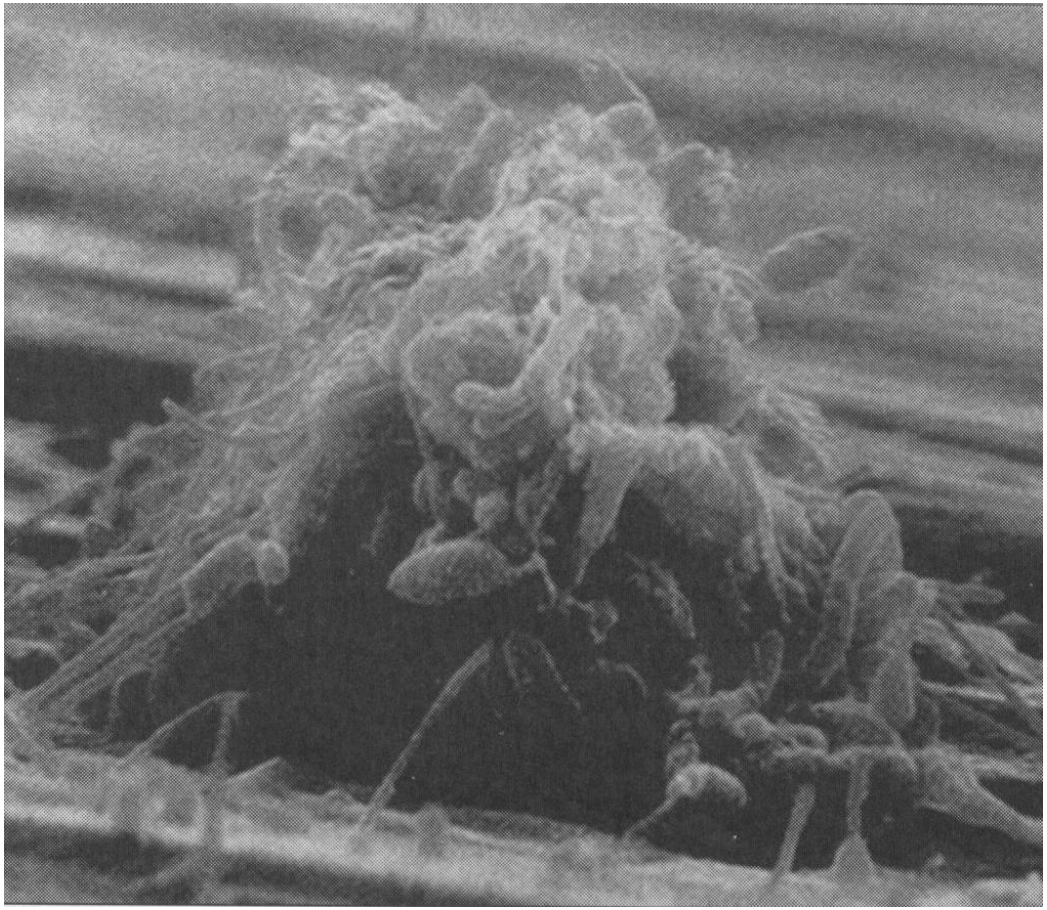
ANTIMICROBIAL RESISTANCE OF MICROORGANISMS

MYCOBACTERIA:	e.g. *Mycobacterium tuberculosis* (cell wall lipids)
VIRUSES:	Enveloped viruses are less resistant because many disinfectants are lipid soluble or solvents (e.g. ethanol)

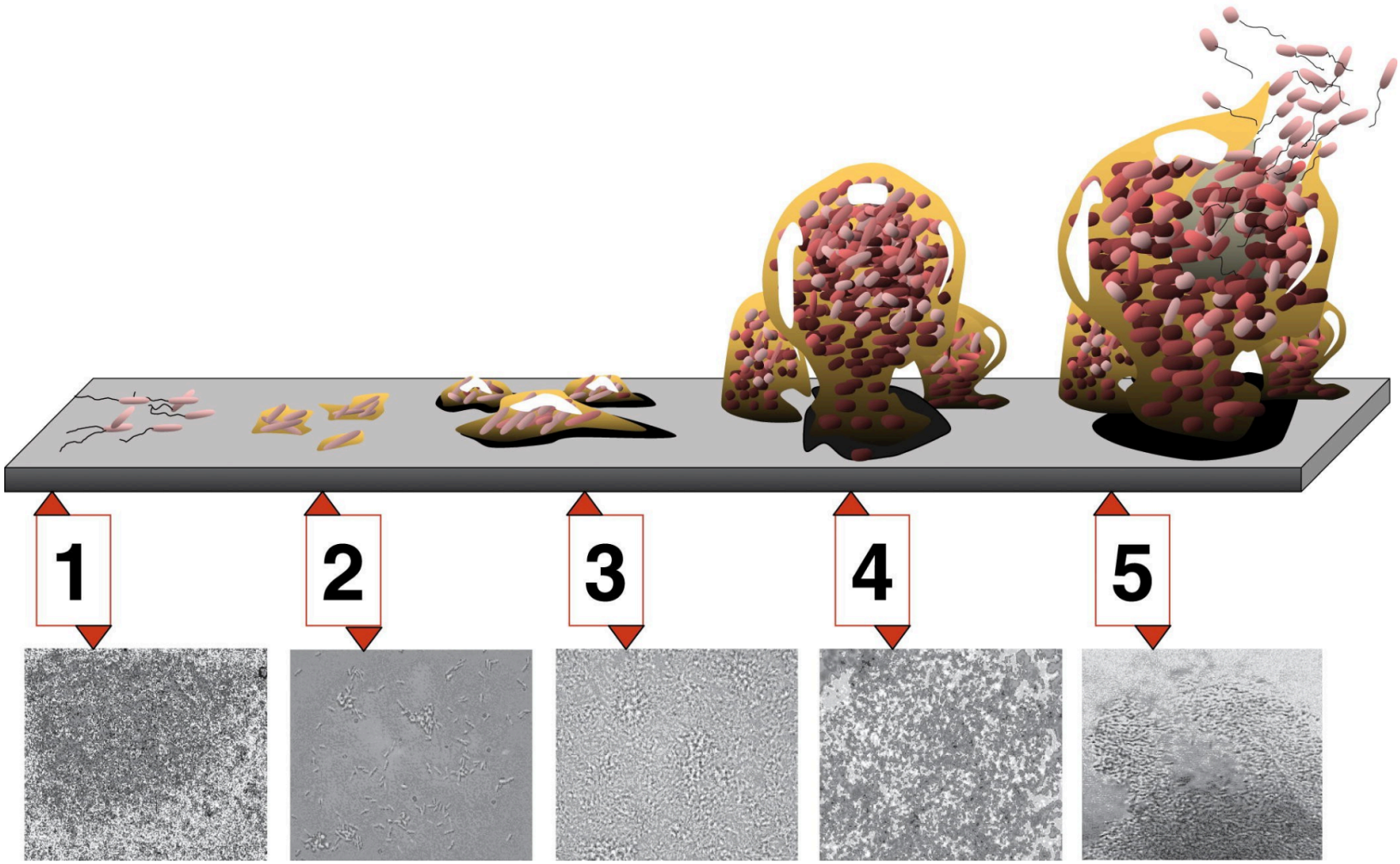
BIOFILM
(Microbial absorption and desorption)



BIOFILM
(Microbial absorption and desorption)



BIOFILM FORMATION



Five stages of biofilm development: (1) Initial attachment, (2) Irreversible attachment, (3) Maturation I, (4) Maturation II, and (5) Dispersion. Each stage of development in the diagram is paired with a photomicrograph of a developing *P. aeruginosa* biofilm. All photomicrographs are shown to the same scale.

D. Davis - From: D. Monroe. "Looking for Chinks in the Armor of Bacterial Biofilms". *PLoS Biology* **5** (11, e307).

SELECTION OF DISINFECTANTS

APPLICATION TECHNIQUES

❶ HPLV: High Pressure – Low Volume

❷ LPHV: Low Pressure – High Volume

❸ CIP: Clean-In-Place

❹ Gel/Foams

❺ Manual

CLASSIFICATION OF DISINFECTANTS ACCORDING TO THEIR ACTIVITY

❶ HIGH ACTIVITY: effective against all microorganisms, including bacterial endospores. E.g. ethylene oxide, 2% glutaraldehyde.

❷ MEDIUM ACTIVITY: effective against tuberculous mycobacteria and highly resistant viruses (Hepatitis viruses, rhinoviruses), non-effective against endospores. E.g. iodine tincture.

CLASSIFICATION OF DISINFECTANTS ACCORDING TO THEIR ACTIVITY

❸ LOW ACTIVITY: non-effective against endospores, tuberculous mycobacteria and nonenveloped viruses. If used correctly, they can kill fungi and bacterial vegetative forms. Low toxicity for humans, economical. E.g. iodophores, quaternary ammonium compounds, chlorhexidine.

CLASSIFICATION OF DISINFECTANTS ACCORDING TO THEIR ACTIVITY

Be careful! Some compounds can be used with different aims:

❶ DISINFECTANTS: applied to non-living surfaces;

❷ ANTISEPTICS: applied to living tissues;

❸ FOOD ADDITIVES: mainly preservatives;

❹ SURFACE DECONTAMINATING AGENTS: applied to food surface (if allowed)

CONDITIONS THAT INCREASE THE EFFECTIVENESS OF MOST DISINFECTANTS

❶ Use on surfaces

❷ Clean surfaces

❸ High temperature

❹ Microbial cells in exponential phase

❺ Gram-positive bacteria

❻ No endospores

❼ Extended contact time

QUATERNARY AMMONIUM COMPOUNDS

Good disinfectants against Gram-positives

(e.g. benzalkonium chloride, cetylpyridinium chloride)

High surfactant activity (foaming capacity and detersion)

Good disinfectant activity, very low toxicity

Excellent solubility

Stable solutions, very economical, deodorant

Suitable for all materials

Not very active against Gram-negatives, not effective against mycobacteria and endospores

Low activity in presence of organic substance, soaps and hard water

QUATERNARY AMMONIUM COMPOUNDS

Advantages

Wetting and penetrating action
Residual activity
Deodorant
Suitable for all materials
Stable
Can be applied manually

Disadvantages

Reduced activity in acid environment
Low activity in hard water and in presence of organic substance
Selective biocide
Foaming activity

PERACETIC ACID

Strong and rapid action, wide spectrum

(1% solution inactivates bacteria and fungi in 5 min, viruses and endospores in 30 min)

Irreversible oxidizer

Active at low temp but unstable at high temp

No toxic residues

Scarcely affected by organic substance

Reacts with aluminium, copper, zinc, bronze, concrete Non-foaming

Frequently used for CIP. Used also for stables.

Used to clean the outer surface of food packaging in clean rooms.

PERACETIC ACID

Advantages

Rapid and intense activity

Wide spectrum

Effective in hard water

Non-foaming

Disadvantages

Pungent and unpleasant odour

Irritating

Very reactive

Unstable at high temp

POLYBIGUANIDES

Very effective against bacteria, less against fungi

Active between pH 5.0 and 8.5

Neutral solutions, can be applied manually

Suitable for all materials but bronze and copper

Incompatible with chlorine (forms gums)

No odour

Moderately foaming

POLYBIGUANIDES

Advantages

Effective against bacteria

Residual activity

Neutral solutions

Low skin sensitisation

Disadvantages

Low activity against fungi

Inactive at alkaline pH

Low activity at acid pH

Inactivated by phosphates and chlorine

Need accurate rinsing

CHLORHEXIDINE (biguanide)

Persistant and atoxic bactericidal

(effective against fungi, bacterial vegetative cells, some enveloped viruses BUT NOT against endospores)

Common antiseptic for skin and mucosae (e.g. mouthwash)

Together with detergents and alcohols, used in presurgery

Residual activity

Low toxicity (but irritating for conjunctiva)

Antiseptic for hand cleaning

CHLORHEXIDINE

Advantages

Residual activity

Low toxicity

Scarcely affected by organic substance

Disadvantages

Selective activity

Irritating for conjunctiva

Mainly used as an antiseptic and not as a disinfectant

GLUTARALDEHYDE

Effective sterilizer

(2% sol. Inactivates bacteria, mycobacteria and viruses in 10 min but endospores in 3-10 hours!)

Rapid and intense activity also in presence of organic substance

Widespread in hospitals, less in the food industry (cost)

Active at neutral or low alkaline pH

Completely inactivated by amines and ammonia

Deodorant

Less irritating than formalin

GLUTARALDEHYDE

Advantages

Rapid and intense activity

Wide spectrum

Effective in presence of organic substance

Deodorant

Noncorrosive

Disadvantages

Low activity at acid pH

Skin sensitisation

Expensive

CHLORINE AND CHLORINE COMPOUNDS

Strong oxidizers, active also against HBV

(effective also at low concentration, medium temp, neutral pH)

INORGANIC: liquids (e.g. hypochlorite) or powders (e.g. chlorinated trisodium phosphate)

ORGANIC: usually powders (e.g. chloramine) that produce salts in solution

pH-dependant activity: formation of hypochlorous acid (biocidal activity inversely proportional to pH)

CHLORINE COMPOUNDS

Unstable in alkaline solutions

Some irritate skin and damage fabric

At high temp and acid pH, they can corrode inox steel

Not suitable for light metals

Versatile and economical

CHLORINE COMPOUNDS

Advantages

Effective and rapid

Wide spectrum

Cost-effective

Versatile

Disadvantages

Irritating, potentially corrosive

Unstable, accidentally toxic

Reduced activity in presence of organic substance

Can have unpleasant odour

IODINE-BASED DISINFECTANTS

General properties

Active at acid pH (2.5-3.5) and much less at pH>5.0

At ordinary concentrations (12.5-25 ppm free iodine) are much less effective than chlorine on endospores

Noncorrosive for inox steel but can corrode light metals, copper, zinc, and aluminium

Can stain plastic and damage rubber (not iodine monochloride)

IODINE-BASED DISINFECTANTS

Iodine tincture

(2% or more iodine in a water-alcohol solution of potassium iodine)

Effective antiseptic

Stains fabric, materials and skin

Can damage epithelia (skin sensibilisation)

IODINE-BASED DISINFECTANTS

Iodophores

Made of 3 components:

❶ iodine

❷ carrier (organic compound/iodine stabilizer, e.g. polymer or non-ionic surfactant)

❸ acid (usually phosphoric)

IODINE-BASED DISINFECTANTS

Iodophores

(e.g. Betadine, Isodine)

Water soluble, stable, slowly release iodine

Effective antiseptics and disinfectants

Don't stain fabric, materials and skin (but can stain plastic and teflon, and damage rubber)

Less irritating than iodine

IODINE-BASED DISINFECTANTS

Iodine monochloride

(acid disinfectants with no surfactants, releasing iodine in solution)

Same antimicrobial activity as iodine

Don't stain fabric, skin and materials

No foam

Easy to rinse (e.g. in CIP)

IODINE-BASED DISINFECTANTS

General applications

Presurgery

Antisepsis (skin wounds)

Disinfection/antisepsis in hospitals and labs

Treatment of compatible Food Contact Surfaces (FCS)

Beer industry (CO_2 tanks)

CIP (iodine monochloride)

IODINE-BASED DISINFECTANTS

Advantages

Wide spectrum
Active at low concentration
Active in hard water
Noncorrosive for inox steel
Versatile

Disadvantages

Low activity against endospores
Low activity at pH>5.0
Foam (except iodine monochloride)
Usually damage plastic and rubber
Unpleasant odour if overdosed

ALCOHOLS

Rapid action and evaporation

(effective on bacteria and fungi

BUT NOT on endospores and nonenveloped viruses)

Protein denaturation and solvent for lipids (damage to membranes and virus envelope)

No residues

Poor antiseptic (coagulate proteins, under which bacteria can grow)

Can be used in the formulation of other disinfectants to increase their activity (tincture)

ALCOHOLS

Ethanol

Recommended concentration = 70% (effective between 60 and 90%) Pure ethanol is less effective because protein denaturation requires water

Isopropanol

Frequently sold as alcohol in supermarkets

Slightly more effective than ethanol

Cheaper and less volatile than ethanol

TABLE 7.6 Germicidal Action of Various Concentrations of Ethanol in Aqueous Solution Against *Streptococcus pyogenes*

Concentration of Ethanol (%)	Time (sec)				
	10	20	30	40	50
100	–	–	–	–	–
95	+	+	+	+	+
90	+	+	+	+	+
80	+	+	+	+	+
70	+	+	+	+	+
60	+	+	+	+	+
50	–	–	+	+	+
40	–	–	–	–	–

Notes: A minus sign indicates no germicidal action (bacterial growth); a plus sign indicates germicidal action (no bacterial growth). The highlighted area represents bacteria killed by germicidal action.

From: Tortora, Funke & Case, Microbiology, an Introduction (2002)

ALCOHOLS

Advantages

Rapid action

No residues

Solvent for lipids

Disadvantages

Selective action

Poor antiseptic

Inflamable

HYDROGEN PEROXIDE

Effective disinfectant, poor antiseptic on open wounds

Very used as antiseptic both in hospitals and at home

Poor antiseptic on open wounds (inactivated by catalase)

However, used to irrigate deep wounds in order to provide oxygen and hamper the growth of strict anaerobes

On non-living surfaces, it can be sporicide at high temp

Used to clean packages in aseptic packaging of foods

Used to disinfect contact lenses

HYDROGEN PEROXIDE

Advantages

Effective

Scarse residual action

Very useful to irrigate (not disinfect) deep wounds

Disadvantages

Poor antiseptic

Rapid inactivation in presence of catalase

CONCLUSIONS

❶ Disinfection aims to eliminate undesired microorganisms

❷ Cleaning and Disinfection should be carried out regularly to prevent biofilm formation

❸ The application technique depends on several factors, such as type and level of dirt, shape and type of material

CONCLUSIONS

❺ The effectiveness of disinfectants is affected by instrinsic and extrinsic conditions

❻ Disinfectants can have different mechanisms of action

❼ To select the best disinfectant for each application, we should consider advantages and disadvantages, as well as the objective of disinfection

THE IDEAL DISINFECTANT

DOES NOT EXIST

PRACTICAL HINTS FOR CLEANING

1

LIST ALL THE CONSTRUCTION MATERIALS

some plastic materials, light metals, rubber, and teflon

cutting boards can be damaged by unsuitable chemicals

(e.g. concentrated acids for light metals, iodine for teflon and rubber, etc.)

2

EVALUATE IF CLEANING WILL BE CARRIED OUT BY SPECIALIZED EXTERNAL STAFF OR BY INTERNAL WORKERS

Formation and internal audits

3

IDENTIFY PRODUCTION AREAS WITH EXTRAORDINARY CLEANING REQUIREMENTS

Special sections in the Cleaning and Disinfection Plan

4

CAREFUL EVALUATION OF THE TECHNICAL SHEETS

Investigate the function of each ingredient in cleaning formulations

5

GENERAL RULES CAN HAVE EXCEPTIONS

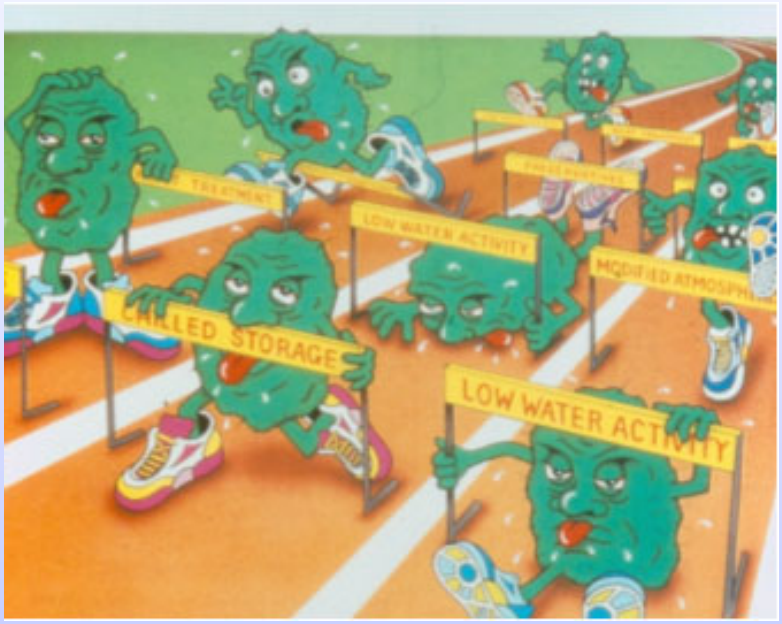
Peracetic acid is less stable at higher temperatures

Pure ethanol is not a good disinfectant

FOOD BIOPRESERVATION

(not just bacteriocins…)

MULTIPLE-HURDLES STRATEGIES FOR FOOD PRESERVATION (HURDLE TECHNOLOGIES)



BIOPRESERVATION: DEFINITION (Stiles, 1996)

BIOPRESERVATION aims to prevent the contamination and growth of undesired microorganisms in foods, by addition of:

a) antimicrobial compounds, naturally present in foods

b) antimicrobial compounds, produced in foods after physical or chemical stimulation, or after protective cultures addition

SINCE THE 90s,

HURDLE TECHNOLOGIES HAVE BEEN CHANGING

THE FOOD MARKET DRAMATICALLY

WIDESPREAD PRODUCTS

WITH HIGH ADDED VALUE

OUR FOODS,

BEFORE AND AFTER HURDLE TECHNOLOGIES

BEFORE…

Dry snacks

NOW ALSO…

Chilled chocolate snacks and milk slices

OUR FOODS,

BEFORE AND AFTER HURDLE TECHNOLOGIES

BEFORE…

Canned sauces

NOW ALSO…

Chilled sauces

OUR FOODS,

BEFORE AND AFTER HURDLE TECHNOLOGIES

BEFORE…

Stock cubes

NOW ALSO…

Gelled stock

OUR FOODS,

BEFORE AND AFTER HURDLE TECHNOLOGIES

BEFORE…

Fresh vegetables

NOW ALSO…

Fresh-cut vegetables

ESSENTIAL OILS (OEs) AS HURDLES IN FOOD PRESERVATION

THREE MAIN GOALS:

- PATHOGENS CONTROL
- FOOD SPOILERS CONTROL
- ANTIOXIDANT ACTIVITY

UNIVERSITÁ DEGLI STUDI DI TERAMO
Facoltà di Bioscienze e tecnologie agroalimentari e ambientali

Plant-based intervention strategies for *Listeria monocytogenes* control in foods

A. Paparella*, A. Serio, C. Chaves López and G. Mazzarrino

Green technologies for controlling *Listeria monocytogenes* in foods: the combined hurdles approach

Free download from:

http://www.formatex.info/microbiology4/vol2/1230-1246.pdf

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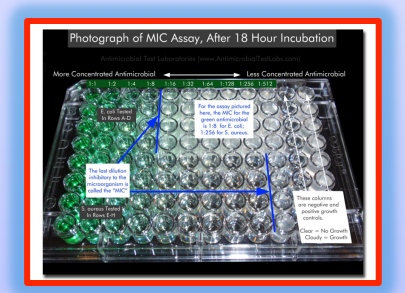
Our protocol

for

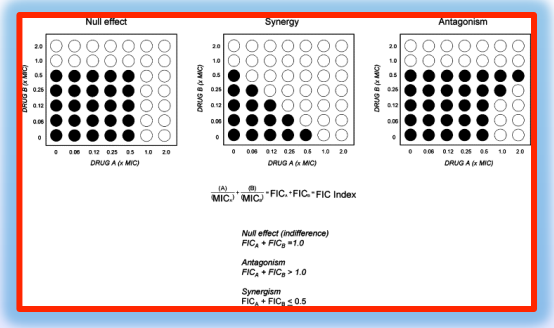
EOs selection

OBJECTIVE		METHOD
FIRST SCREENING		DISK DIFFUSION
SCREENING FOR A HIGH NUMBER OF SAMPLES		AGAR WELL TEST
MINIMUM INHIBITORY CONCENTRATION (MIC)		BROTH DILUTION MICRODILUTION IN BIOSCREEN (AUTOMATIC TURBIDIMETRY)
TIME-KILL KINETICS		VIABLE COUNT AUTOMATIC TURBIDIMETRY

OBJECTIVE

METHOD

INTERACTION TEST

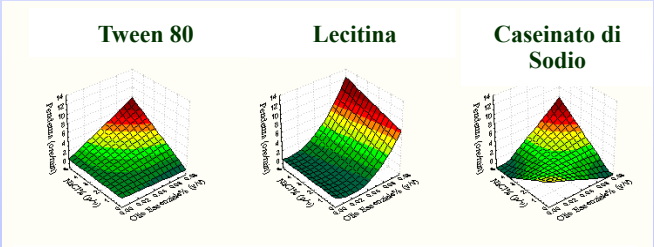


BROTH DILUTION CHECKERBOARD

MECHANISM OF ACTION

e.g. EPR, FLOW CITOMETRY

DEVELOPMENT OF EMULSIONS



STABILITY TEST, RHEOLOGY

IN SITU STUDIES

CHALLENGE STUDIES

SHELF-LIFE STUDIES

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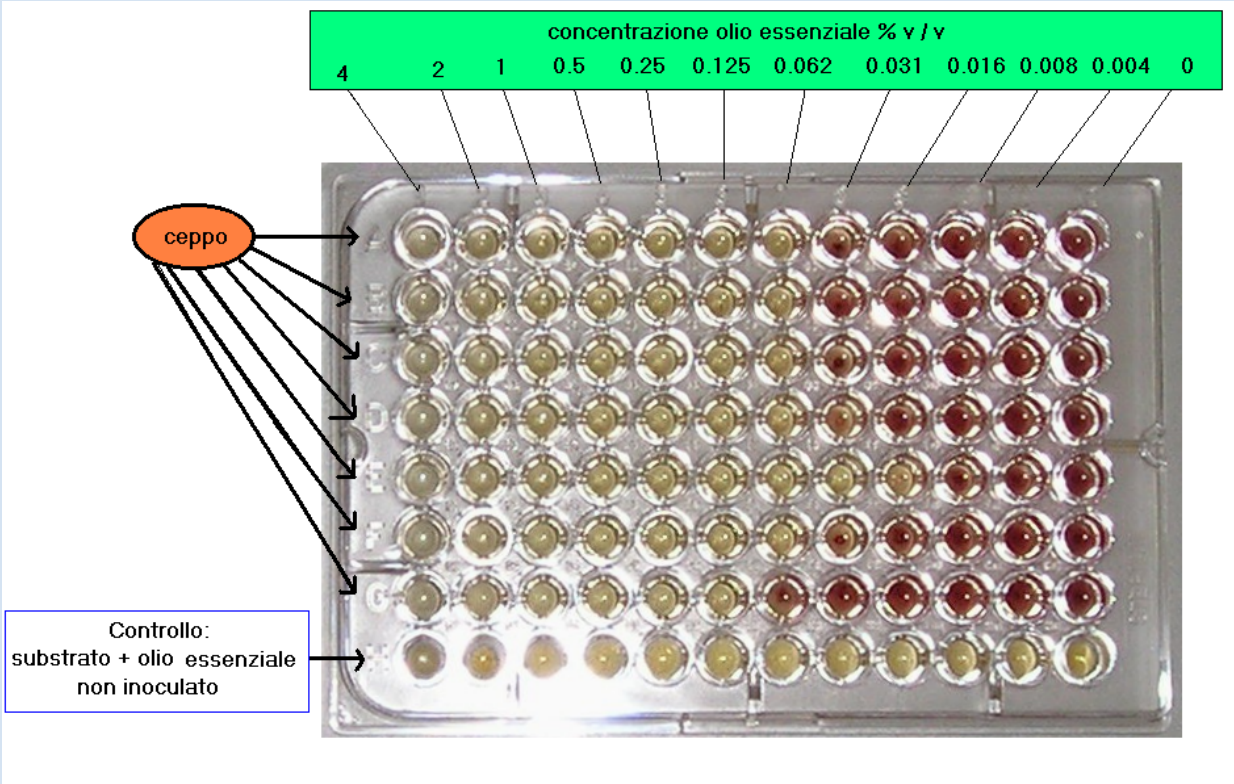
FOOD BIOPRESERVATION: ACTIVITIES

1. Mechanism of action of EOs on bacterial cells
2. Effect of EOs on cells physiology
3. Plant extracts
4. Chitosan
5. Olive mill wastewater
6. Ozone

Evaluation of the antimicrobial activity of *Origanum vulgare* OE against *L.monocytogenes* by 2,3,5-triphenyltetrazolium chloride



FOOD BIOPRESERVATION:

PATHOGENS CONTROL

Mechanisms of action

Oregano and thyme EOs act on cytoplasmic membrane, while cinnamon has a weaker action on *L. monocytogenes* cells.

Available online at www.sciencedirect.com
ScienceDirect
Food Control 19 (2008) 1174–1182
FOOD CONTROL
www.elsevier.com/locate/foodcont

Flow cytometric assessment of the antimicrobial activity of essential oils against *Listeria monocytogenes*

Antonello Paparella [a,*], Lorenzo Taccogna [a], Irene Aguzzi [b], Clemencia Chaves-López [a], Annalisa Serio [a], Fulvio Marsilio [b], Giovanna Suzzi [a]

[a] *Dipartimento di Scienze degli Alimenti, Università degli Studi di Teramo, Via C.R. Lerici, 1, 64023 Mosciano Stazione, TE, Italy*
[b] *Dipartimento di Scienze Biomediche Comparate, Università degli Studi di Teramo, Piazza Aldo Moro 45, 64100 Teramo, TE, Italy*

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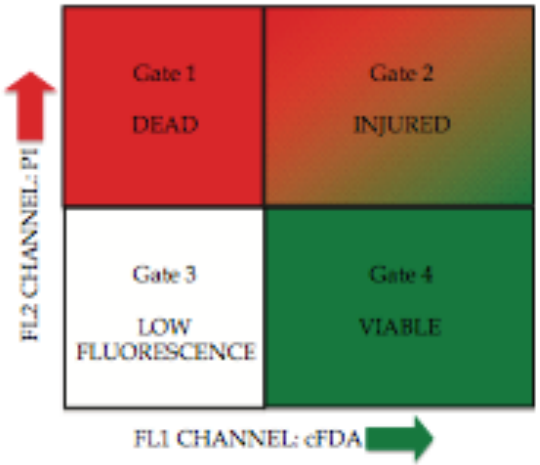

Fig. 1. Microbial subpopulations, after exposure to essential oils, evaluated by flow cytometry (dyes: carboxyfluorescein diacetate cFDA; propidium iodide PI)

Clear discrimination among viable, injured and dead cells

Quantification of injured cells is particularly interesting for food microbiologists, as this subpopulation might be critical if cell recovery becomes possible, e.g. in temperature abuse conditions during food storage.

Mechanisms of action

Flow Cytometry Applications in Food Safety Studies

Antonello Paparella, Annalisa Serio and Clemencia Chaves López

Flow cytometric assessment may provide an efficient tool to evaluate microorganism adaptation to EOs, as well as to study interactions between EOs and food ingredients, in order to define conditions which maximise EOs potential for food biopreservation.

Mechanisms of action

Letters in Applied Microbiology

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ORIGINAL ARTICLE

Electronic paramagnetic resonance investigation of the activity of *Origanum vulgare* L. essential oil on the *Listeria monocytogenes* membrane

A. Serio[1], M. Chiarini[1], E. Tettamanti[2] and A. Paparella[1]

1 Department of Food Science, University of Teramo, Mosciano Sant'Angelo (TE), Italy
2 Department of Biomedical Comparative Sciences, University of Teramo, Piazza Aldo Moro, Teramo (TE), Italy

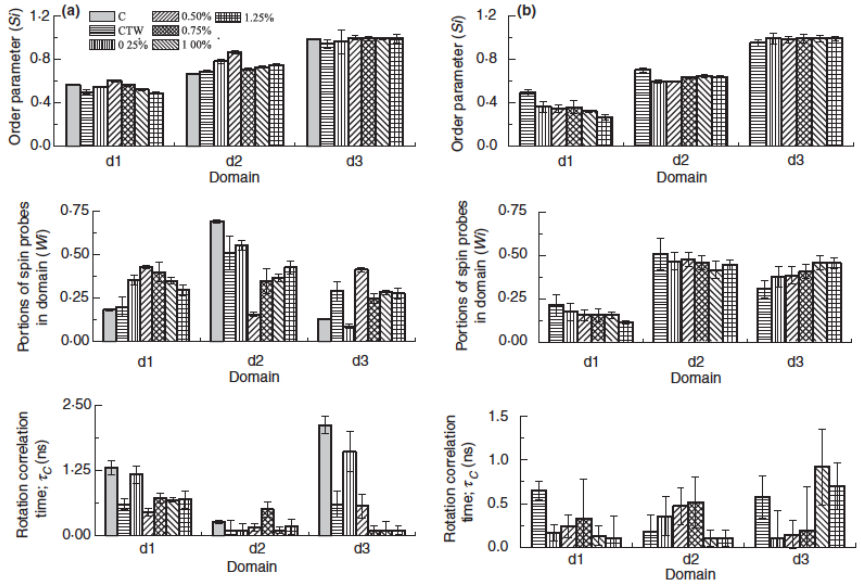

Figure 3 Order parameter (plot Si), rotational correlation time (plot τc) and weight (plot Wi) of three coexisting domains (d1, d2, d3) in the membrane of *Listeria monocytogenes* ATCC 7644 cells. Data obtained by computer fitting of the experimental EPR spectra of samples C (Cells exposed to 0% EO), CTw (0% EO plus 0·25% Tween 80), 0·25, 0·50, 0·75, 1·00 and 1·25% EO. The error bars were determined by the fitting program. Plot (a) for samples treated in PBS; Plot (b) for samples treated in PBS and 30 mmol l^{-1} NaN_3.

Changes in membrane fluidity and order.

The cells spend energy to impair EO entrance in the membrane, at least up to a critical concentration.

Increase in Lag length of treated cells suggests a cell damage recovery.

SCALING UP FROM LAB SCALE TO IN SITU STUDIES

10 to 100-fold increase in biopreservatives %

SUCCESS DEPENDS ON BOTH MICROBIAL TARGET SENSITIVITY AND SENSORY CHARACTERISTICS OF FOODS

Generally, Gram positives are more sensitive

Design of Experiment for reducing biopreservative dose

LWT - Food Science and Technology 58 (2014) 116–123

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Application of Central Composite Design to evaluate the antilisterial activity of hydro-alcohol berry extract of *Myrtus communis* L.

Annalisa Serio, Clemencia Chaves-López, Maria Martuscelli, Giovanni Mazzarrino, Carla Di Mattia, Antonello Paparella*

Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Via C.R. Lerici 1, 64023 Mosciano Stazione, TE, Italy

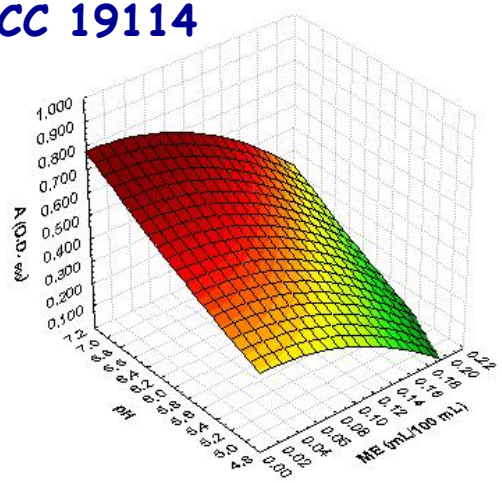
Table 1
Composition of the Central Composite Design and results obtained for λ (Lag phase length in h) and A (maximum growth value as $O.D._{600\ nm}$) for the six strains tested after growth data modelling according to Gompertz equation modified by Zwietering et al. (1990).

RUN	Parameters			ATCC 19114		ATCC 7644		2		3		5		7	
	NaCl (g/100 mL)	pH	ME (mL/100 mL)	λ	A	λ	A	λ	A	λ	A	λ	A	λ	A
1	1.50	6.50	0.156	7.79a	0.48A	7.45a	0.45A	7.50a	0.45A	9.66b	0.40A	3.99c	0.81B	7.68a	0.44A
2	0.50	6.50	0.156	7.83a	0.52A	6.51b	0.48B	6.52b	0.46B	8.22a	0.64C	6.12b	0.64C	6.03b	0.54A
3	1.50	6.50	0.078	8.00a	0.61A	6.34b	0.50B	6.08b	0.49B	9.45c	0.59A	7.32a	0.57A	7.58a	0.52B
4	0.50	6.50	0.078	7.73a	0.62A	8.27a	0.46B	6.10b	0.47B	8.56c	0.63B	6.20a	0.62B	7.55a	0.56C
5	1.50	5.50	0.156	9.62a	0.29A	4.83b	0.44B	6.77c	0.33A	10.02a	0.29A	6.09c	0.47B	8.20d	0.27A
6	0.50	5.50	0.156	9.47a	0.30A	9.08a	0.20B	8.58b	0.23B	8.30b	0.31A	6.91c	0.39C	8.46b	0.26AB
7	1.50	5.50	0.078	10.15a	0.35A	8.37b	0.28B	7.76c	0.33AB	9.65d	0.39A	7.29c	0.41C	8.64b	0.32A
8	0.50	5.50	0.078	9.31a	0.40A	6.97b	0.34B	7.09b	0.36B	10.28c	0.44A	6.36b	0.43A	7.72b	0.35B
9	1.00	6.00	0.117	9.33a	0.35A	7.08b	0.37A	7.04b	0.39A	9.37a	0.39A	6.67b	0.51B	8.34d	0.31C
10	1.00	7.00	0.117	8.18a	0.58A	6.50b	0.65B	6.51b	0.61B	10.03c	0.64B	8.09a	0.74C	8.21a	0.61B
11	1.00	5.00	0.117	12.90a	0.27A	8.06b	0.27A	11.52d	0.25A	12.31a	0.31B	11.47d	0.35B	11.02d	0.30B
12	1.00	6.00	0.195	–	–	7.26a	0.32A	7.55a	0.29A	12.41b	0.11B	–	–	9.22c	0.23A
13	1.00	6.00	0.039	8.06a	0.58A	6.88b	0.47B	6.81b	0.46B	9.77c	0.52B	6.23a	0.28C	7.4b	0.53D
14	2.00	6.00	0.117	9.89a	0.32A	8.18b	0.32A	8.48b	0.30A	9.86a	0.35A	7.24c	0.48B	8.74b	0.32A
15	0.00	6.00	0.117	8.07a	0.42A	6.56b	0.38A	6.48b	0.40A	6.73b	0.53B	6.95a	0.44C	7.31c	0.39A
16	1.00	6.00	0.117	9.50a	0.37A	7.11b	0.37A	7.21b	0.37A	9.32a	0.37A	6.44c	0.51B	7.97d	0.34A
17	1.00	6.00	0.117	9.47a	0.37A	7.05b	0.37A	7.08b	0.38A	6.64b	0.36A	7.06b	0.49B	7.98c	0.34A
Control	0.00	7.00	0.000	8.27a	0.70A	6.15b	0.72A	6.11b	0.64B	8.10a	0.66B	6.07b	0.64B	7.98a	0.73A

*ME = *Myrtus communis* berry extract.
–, No growth.
Significant differences ($p \leq 0.05$) in the same row are indicated by small (for λ) and capital (for A) letters.

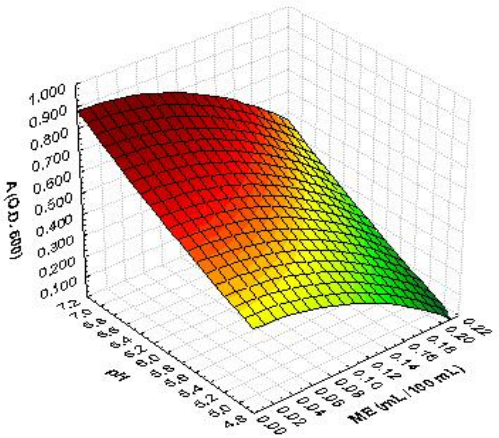
Fig. 3. Response surface plot for maximum growth value *A* of *L. monocytogenes* ATCC 19114 and strains 3 and 7 as a function of *Myrtus communis* berry extract (ME) and pH (NaCl was fixed at the intermediate concentration of 1.00 g/100 mL).

ATCC 19114

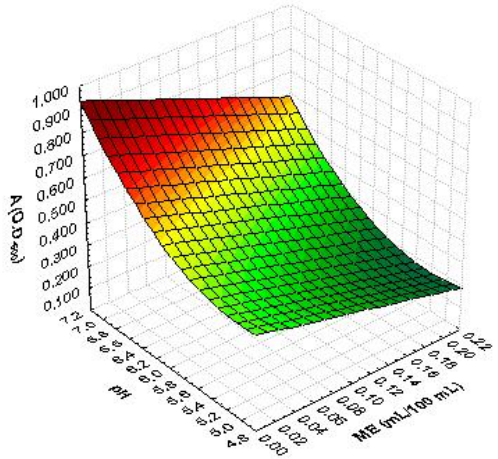


Additive effect between myrtle extract and pH

3



7



The outcome of this study could be particularly useful for potential industrial applications. In fact, compared with essential oils, hydro-soluble extracts do not require the presence of emulsifiers, thus facilitating applications in manufacturing environments. ME at sub-lethal concentrations was effective in reducing or containing cell growth, and therefore in reducing the risk deriving from *L. monocytogenes*. Moreover, the efficacy of very low ME concentrations, in combination with salt and pH, can be valuable to define antilisterial strategies, potentially avoiding sensory changes in foods.

Interactive effect between myrtle extract and pH

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Salmonella enterica and *Listeria monocytogenes* inactivation dynamics after treatment with selected essential oils

Giovanni Mazzarrino [a], Antonello Paparella [a], Clemencia Chaves-López [a], Angelo Faberi [b], Manuel Sergi [a], Costantino Sigismondi [a], Dario Compagnone [a], Annalisa Serio [a, *]

INACTIVATION DYNAMICS: 21 EOs vs. 2 antibiotics against *S.enterica* and *L.monocytogenes*

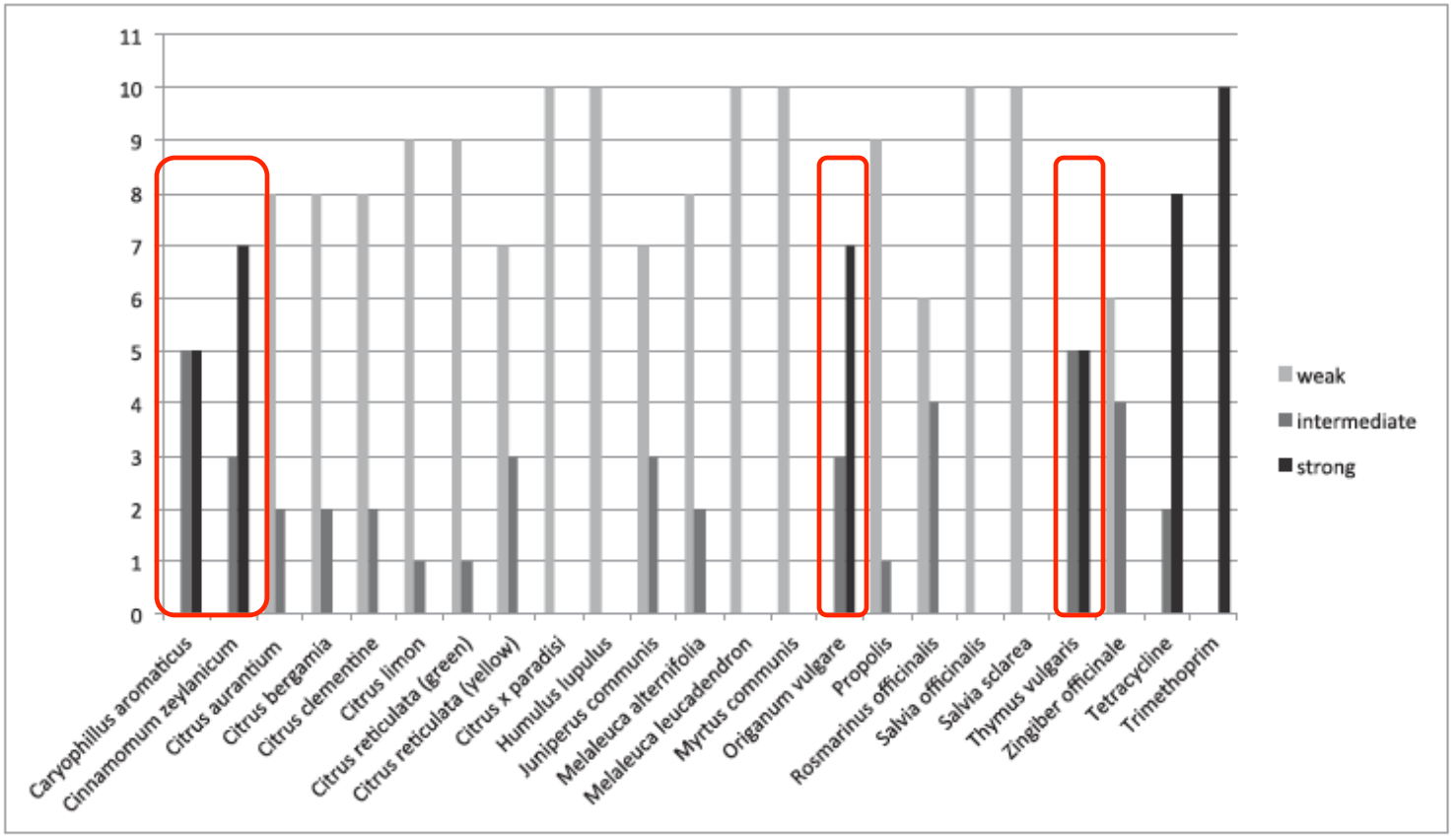

Fig. 1. Antimicrobial activity of 21 Essential Oils, tetracycline and trimethoprim on *Salmonella enterica* (a) and *Listeria monocytogenes* (b) strains, according to Disk Diffusion results. Weak activity = inhibition halo ≤12 mm; intermediate activity = inhibition halo ≥12.1 mm and <20.0; strong activity = inhibition halo ≥20.1 mm.

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INACTIVATION DYNAMICS: MICs OF SELECTED EOs

Salmonella enterica and *Listeria monocytogenes* inactivation dynamics after treatment with selected essential oils

Giovanni Mazzarrino [a], Antonello Paparella [a], Clemencia Chaves-López [a], Angelo Faberi [b], Manuel Sergi [a], Costantino Sigismondi [a], Dario Compagnone [a], Annalisa Serio [a, *]

Minimum Inhibitory Concentration (MIC) (μL/mL) of selected essential oils against *Salmonella enterica* and *Listeria monocytogenes*.

Essential oils	*Salmonella* S1	*Salmonella* S4	*Salmonella* S.E.44/06	*Salmonella* S.E. 44/5/9-3	*L. monocytogenes* ATCC 7644	*L. monocytogenes* LM 4	*L. monocytogenes* LM 17	*L. monocytogenes* LM 19
Caryophillus aromaticus	0.6aA	2.5aB	2.5aB	2.5aB	2.5aB	2.5aB	1.2aC	1.2aC
Cinnamomum zeylanicum	2.5bA	2.5bA	2.5aA	2.5aA	0.6bB	2.5aA	2.5bA	2.5bA
Origanum vulgare	1.2cA	1.2cA	0.6bB	1.2bA	0.6bB	1.2bA	0.6cB	0.6cB
Melaleuca alternifolia	20.0dA	20.0dA	10.0cB	20.0cA	10.0cB	10.0cB	10.0dB	10.0dB
Thymus vulgaris	5.0eA	5.0eA	2.5aB	5.0dA	5.0dA	5.0dA	5.0eA	2.5bB
Juniperus communis	>40	>40	>40	>40	>40	>40	>40	>40
Melaleuca leucadendron	>40	>40	5.0d	>40	>40	>40	>40	>40
Myrtus communis	>40	>40	5.0d	>40	>40	>40	>40	>40
Salvia officinalis	>40	>40	2.5a	>40	>40	>40	>40	>40
Salvia sclarea	>40	>40	2.5a	>40	>40	>40	>40	>40

Different small letters in the same column indicate significant differences ($P < 0.05$) between the essential oils treatment; different capital letters in the same row indicate significant differences ($P < 0.05$) on oil sensitivity between the strains.

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EVOLUTION OF INACTIVATION DYNAMICS AT INCREASING EOs CONCENTRATIONS

Salmonella enterica and *Listeria monocytogenes* inactivation dynamics after treatment with selected essential oils

Giovanni Mazzarrino [a], Antonello Paparella [a], Clemencia Chaves-López [a], Angelo Faberi [b], Manuel Sergi [a], Costantino Sigismondi [a], Dario Compagnone [a], Annalisa Serio [a, *]

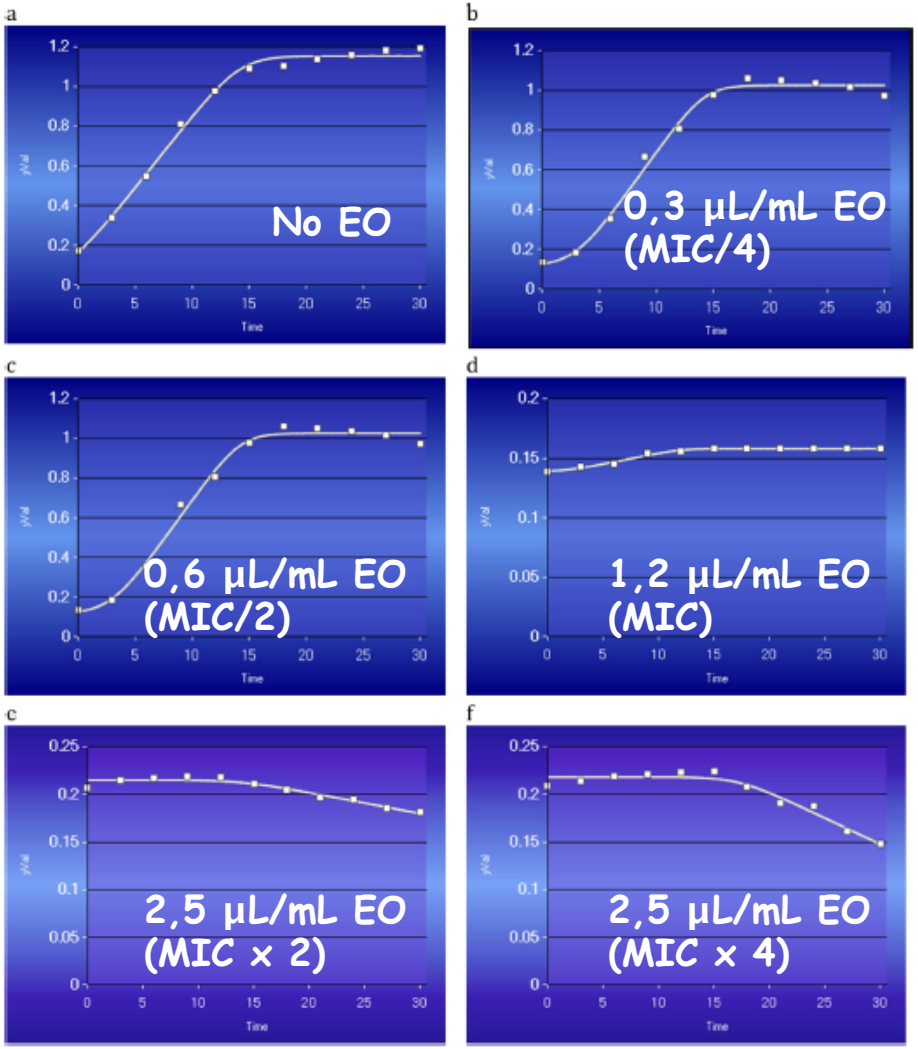


Fig. 2. Growth/inhibition dynamics of *Salmonella* Derby S1 in presence of different concentration of *Origanum vulgare* essential oil. a) Growth in control conditions (no essential oil); b) 0.3 µL/mL EO (MIC/4); c) 0.6 µL/mL EO (MIC/2); d) 1.2 µL/mL EO (MIC value); e) 2.5 µL/mL EO (MIC × 2); f) 5.0 µL/mL EO (MIC × 4).

Salmonella Derby S1

treated with

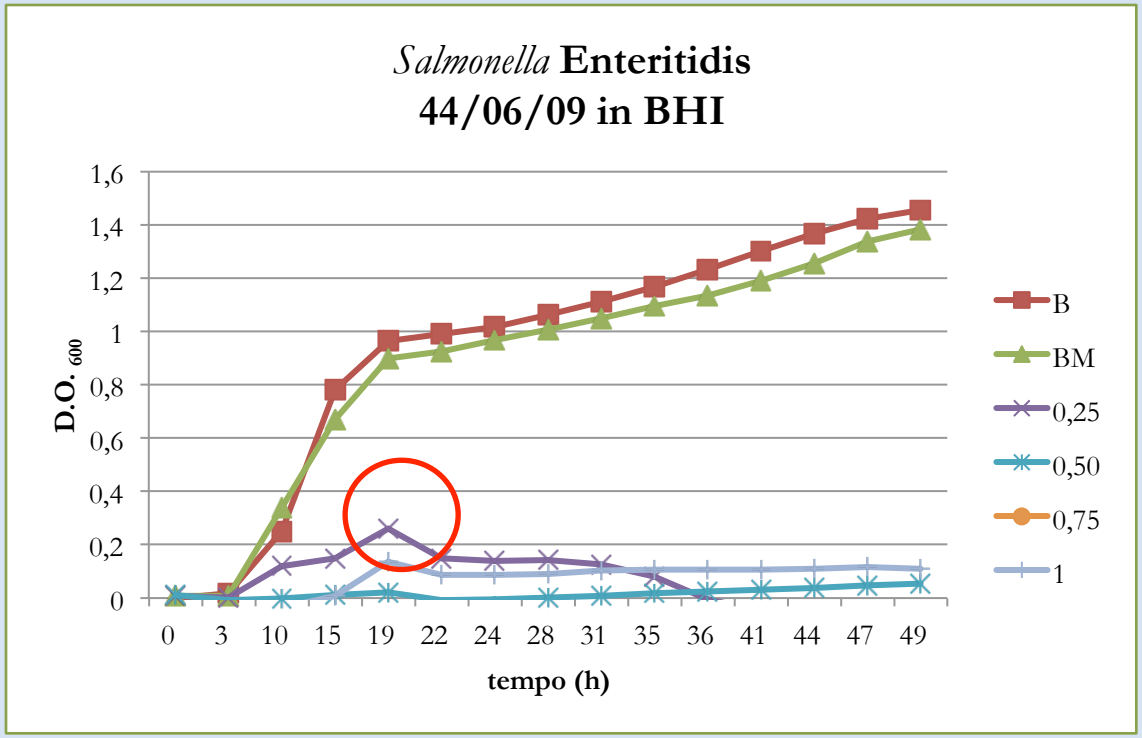
Origanum vulgare EO

UNIVERSITÀ DEGLI STUDI DI TERAMO

Facoltà di Bioscienze e tecnologie agroalimentari e ambientali

Salmonella Enteritidis growth inhibition in liquid medium (B: BHI; BM: Modified BHI) during 48 hours after treatment with OLIVE MILL POLYPHENOLS (values in %)

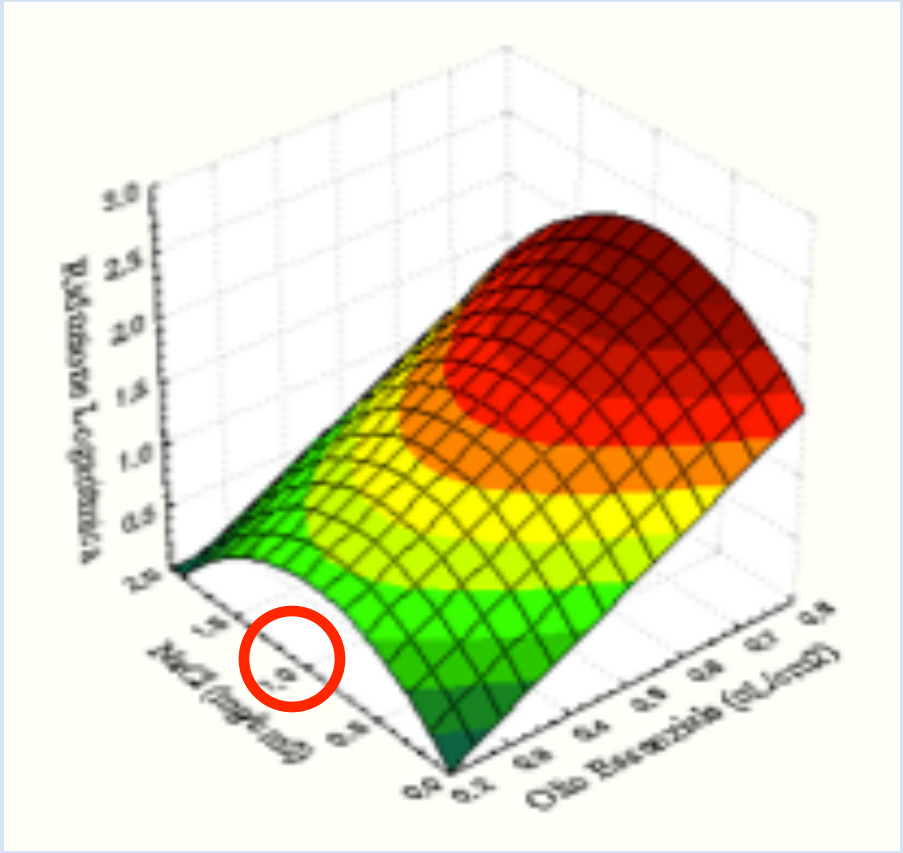
(Paparella A., Serio A., Chaves López C., 2012)



FOOD BIOPRESERVATION:

SHELF-LIFE EXTENSION

DEVELOPMENT OF EO EMULSIONS FOR SURFACE TREATMENTS OF MEAT PRODUCTS IN CLEAN ROOMS (REAL SYSTEM)



Effect of OE-NaCl interaction on log decrease of *L. monocytogenes* in cured pork coppa

SPECIFIC MARKERS FOR SPECIFIC FOODS: SPB IN MARINE FISH

Food Control 39 (2014) 111–118

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Food Control

journal homepage: www.elsevier.com/locate/foodcont

A survey on bacteria isolated as hydrogen sulfide-producers from marine fish

Annalisa Serio, Giuseppe Christian Fusella, Clemencia Chaves López, Giampiero Sacchetti, Antonello Paparella*

Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Via C.R. Lerici 1, 65023 Mosciano Stazione, TE, Italy

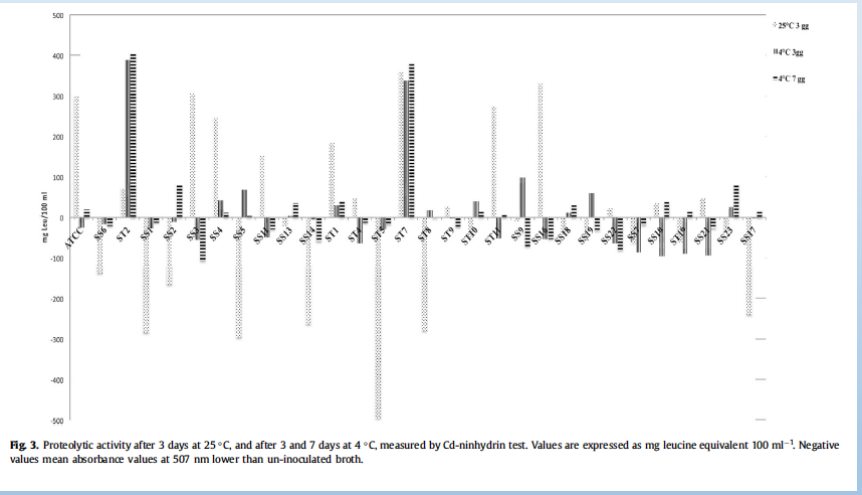


Fig. 3. Proteolytic activity after 3 days at 25 °C, and after 3 and 7 days at 4 °C, measured by Cd-ninhydrin test. Values are expressed as mg leucine equivalent 100 ml^{-1}. Negative values mean absorbance values at 507 nm lower than un-inoculated broth.

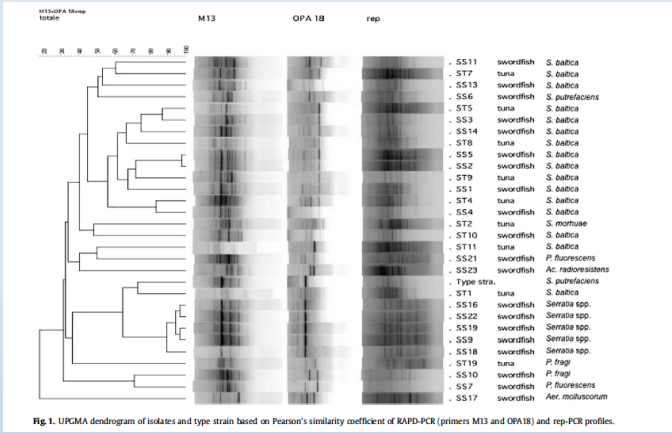


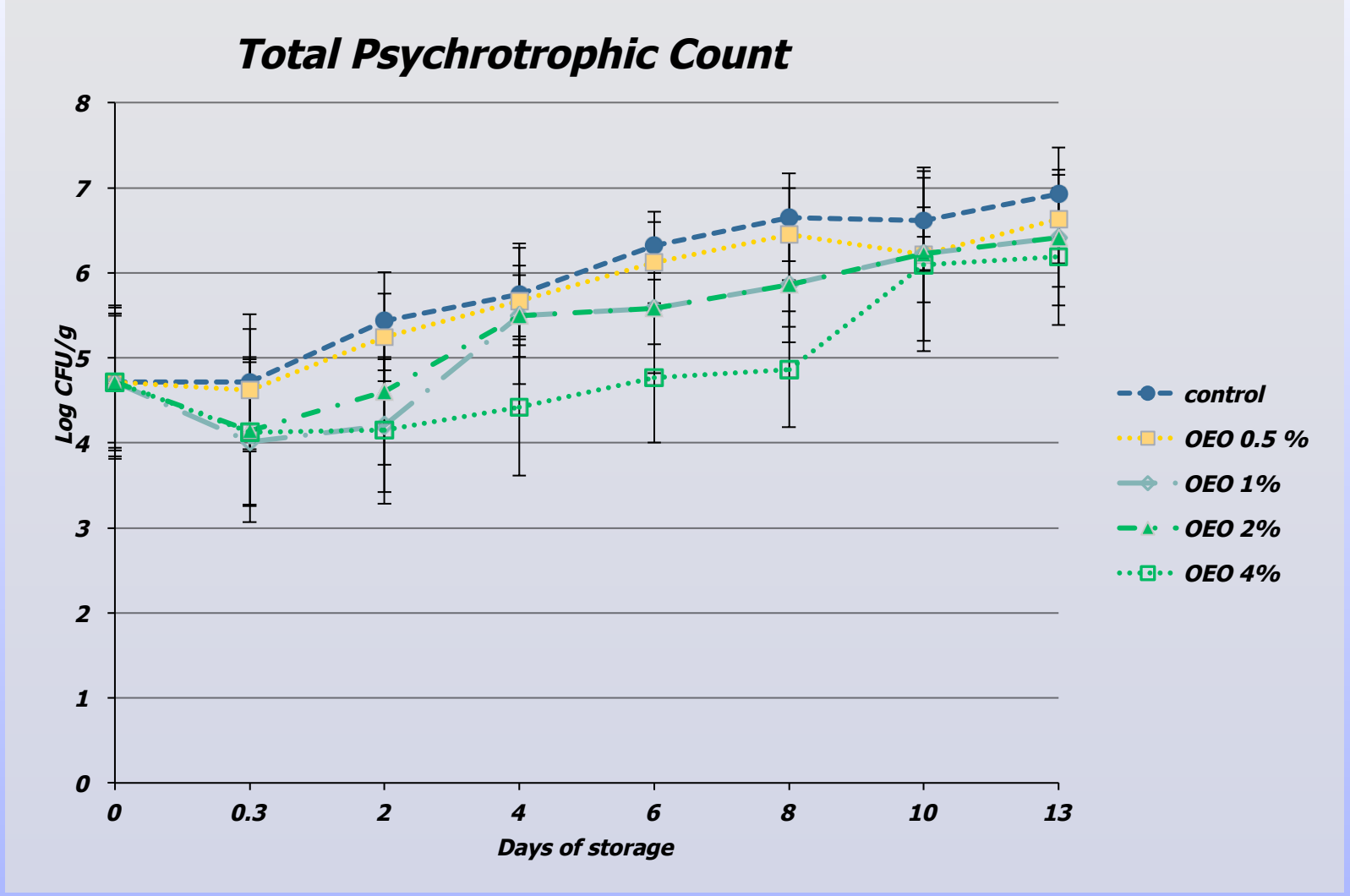
Fig. 1. UPGMA dendrogram of isolates and type strain based on Pearson's similarity coefficient of RAPD-PCR (primers M13 and OPA18) and rep-PCR profiles.

First of all, *Shewanella* genus has been reconsidered in the last decades, and our data support the need to deepen those studies and to define species-specific characteristics. In particular, until a few decades ago, all shewanellas producing hydrogen sulfide were identified as *S. putrefaciens*, even though some strains are not producers.

In conclusion, *Shewanella* spp. showed interesting physiological and metabolic traits *in vitro*, as it grew rapidly at 4 and 8 °C, produced TMA, H_2S and biogenic amines, and showed proteolytic activity also at low temperatures, therefore potentially being able to modify texture and sensory characteristics of finfish. Moreover, extracellular DNAse activity and the capability to grow also in presence of high salt concentrations may provide a competitive advantage and a greater adaptability, which can represent a limiting factor for the storage of both fresh and transformed fish products.

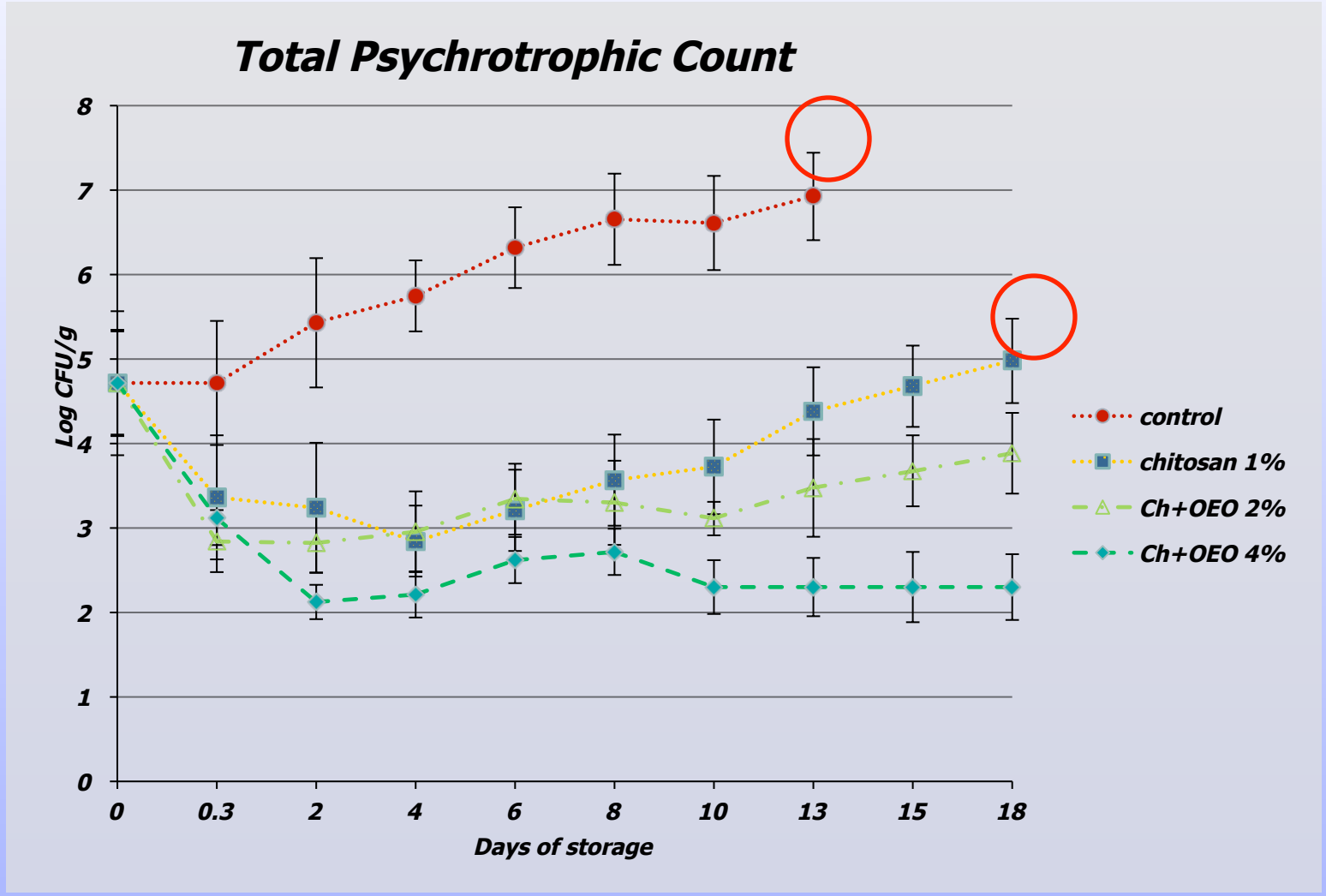
MULTIPLE-HURDLES STRATEGIES FOR SL EXTENSION IN MAP PORK AT 4°C: TOTAL PSYCHROTROPHIC COUNT

OE *Origanum vulgare*



MULTIPLE-HURDLES STRATEGIES FOR SL EXTENSION IN MAP PORK AT 4°C: TOTAL PSYCHROTROPHIC COUNT

OE *Origanum vulgare* + CHITOSAN 1%



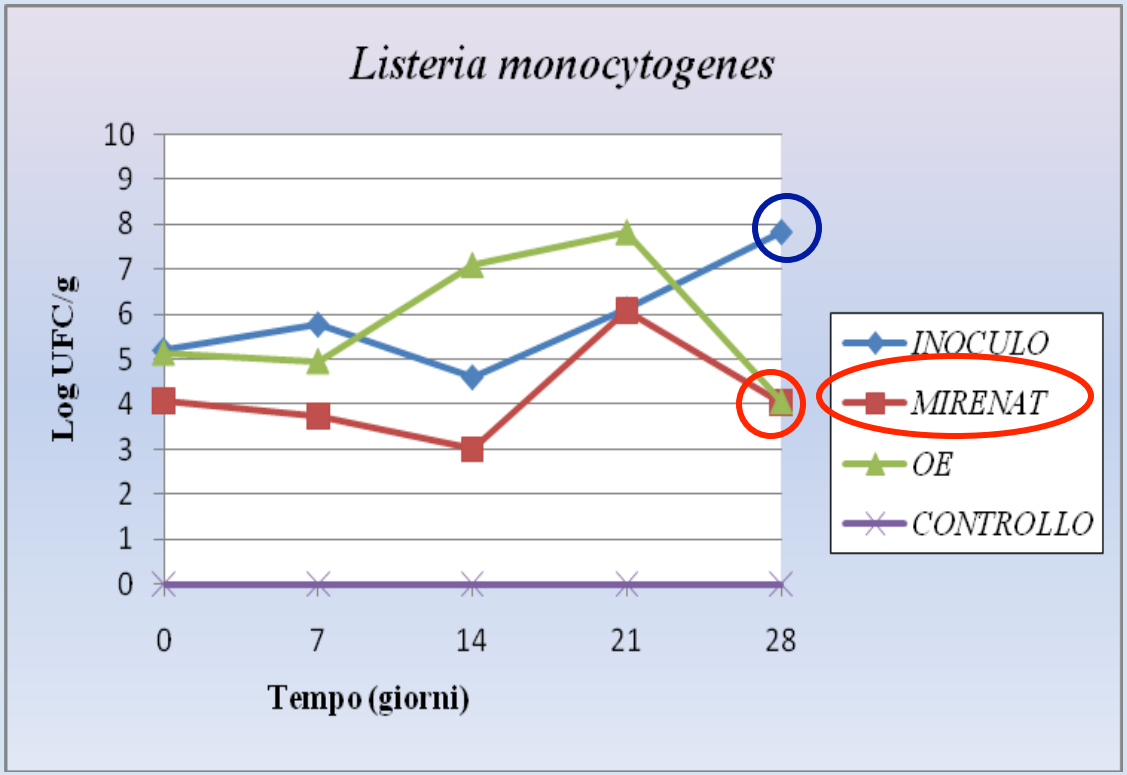
EMERGING

"GREEN" TECHNOLOGIES

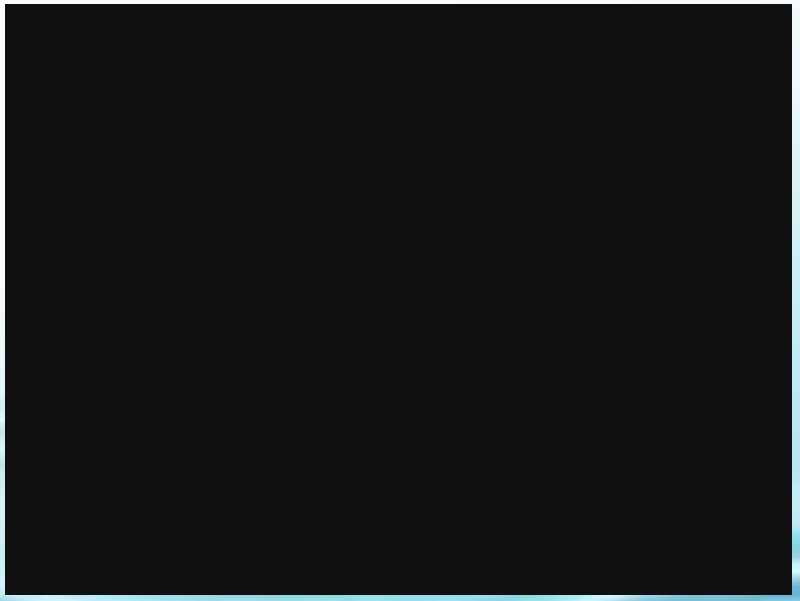
Surface decontamination:

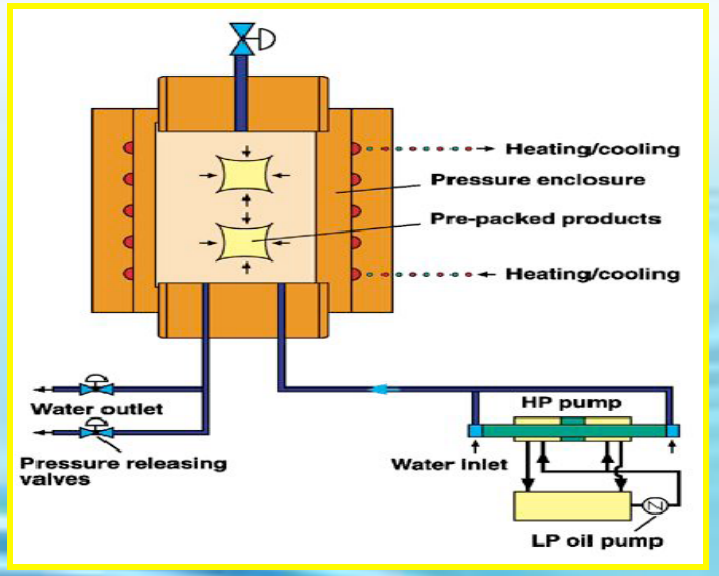
Effect of surface treatments with 4% oregano EO or 4% Mirenat-N (N -lauroyl-L-arginine ethyl ester) on *L. monocytogenes* ATCC 19114 inoculated on Caciotta cheese rind, at 10°C

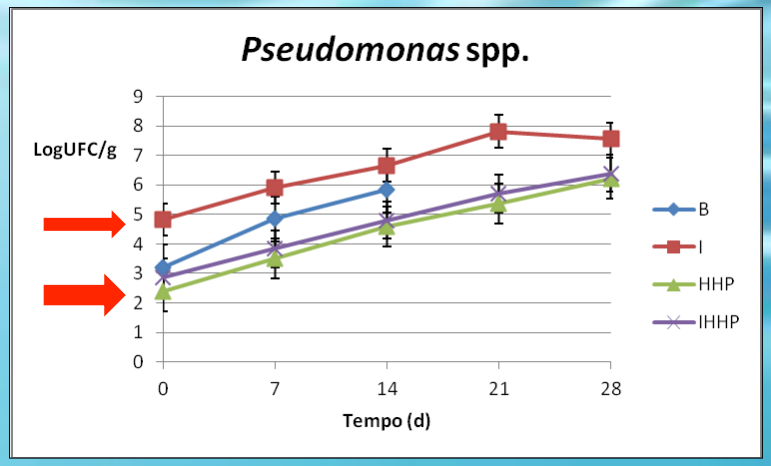
(Serio A., Chaves López C., Paparella A., 2012)



HIGH HYDROSTATIC PRESSURE (HHP)







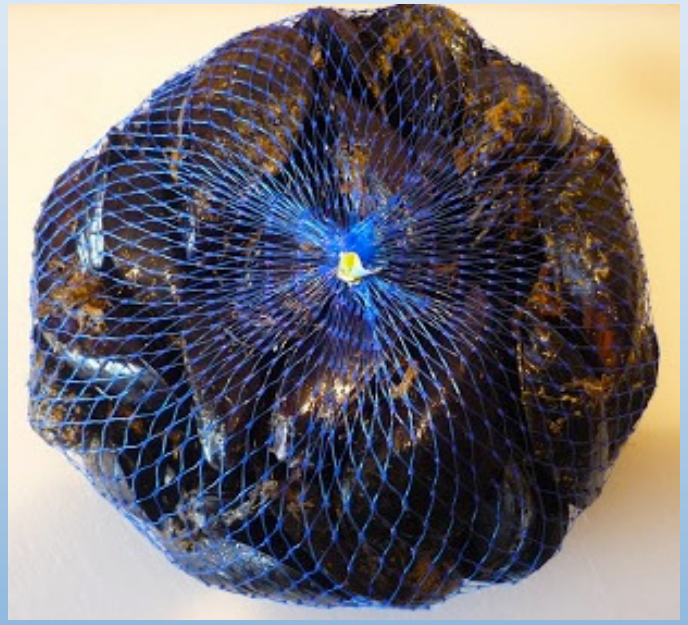
HHP TREATMENT: 600 MPa for 8 minutes

HYPOBARIC PACKAGING FOR LIVE MUSSELS

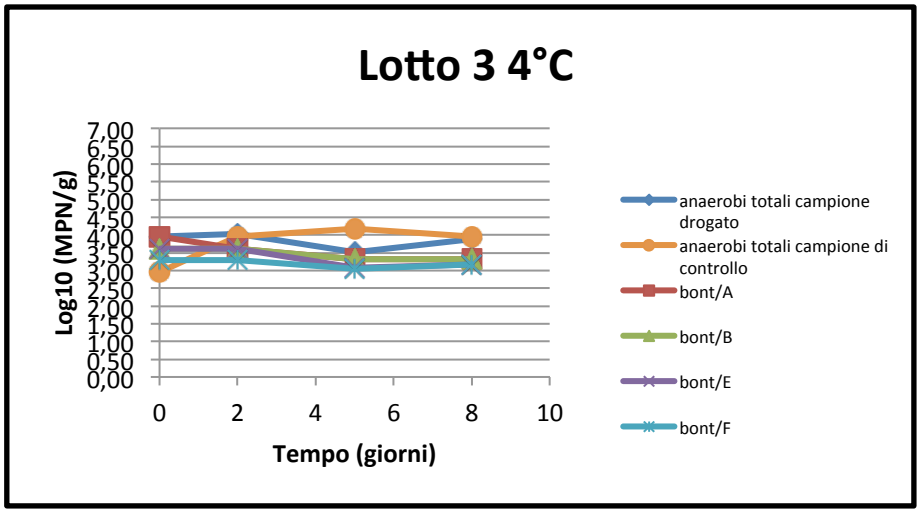
Microbial Challenge studies on *Clostridium botulinum* (cooperation with ISS)

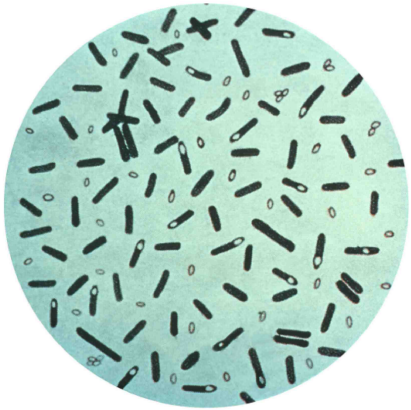
Development and validation

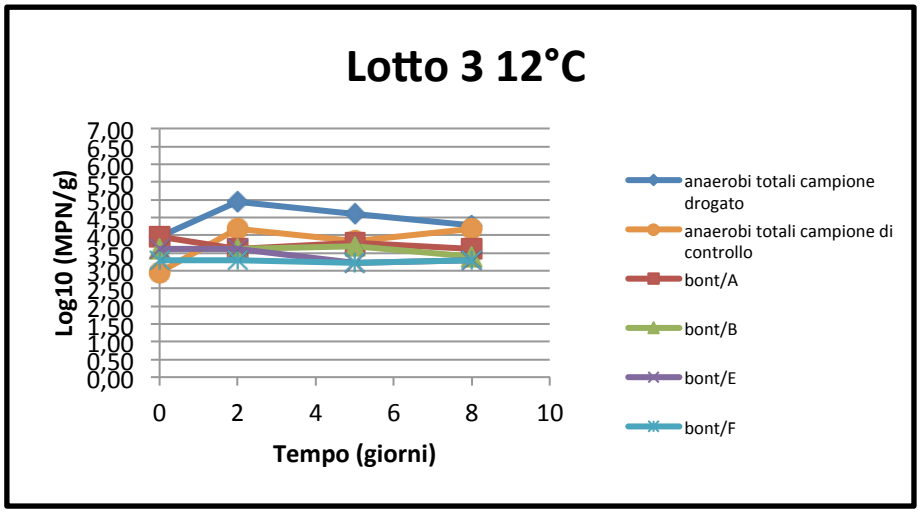
of new food products



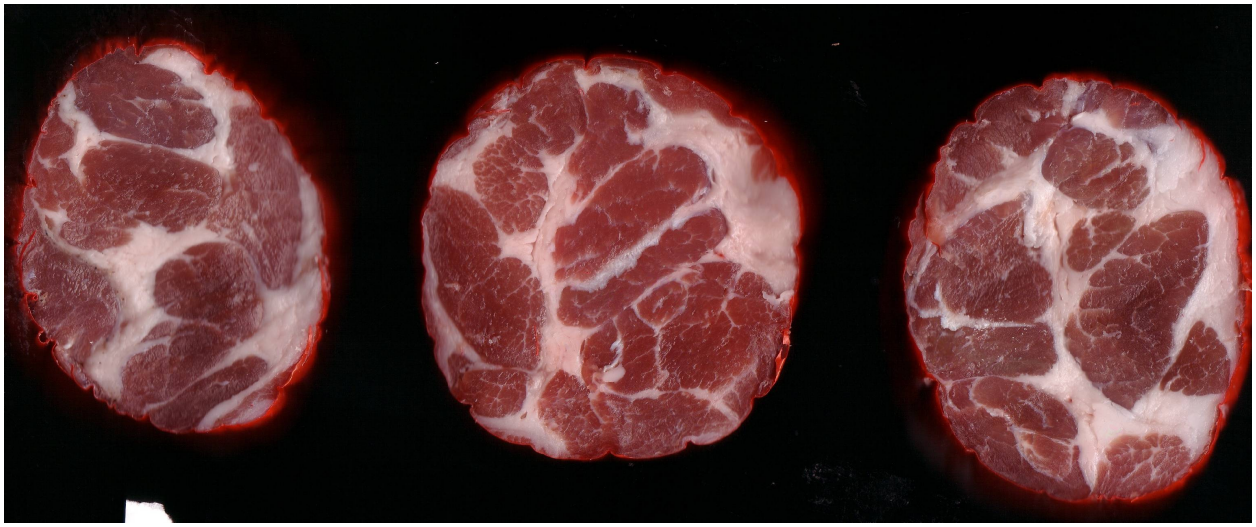
Challenge study in hypobaric packed mussels: *Cl. botulinum* inoculum (6 strains: 1 A + 2 B + 2 E + 1 F) MPN – Real-Time PCR







VACUUM IMPREGNATION FOR NITRITE-FREE MEATS



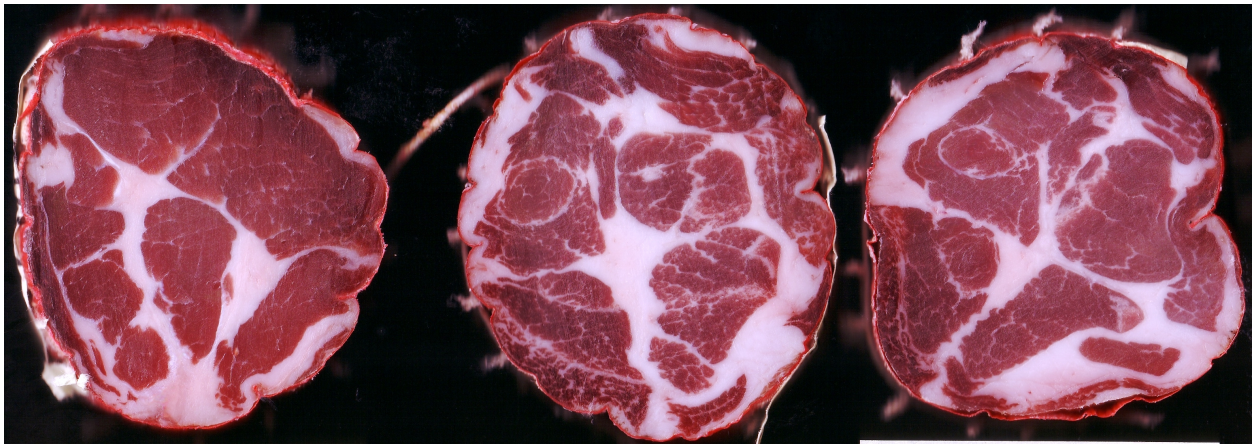
End drying

Dry curing (salt +nitrate)

Dry curing (salt + nitrate + lactate)

Brining under pulsed vacuum (only salt)

TECNOLONZA PROJECT (FIT Min. Att. Prod. 2007)



120 days curing

THANKS TO OUR GROUP

- Antonello PAPARELLA Professor
- Clemencia CHAVES LOPEZ Researcher
- Annalisa SERIO Researcher
- Chiara ROSSI Junior Researcher
- Serena D'AMATO Ph.D. Student
- Francesca MAGGIO Ph.D. Student

CONCLUSIONS

- EOs ARE EFFECTIVE HURDLES AGAINST BOTH PATHOGENS AND SPOILERS
- APPLICATION IN REAL SYSTEMS NEEDS STRATEGIES FOR DOSE REDUCTION
- GREEN TECHNOLOGIES ARE STRATEGIC OPTIONS FOR FOOD INNOVATION
- NEW IDEAS MAY DERIVE FROM OBSERVATION OF NATURE

Nihil ab rerum natura sine aliqua occultiora causa gigni

Pliny the Elder, *Naturalis Historia*, Book XXII (i)

Nothing is created by Nature without some more hidden reason


UNITE
UNIVERSITÀ
DEGLI STUDI
DI TERAMO
Thank you for your attention!
apaparella@unite.it
Facebook: Antonello Paparella Unite