PathogenCombat
for safe food

New protective and probiotic cultures to prevent and control foodborne pathogens along the food chain



Francesca Gaggìa, University of Bologna

Nancy Carlini, Probiotical SpA

Cork, 9 March 2009

PathogenCombat
for safe food

Introduction

Food safety is of fundamental importance to the European:

CONSUMER FOOD INDUSTRY ECONOMY




Despite significant investment in food safety, the incidence of foodborne disease is still on the rise in the EU.


PathogenCombat
for safe food

Food-borne diseases are caused by ***pathogenic microbes*** such as bacteria, viruses, and parasites, or their toxins present in contaminated foods.



The severity of these infections can vary from mild symptoms to life-threatening conditions.



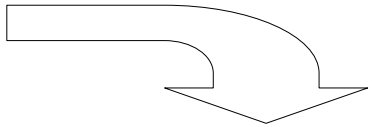
PathogenCombat
for safe food

Many microbes of this sort are commonly found in the intestines of *healthy food producing animals*.



FROM FARM...

CONTAMINATION ROUTES





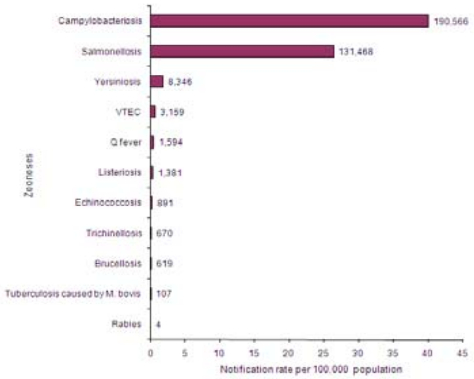

...TO FORK

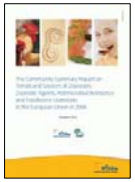
PathogenCombat

European Centre for Disease Prevention and Control

European Food Safety Authority

Reported notification zoonoses rates in confirmed human cases in the EU, 2008





d Quality and Safety

PathogenCombat for safe food

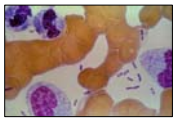
Common pathogens found in the food chain

Campylobacter jejuni

Salmonella enteritidis

Listeria monocytogenes

Escherichia coli



Yersinia enterocolitica

Food Quality and Safety

PathogenCombat

efsa
European Food Safety Authority

Table OUT4. Causative agents in food-borne outbreaks in the EU, 2008

Causative agent	2008				2007 [1]			
	N	%	Verified outbreaks (n)	Possible outbreaks (n)	N	%	Verified outbreaks (n)	Possible outbreaks (n)
Salmonella	1,888	35.4	490	1,398	2,253	39.3	517	1,736
Unknown	1,380	25.9	53	1,327	1,496	25.9	492	992
Viruses	697	13.1	38	659	675	11.8	104	571
Campylobacter	488	9.2	21	467	465	8.1	29	436
Bacterial toxins	525	9.8	159	366	464	8.1	411	53
Other causative agents	167	3.1	68	99	206	3.6	154	52
Escherichia coli, pathogenic	75	1.4	10	65	65	1.1	26	39
Parasites	70	1.3	38	32	58	1.0	35	23
Yersinia	22	0.4	2	20	20	0.3	2	20
Other bacterial agents	20	0.4	11	9	41	0.7	14	27
EU Total	5,332	100	890	4,442	5,733	100.0	1,784	3,949

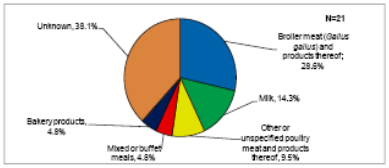
1. 2007 data has been updated in comparison to published data following recent communication received from a MS.

ecdc
European Centre for Disease Prevention and Control

PathogenCombat for safe food

Major sources of infection

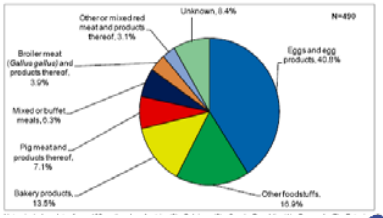
Figure OUT19. Distribution of implicated foodstuffs in verified Campylobacter outbreaks in the EU, 2008



Note: Includes data from 21 outbreaks: Austria (1), Belgium (1), Denmark (1), Finland (1), France (11), Germany (1), Netherlands (3), Poland (1), Spain (1).

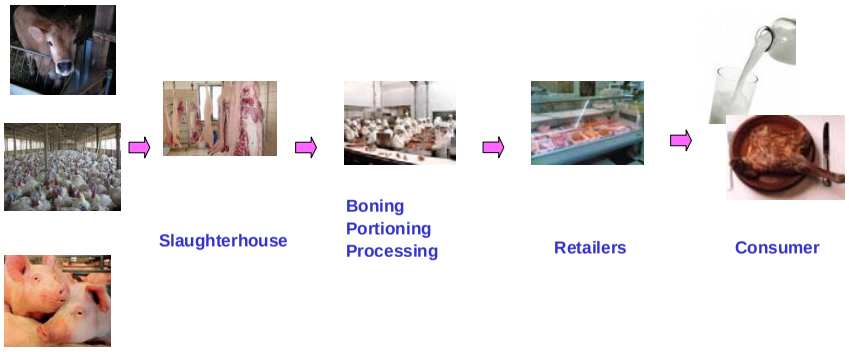
efsa
European Food Safety Authority

Figure OUT14. Distribution of implicated foodstuffs in verified outbreaks caused by Salmonella in the EU, 2008



ecdc
European Centre for Disease Prevention and Control

PathogenCombat
for safe food



Farm

Slaughterhouse

Boning
Portioning
Processing

Retailers

Consumer

CONTAMINATION ROUTE ALONG THE FOOD CHAIN

Food Quality and Safety

PathogenCombat
for safe food

Protective and probiotic cultures

A strategy to reduce foodborne pathogens

The use of **protective** and **probiotic cultures** may be a useful and effective strategy to prevent or reduce the incidence of pathogens in the food chain, improve hygiene, food safety and consumer health.

Cultures

Pathogens

Food Safety

Consumer Health

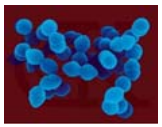
Food Quality and Safety

PROTECTIVE CULTURES:

Cultures added to food products to inhibit pathogens and/or prolong the shelf-life

Mainly Lactic Acid Bacteria

and/or their antibacterial metabolites



PathogenCombat
for safe food

Metabolic products of lactic acid bacteria with antimicrobial properties

Product	Main target organism
Organic acids - lactic acid - acetic acid	 - Putrefactive and Gram-negative bacteria, some fungi - Putrefactive bacteria, clostridia, some yeasts & fungi
Hydrogen peroxide	Pathogens and spoilage organisms, especially in protein-rich foods
Enzymes - lactoperoxidase system with H_2O_2 - lysozyme	 - Pathogens & spoilage bacteria (milk and dairy products) - Undesired Gram-positive bacteria
Low-molecular metabolites - reuterin (3-OH-propionaldehyde) - diacetyl - fatty acids	 - Wide spectrum of bacteria, moulds and yeasts - Gram-negative bacteria -Different bacteria
Bacteriocins -Nisin - Other	 Some LAB and Gram-positive bacteria, notably endospore-formers Gram-positive bacteria, inhibitory spectrum according to producer strain and bacteriocin type

PathogenCombat
for safe food

Protective culture applications in dairy products

- Fresh fermented product and Soft cheeses, smear type cheeses
  - Control of spoilage (yeasts-moulds) microorganisms and *Listeria*


Yoghurt


Mascarpone


Anthotyro


Brie

- Semi hard & hard cheeses
  - Control of undesirable spoilage bacteria


Cheddar


Gouda


Kasseri


Pecorino

PathogenCombat
for safe food

Protective culture applications in meat products

- Control of *Listeria, Campylobacter, Salmonella*

- Cured meat, dry or semi dry
- Cooked or ground meat


Beef jerky


Bresaola

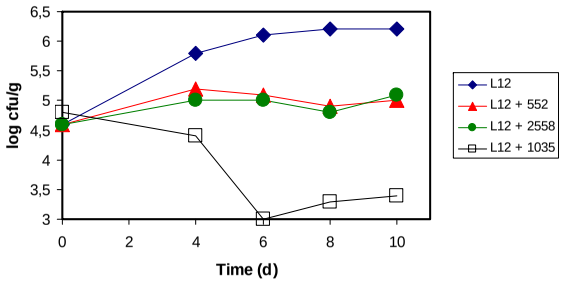

Semi dry sausages


Cured meats


Ground meat

Food Quality and Safety

PathogenCombat
for safe food



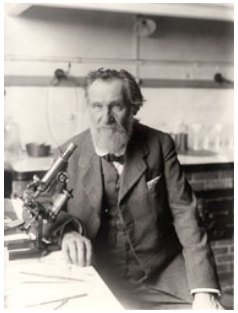
BFE 552: *Lactobacillus sakei* (non-bacteriocin producer)
BFE 2558: *Lactobacillus sakei* (non-bacteriocin producer)
BFE 1035: *Leuconostoc carnosum* (bacteriocin producer)

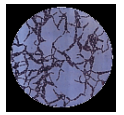
Influence of bacteriocin and non-bacteriocin producing lactic acid bacteria on *Listeria monocytogenes* L12 in a mild tuna salad (pH 5.5) at 10°C (Gores and Schillinger, 2004)

PathogenCombat
for safe food

PROBIOTIC CULTURES

"probiotics are living microorganisms which, upon ingestion in certain numbers, exert health effects beyond inherent basic nutrition."
(FAO/WHO, 2001)




Lactobacillus GG


Lb. acidophilus NCFM


Lb. casei Shirota

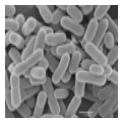

Bifidobacterium Bb12

Mainly:

- Lactic Acid Bacteria (LAB)
- *Bifidobacteria*

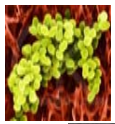
LAB and bifidobacteria are the best candidates for applications

- Have been used since the beginning of history as starter cultures
- Present in almost all fermented foods-vegetables, meat products, dairy products
- Are part of the natural microbiota of both farm animals and humans
- Have a long history of consumption and safe use

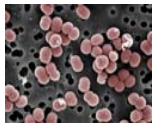


Lactobacillus

Bifidobacterium

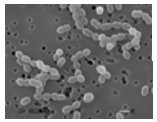


Lactococcus



Pediococcus

Streptococcus



Leuconostoc

Probiotic cultures: beneficial effects in human

- **Competition vs. pathogens**
- **Inflammatory bowel diseases**
- **Production of antimicrobial substances**
- **Alleviation of lactose intolerance**
- **Alleviation of viral / antibiotics associated diarrhoea**
- **Enhancement of immune system**
- **Anticarcinogenic activity**
- **Production of vitamins**

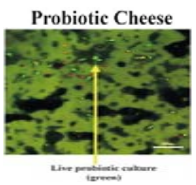
PathogenCombat for safe food

Probiotics & Food

The most developed probiotic containing foods are:

FERMENTED DAIRY PRODUCTS

- above all yogurts
- now → increase of probiotic cheese



Cheese matrix and its high fat content allow a **better resistance during gastric transit**

NEW non-dairy probiotic foods




- Baby foods
- Confectionery (chocolate bars, mousse..)
- Drinks (*e.g.* sugar beet juice)
- Cereal products
- Meat and Fish

PathogenCombat for safe food

Probiotic cultures at Primary Production

- Improved growth of farm animals
  - ❖Through control of subclinical gut infections, a similar mechanism to that of antibiotic growth promoters.
- Improved utilisation of food
  - ❖by increasing the efficiency of the existing digestive processes
  - ❖ promoting the digestion of previously indigestible substances.
- Reduced intestinal upsets
  - ❖Symptoms include scouring or diarrhoea, loss of appetite and poor digestion of food
- Improved health
  - ❖Generated by increasing resistance to infectious diseases either by direct antagonism or by stimulating immunity
- Establishment/Re-establishment of microbiota
  - ❖establishment of healthy gut microbiota in immature animals
  - ❖re-establishment of gut microbiota following antibiotic use

Food Quality and Safety

PathogenCombat
for safe food

Probiotics & Animals



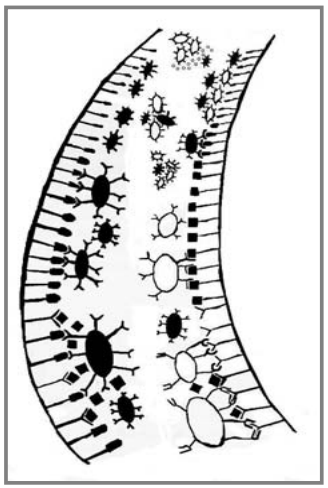
➢ **Increased use of probiotics in animal feeding**

- *Reduction of pathogens in the food chains*
- *Contribution on the production of organic meat*

Food Quality and Safety

PathogenCombat
for safe food

THE GUT AS A BACTERIAL HABITAT



Maxwell and Stewart, 1995

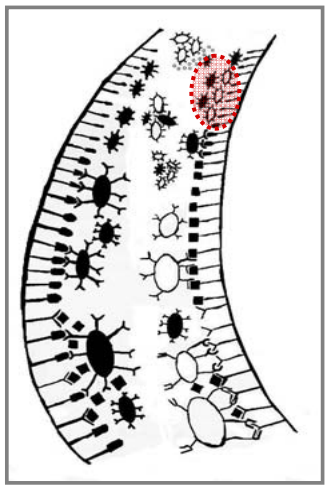
Modulation of the gut microbiota

SCHEMATIC REPRESENTATION OF THE ADHESION OF PATHOGENS (BLACK OVAL) AND THEIR ENTEROTOXINS (BLACK SQUARE) TO GUT RECEPTORS AND SOME MECHANISMS BY WHICH PROBIOTIC BACTERIA (WHITE OVAL) MAY DECREASE PATHOGENITY

Food Quality and Safety

PathogenCombat
for safe food

THE GUT AS A BACTERIAL HABITAT



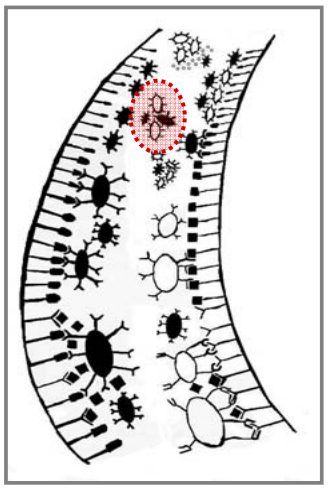
COMPETITIVE ESCLUSION
Adhesin-mediated attachment
of probiotics excludes pathogens

Maxwell and Stewart, 1995

Food Quality and Safety

PathogenCombat
for safe food

THE GUT AS A BACTERIAL HABITAT



COMPETITIVE ESCLUSION
Adhesin-mediated attachment
of probiotics excludes pathogens

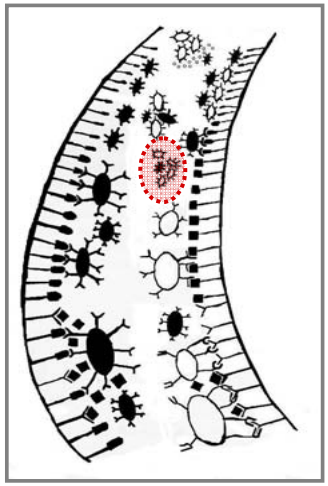
AGGREGATION
Probiotics aggregate with pathogens,
leading to expulsion from the gut

Maxwell and Stewart, 1995

Food Quality and Safety

PathogenCombat
for safe food

THE GUT AS A BACTERIAL HABITAT



Maxwell and Stewart, 1995

COMPETITIVE ESCLUSION
Adhesin-mediated attachment
of probiotics excludes pathogens

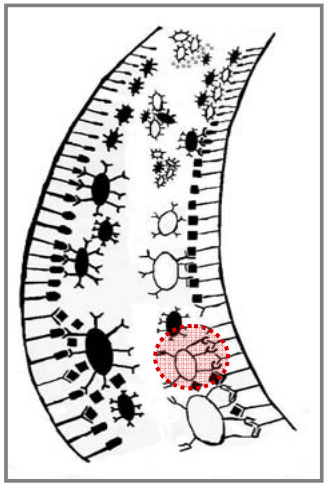
AGGREGATION
Probiotics aggregate with pathogens,
leading to expulsion from the gut

NUTRIENT COMPETITION
Probiotics compete with pathogens
for essential nutrients

Food Quality and Safety

PathogenCombat
for safe food

THE GUT AS A BACTERIAL HABITAT



Maxwell and Stewart, 1995

COMPETITIVE ESCLUSION
Adhesin-mediated attachment
of probiotics excludes pathogens

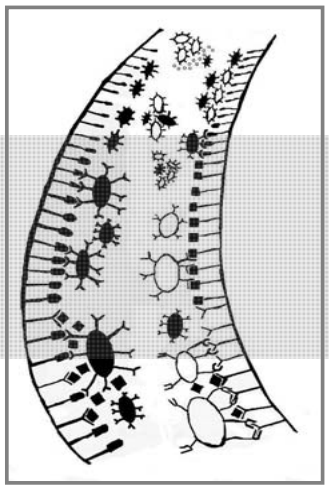
AGGREGATION
Probiotics aggregate with pathogens,
leading to expulsion from the gut

NUTRIENT COMPETITION
Probiotics compete with pathogens
for essential nutrients

MASKING
Masking of intestinal receptors for
enterotoxins by probiotic adhesine

PathogenCombat
for safe food

THE GUT AS A BACTERIAL HABITAT



Maxwell and Stewart, 1995

COMPETITIVE ESCLUSION
Adhesin-mediated attachment
of probiotics excludes pathogens

BARRIER EFFECT

AGGREGATION
Probiotics aggregate with pathogens,
leading to exclusion from the gut

NUTRIENT COMPETITION
Probiotics compete with pathogens
for essential nutrients

MASKING
Masking of intestinal receptors for
enterotoxins by probiotic bacteria

PathogenCombat
for safe food

RTD III: CONTROL and PREVENTION
WP10 - Protective & probiotic cultures

The team

- Federal Research Institute of Nutrition and Food (MRI-Germany) Partner 5
- DANISCO (Denmark) Partner 6
- Agricultural University of Athens (AUA-Greece) Partner 18
- University of Bologna (UNIBO-Italy) Partner 19

MRI Max Rubner-Institut

DANISCO
First you add knowledge...

Γεωπονικό Πανεπιστήμιο Αθηνών
Agricultural University of Athens

UNIVERSITÀ DI BOLOGNA A.D.1088

Food Quality and Safety

PathogenCombat for safe food

RTD III: CONTROL and PREVENTION

WP10 - Protective & probiotic cultures

Building up a collection of LAB and Bifidobacteria for application in many stages of the food production chain

- Inhibition of a variety of pathogens common in the food industry
- Ability to survive under food processing conditions or gastric system of animals or humans
- Safety for consumption by farm animals or humans

Strains with this potential can then be considered as **protective** and **probiotic cultures**, e.g. multifunctional cultures to be incorporated in the development of **prevention strategies** for foodborne pathogens throughout the **food chain**

PathogenCombat for safe food

Task 10.1. Screening for antimicrobial activity

Stage 1- Screening against non-pathogen strains

- **H**igh **T**hroughput **S**creening Technology

847 strains

- 94 Enterococci
- 505 LAB
- 212 Bifidobacteria
- 37 Unidentified isolates

→

- *L. innocua*
- *E. coli*
- *S. cerevisiae*

↓

Selected 92 strains

Stage 2 – Screening against pathogens

- Well diffusion & spot on lawn assays

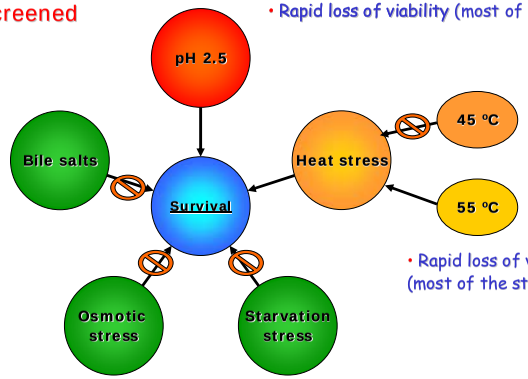
- *L. monocytogenes & M. avium subsp. paratuberculosis*
- *E. coli & C. jejuni*
- *P. nordicum & S. cerevisiae*

PathogenCombat
for safe food

Task 10.2: Survival ability in GI & Food processing conditions

• 70 strains screened

• Rapid loss of viability (most of the strains)



• Rapid loss of viability (most of the strains)

PathogenCombat
for safe food

Task 10.3 Identification & determination of antibiotic resistance

✓ *Identification*

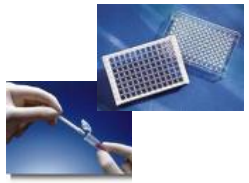
• Fermentation pattern

• Species-specific PCR
• 16s rRNA sequencing

• DNA-DNA hybridisation

✓ *Measurement of the Minimal Inhibitory Concentration*

- TETRACYCLINE
- TRIMETHOPRIM
- CEFUROXIME
- KANAMYCIN
- CHLORAMPHENICOL
- VANCOMYCIN
- AMPYCILLIN
- STREPTOMYCIN
- ERYTHROMYCIN

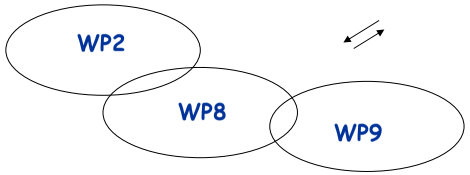


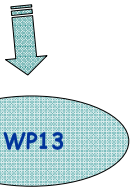
PathogenCombat
for safe food

WP10

Collection of 23
selected strains
as best candidates for applications

Lactobacillus spp.
Pediococcus spp.
Enterococcus spp.
Leuconostoc spp.
Bifidobacterium spp.





Additional studies:

- Functional genomic
- Matrix interactions
- Pathogen interactions
- Host-interaction

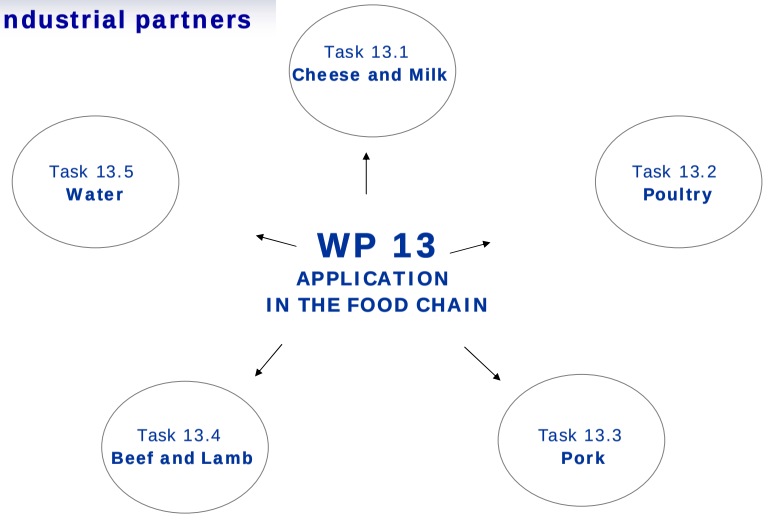
} functional cell lines

- **Application in the food chain**

Food Quality and Safety

- ✓ **25 Partners**
- **10 SMEs**
- **2 Industrial partners**

PathogenCombat
for safe food



Food Quality and Safety

PathogenCombat
for safe food

FOOD PROCESSING COMPANIES

CHEESE and MILK

p. 27 GRANAROLO-I
P. 28 PITTAS-Cyprus
P. 31 BERGPRAGHT-D

POULTRY

P. 29 CAGB-E
P. 33 ZIEGLER-D

BEEF AND LAMB

P. 46 COLEAR-E
P. 47 MARLOSA-E

PORK

P. 29 CAGB-E
P. 34 JAMSA-E

PathogenCombat
for safe food

TRANSFER OF KNOWLEDGES

- Culture independent techniques for pathogen detection in food and water
- Protective and probiotic cultures in feed and food produced at industrial level
- Predictive models to improve food safety
- New cleaning and disinfection methods
- Novel processing technologies to reduce pathogens
- Good hygienic practice at farm level
- Development and implementation of the Food Safety Management System

WP 13

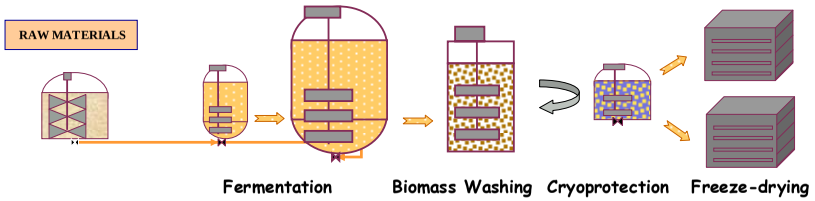
Food Quality and Safety

PathogenCombat
for safe food

Industrial production of 5 promising protective and probiotic strains

- *Lactobacillus plantarum* PCA 236
- *Bifidobacterium longum* PCB 133
- *Lactobacillus plantarum* PCS 20
- *Leuconostoc pseudomesenteroides* PCK 18
- *Lactobacillus pentosus* PCD 101

PROBIOTICAL
MOFIN ALCE GROUP



Food Quality and Safety

PathogenCombat
for safe food

MRI
Max Rubner-Institut

Bergprachi Milchwerk

Application of Protective cultures in cheese ripening

- The experiments included the inoculation of the unripened Camembert cheeses with *Listeria monocytogenes* and protective cultures.

A reduction on *Listeria* growth when a nisin-producing protective culture was inoculated on the "green" Camembert cheese.

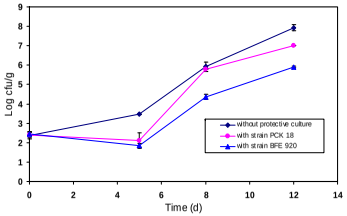


Figure 1. Comparison of the effect of two protective cultures

Food Quality and Safety

Application of probiotics in Sheep Milk Yogurt

1. **Industrial feasibility of probiotic yogurt with *L. plantarum* PCA 236 was validated**
2. **Scale - up of the production process was verified by semi-industrial production trials at the SME for PCA 236**
3. **Analysis confirmed very good stability of the PCA 236 strain after 40 days in refrigerated condition**

ACHIEVEMENT

New probiotic sheep milk yogurt with potential beneficial effects and improved protection against pathogens.

- Organoleptic characteristics of the existing sheep yogurt were maintained

PathogenCombat
for safe food

Application of probiotics in animal feed trials

- **APPLICATION in GOATS**
- **APPLICATION in POULTRY**
- **APPLICATION in TURKEYS** in Progress

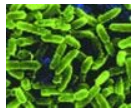
PathogenCombat for safe food

GOATS FEEDING TRIALS

PROBIOTICAL MOFIN ALCE GROUP

Pittas

Γεωπονικό Πανεπιστήμιο Αθηνών
Agricultural University of Athens



L. plantarum PCA 236
[lyophilised powder]

- Standard chemical analysis
- Standard microbiological analysis
  - Probiotic recovery
  - General fecal flora
- Milk quality/quantity

Food Quality and Safety

PathogenCombat for safe food

ACHIEVEMENTS

No adverse effects observed due to probiotic administration
- –No effect on appetite, movement.
- –Animals calm, no sign of any negative effect
- –Lively behavior, shiny hair

Microbiological analysis of feces:

- **Detection of the probiotic *L. plantarum* PCA236**
- **Statistically significant increase in total LAB**
- **Decrease of fecal clostridia counts**

MILK in probiotic group:
- Increased milk production (15-20%)
- Significant increase in polyunsaturated fatty acids

Food Quality and Safety

PathogenCombat for safe food

ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA

PROBIOTICAL MOFIN ALCE GROUP

POULTRY FEEDING TRIALS

1. Probiotics
   - ❖ Trials with 2 best strains active against *C. jejuni*
2. Prebiotics
   - ❖ Trials with 2 prebiotic compounds: FOS and GOS

3. SYNBIOTIC TRIAL

- ❖ ***B. longum* PCB 133 produced by *Probiotical S.p.A*, microencapsulated,**
- ❖ **GALACTO-OLIGOSACCHARIDE [30gr/Kg of feed]**

Detection in fecal samples of C. jejuni and probiotics by RTi –PCR and traditional methods

Food Quality and Safety

PathogenCombat for safe food

In vivo study workflow

1. Probiotics
2. Prebiotics
3. Synbiotic

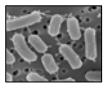
Administration by feed for 15 days

↓

Fecal samples: T_0-T_{15}-T_{20}

↓

Microbiota analysis



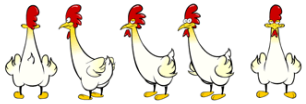
Culture-dependent techniques → to assess microorganisms viability in faeces

Culture-independent technique → DNA extraction → Molecular analysis → **Real Time PCR**

Food Quality and Safety

PathogenCombat
for safe food

ACHIEVEMENTS



- ✓ Colonization of Intestinal Tract of Poultry by *B. longum* PCB 133
  - → PROBIOTIC EFFECT
- ✓ Significant Increase level of Bifidobacterium spp.
  - → PREBIOTIC EFFECT
- ✓ Significant decrease level of C. jejuni
  - → COMPETITIVE EFFECT
- ✓ Production of a **PATENT** for the new synbiotic formula (in progress)

PathogenCombat
for safe food

Contact:

WWW.PATHOGENCOMBAT.COM