4PROTECTION

Special Protective Cultures

A COMPANY OF SACCO SYSTEM

SACCO

SACCO system

4PROTECTION
Special Protective Cultures

NATURAL CARE

INDEX

4PROTECTION, THE NATURAL GUARD FOR YOUR PRODUCT IDENTITY

INTRODUCTION

No additives, no preservatives, 100% natural are the most prevalent trends that also guide the choices of consumers; safety and durability and high quality standard level of foods is as important as ever.
Sacco has the right ingredients for the success of your products and the satisfaction of your customers.

4Protection Special Cultures help to enhance the quality and protect your brand image, allow the product to get to the end of shelf life ensuring a structural and sensorial stability, help to maintain freshness and do not change the taste, aroma and texture.
Your ally for a much more genuine product till the consumer table.

Many of the selected strains used were chosen among probiotic microorganisms which has been studied and shown to be effective through specific studies, microbiological tests and sensorial analysis of the products.

NATURAL CARE

WHAT IS 4PROTECTION LINE AND WHY USE IT

Since 1998 Sacco has selected yeasts and bacteria for protection against spoiling unwanted microorganisms in dairy products such as yogurt, fermented milk, fresh cheese, semi-hard cheese, meat and fish. The cultures of 4Protection Lines help to control and preserve the final product from alterations, fighting in a completely natural way any possible unwished bacteria and thereby maintaining a "**clean label**" product.

HOW 4PROTECTION LINE WORKS

Today it is known that microorganisms produce a diverse range of microbial defense molecules including exotoxins, lytic agents, metabolic by-products and bacteriocins (from EFFCA position PFC-2016).
The process is based on a competitive effect for space against microorganisms in general, including pathogens, on the production of anti-microbial metabolites such as organic acids and peptides with specific mode-of-action.

The selected 4Protection ferments do not acidify, nor alter the organoleptic characteristics of the product and are easily adapted even at refrigeration temperatures.

The different applications are studied as a function of the characteristics of the technological process and of the desired performance of the products. Sacco's technologists are committed to working alongside our customers to find the best solutions and production process, working together with clients offering a product and a customized service.
4Protection line is compatible and complementary to all the Sacco's starter cultures, they are used by direct inoculation or surface treatment.
Sacco is glad to help customers in finding the best solutions for their specific purpose, according with the characteristics of the products, the technological process and the activity needed from the use of our protective cultures.

4PROTECTION LINE FOR DAIRY

Sacco has 4 lines of products dedicated to the protection of dairy:

Anti indigenous yeasts and moulds	**AYM**
Anti Listeria monocytogenes	**AL**
Anti Clostridia	**AC**
Anti Other Spoilage Microorganisms	**AOSM**

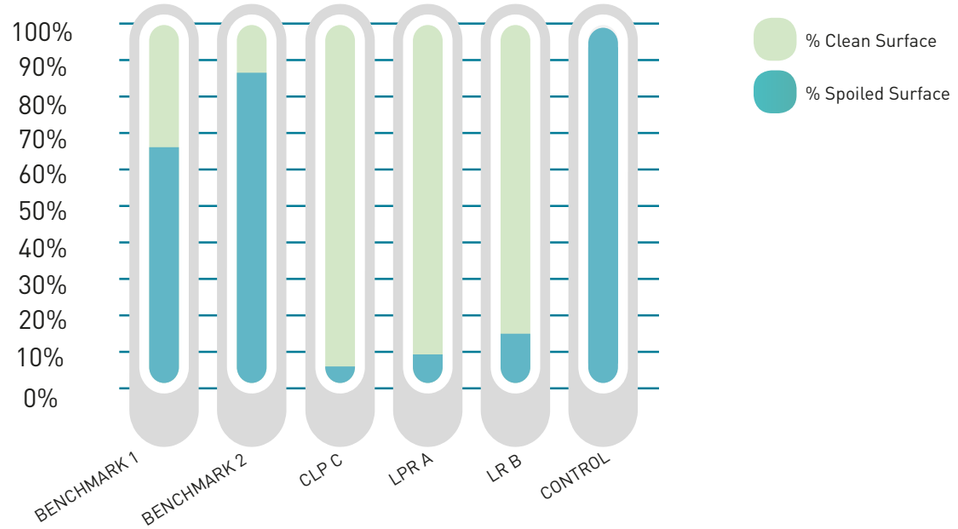
The 4Protection Line helps to improve the products quality and the brand image, reducing non-compliant products, food waste and therefore business costs.

AYM
ANTI YEAST
AND MOULDS

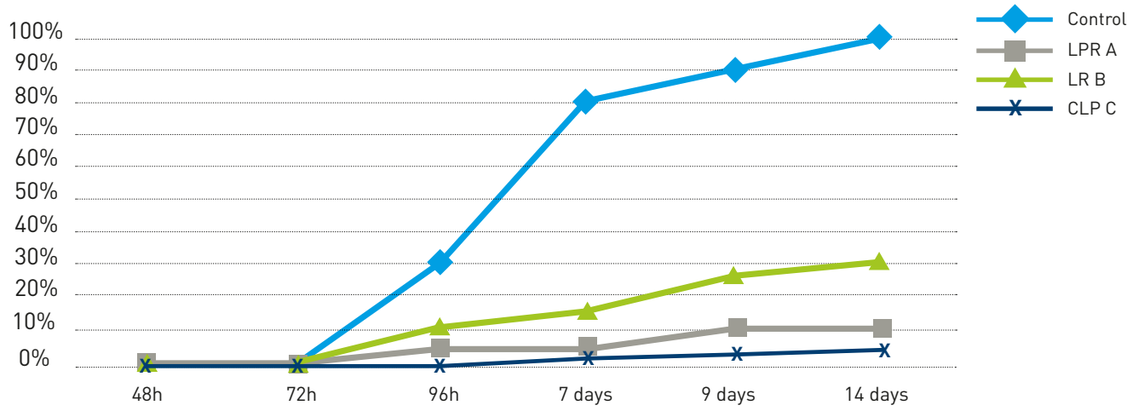
4Protection AYM allows products to reach the end of their shelf life, ensuring structural and sensorial stability, helps to maintain their freshness and does not change their taste, aroma and texture.

Product	Applications
LPR A	Yogurt, fresh fermented products, fresh cheese, soft cheese, semi hard cheese and hard cheese
LR B	Yogurt, fresh fermented products, fresh cheese, soft cheese, semi hard cheese and hard cheese
CLP C	Fresh fermented products, fresh cheese, soft cheese, semi hard and hard cheese

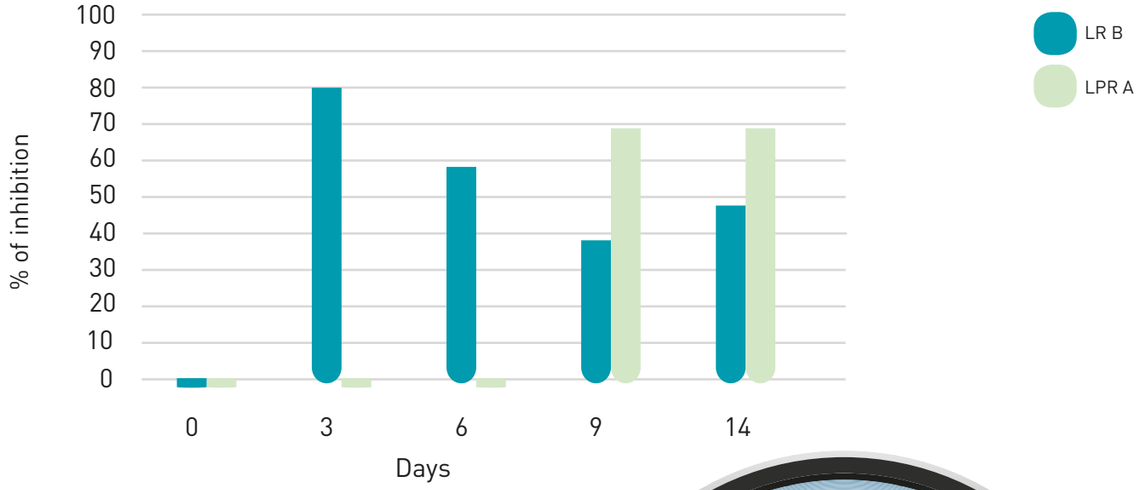
Yogurt surface spoiled by moulds after 6 days at 5°C



Spoiled yogurt surface percentage during time



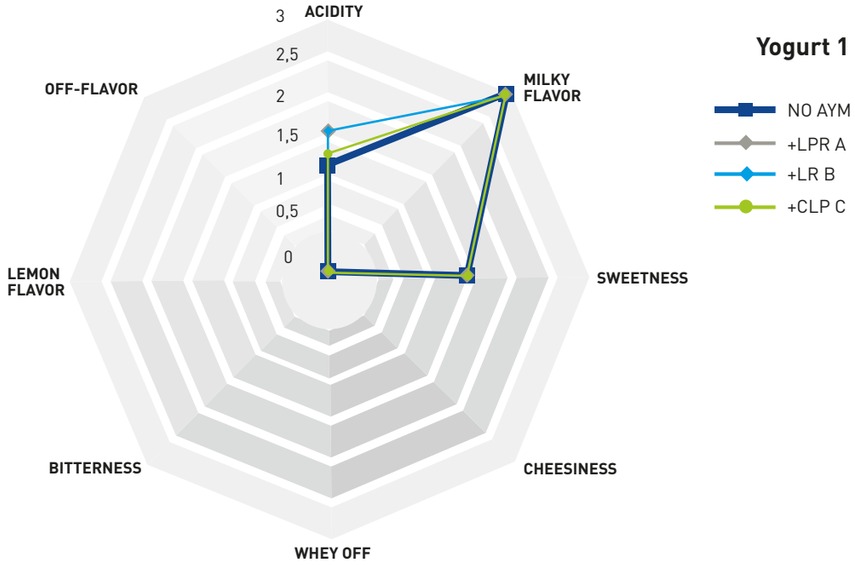
Yeast inhibition capability

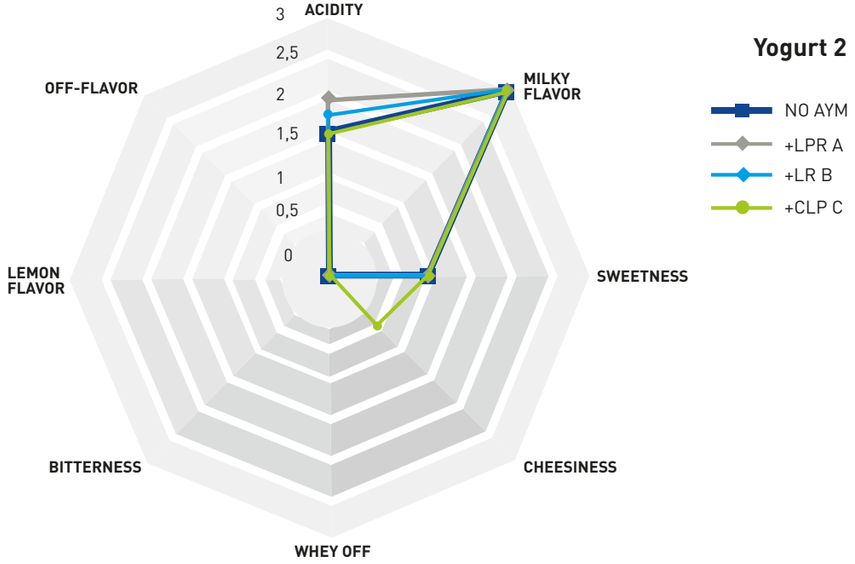


Inhibition capability of AYM cultures compared to the control

Challenge test evidences an immediate strong inhibition effect of our LR B while LPR A shows an increasing inhibition effect during time

Sensory evaluation



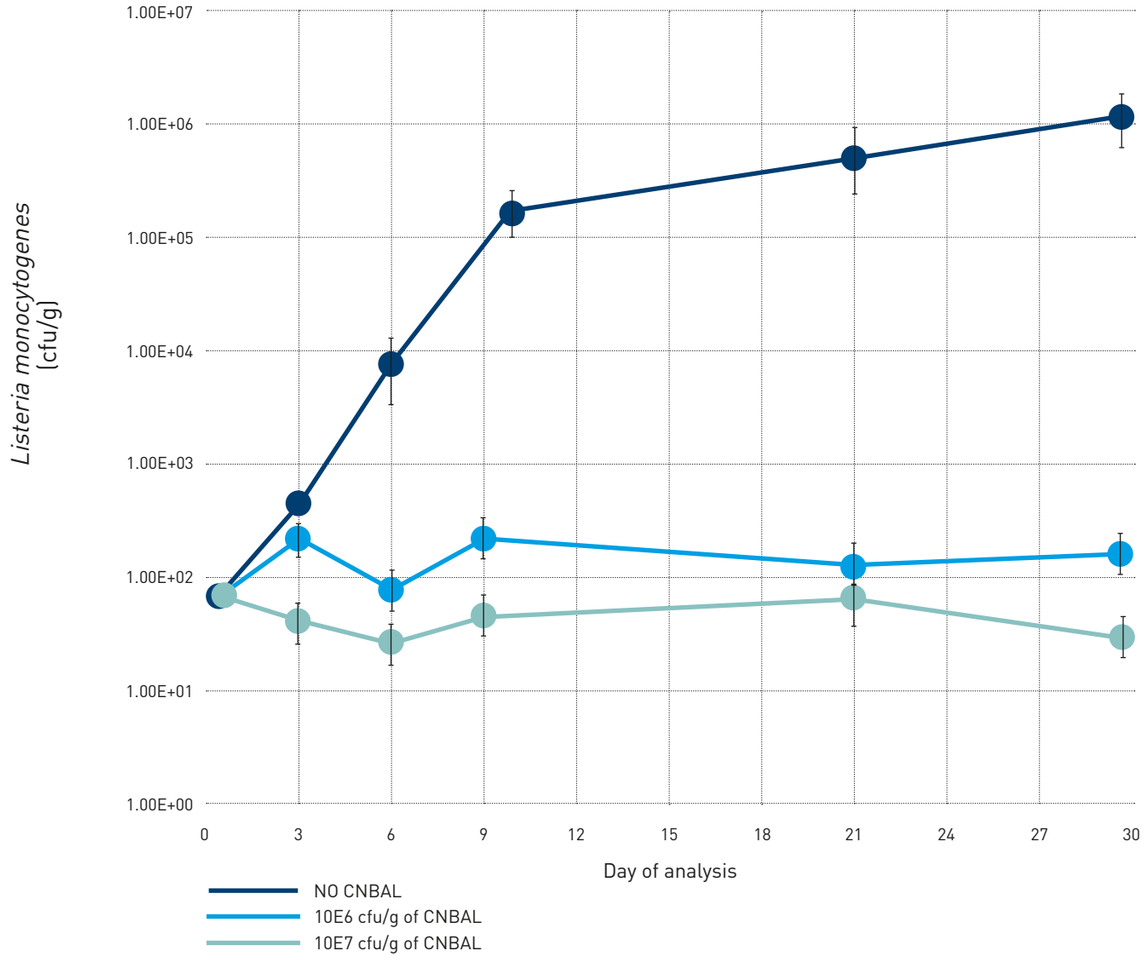


AL – Anti *Listeria monocytogenes*

4Protection AL reduces the growth of *Listeria monocytogenes*, increasing the safety of the product throughout its shelf life.

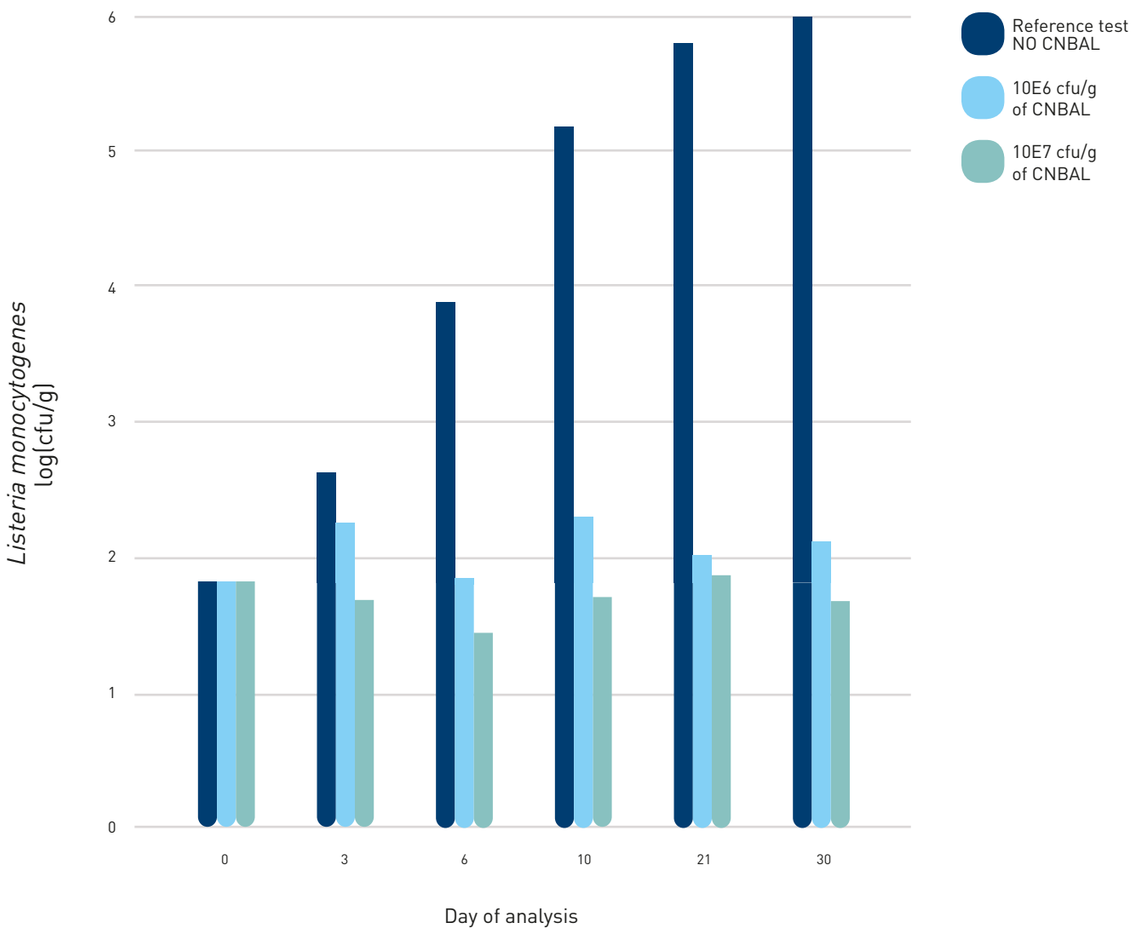
Product	Applications
LPAL	Soft cheese
CNBAL	Cheese ripened at low temperature and without sugar, like semi hard and hard cheese, gorgonzola, blue cheese

Counts of *Listeria monocytogenes* in cheese



Counts of *Listeria monocytogenes* in cheese. Day "0" is the day of inoculation with *L. monocytogenes*. The values given are averages of duplicate sampling of three batches. Light blue line indicate low dosage of protective culture 10E6 cfu/g and light green line indicate high dosage 10E7 cfu/g. The culture CNBAL inhibits the growth of *L. monocytogenes.*
The higher concentration of the culture, the better inhibition

Log10 growth of *Listeria monocytogenes* in samples of cheese



Counts of *Listeria monocytogenes*, given as log(cfu/g), in cheese. Day "0" is the day of inoculation with *L. monocytogenes*.
The values given are averages of duplicate sampling of three batches

Articles and studies:

- **Evidence on inhibition of *Listeria monocytogenes* by divercin V41 action**- Richard, Brillet, Pilet, Prévost, Drider (Letters in Applied Microbiology 2003)

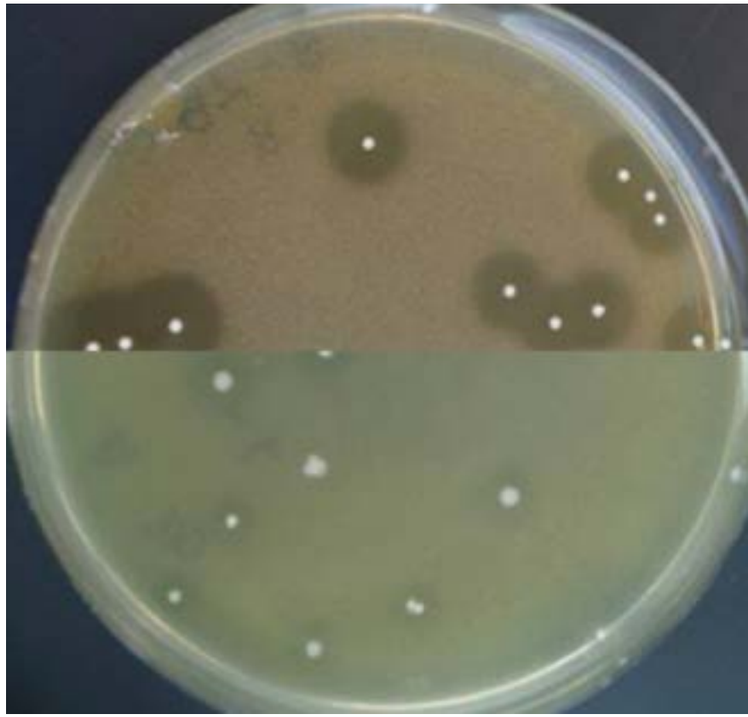
- **Triton X-114 phase partitioning for the isolation of a pediocin-like bacteriocin from *Carnobacterium* divergens** – Métivier, Boyaval, Duffes, Dousset, Compoint, Marion (Letters in Applied Microbiology 2000)

- **Delineation of key amino acid side chains and peptide domains for antimicrobial properties of divercin V41, a pediocin-like bacteriocin secreted by *Carnobacterium* divergens V41** – Bhugaloo-Vial, Douliez, Mollé, Dousset, Boyaval, Marion (Applied and Environmental Microbiology, 1999)

- **Enumeration of *Carnobacterium divergens* V41, *Carnobacterium piscicola* V1 and *Lactobacillus brevis* LB62 by in situ hybridization-flow cytometry** – Connil, Dousset, Onno, Pilet, Breuil, Montel (Letters in Applied Microbiology 1998)

- **Divercin V41, a new bacteriocin with two disulphide bonds produced by *Carnobacterium divergens* V41: primary structure and genomic organization** – Métivier Pilet, Dousset, Sorokine, Anglade Zagorec, Piard, Marion, Cenatiempo, Fremaux (Microbiology 1998)

- **Purification and Amino Acid Sequences of Piscicocins V1a and V1b, two class IIa Bacteriocins Secreted by *Carnobacterium piscicola* V1 that display significantly different levels of specific inhibitory activity** – Bhugaloo-Vial, Dousset, Metivier, Sorokine, Anglade, Boyaval, Marion (Applied and Environmental Microbiology, 1996)



Evidence of bacteriocine production - (Halo size)

For a better understanding of the articles, the strains V41 and SF668 are present in CNBAL product

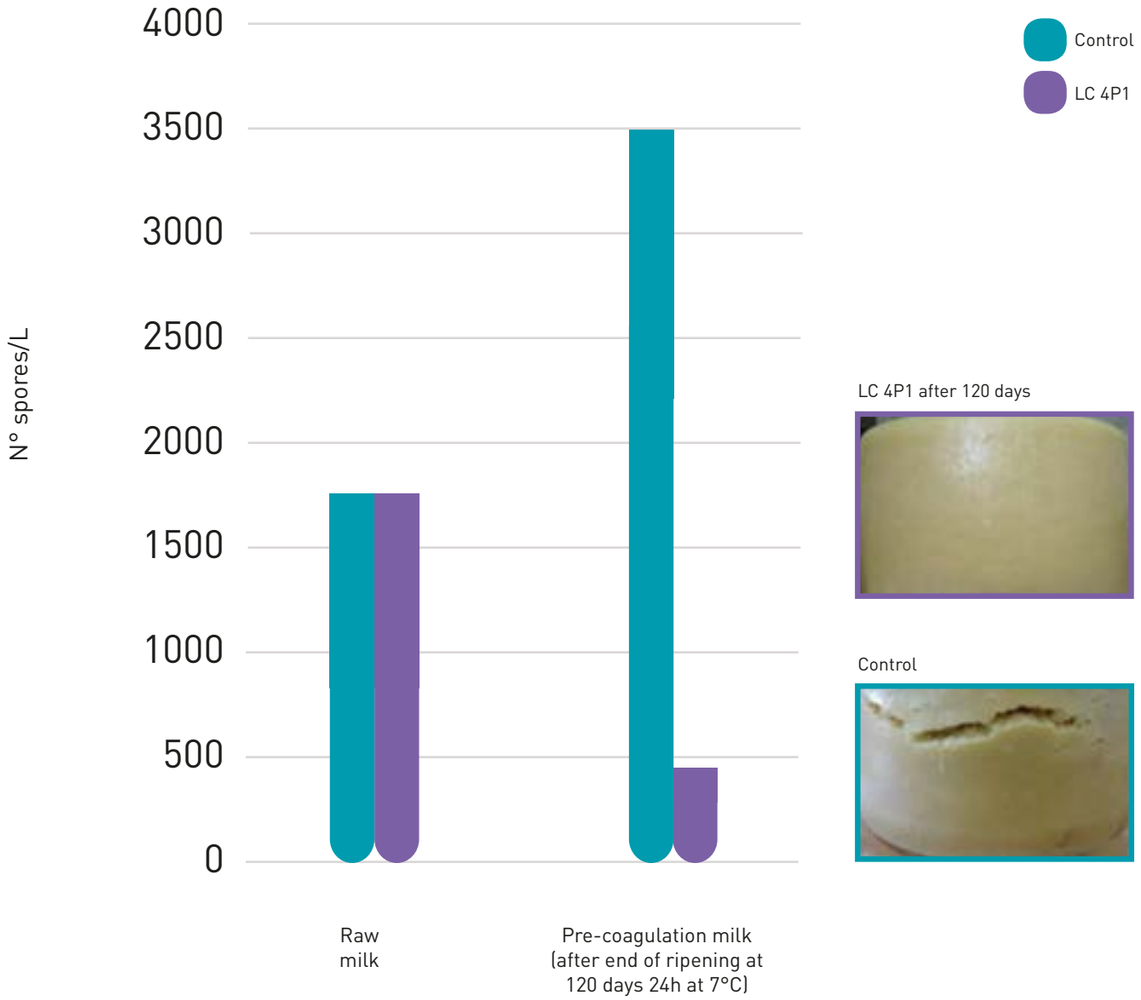
ABSTRACT

AC – Anti Clostridia

4Protection AC acts on Clostridia avoiding the altered aroma, unpleasant smell and ensuring a more consistent and elastic texture and thus a finished product without defects.

Product	Applications
LC 4P1	Semi soft, semi hard and hard cheese
LCP 4P2	Smear ripened cheese (typical flavour)
MO N4P01	Semi soft, semi hard and hard cheese
MO N4P02	Semi soft, semi hard and hard cheese
DY 4P13	Semi soft and semi hard cheese

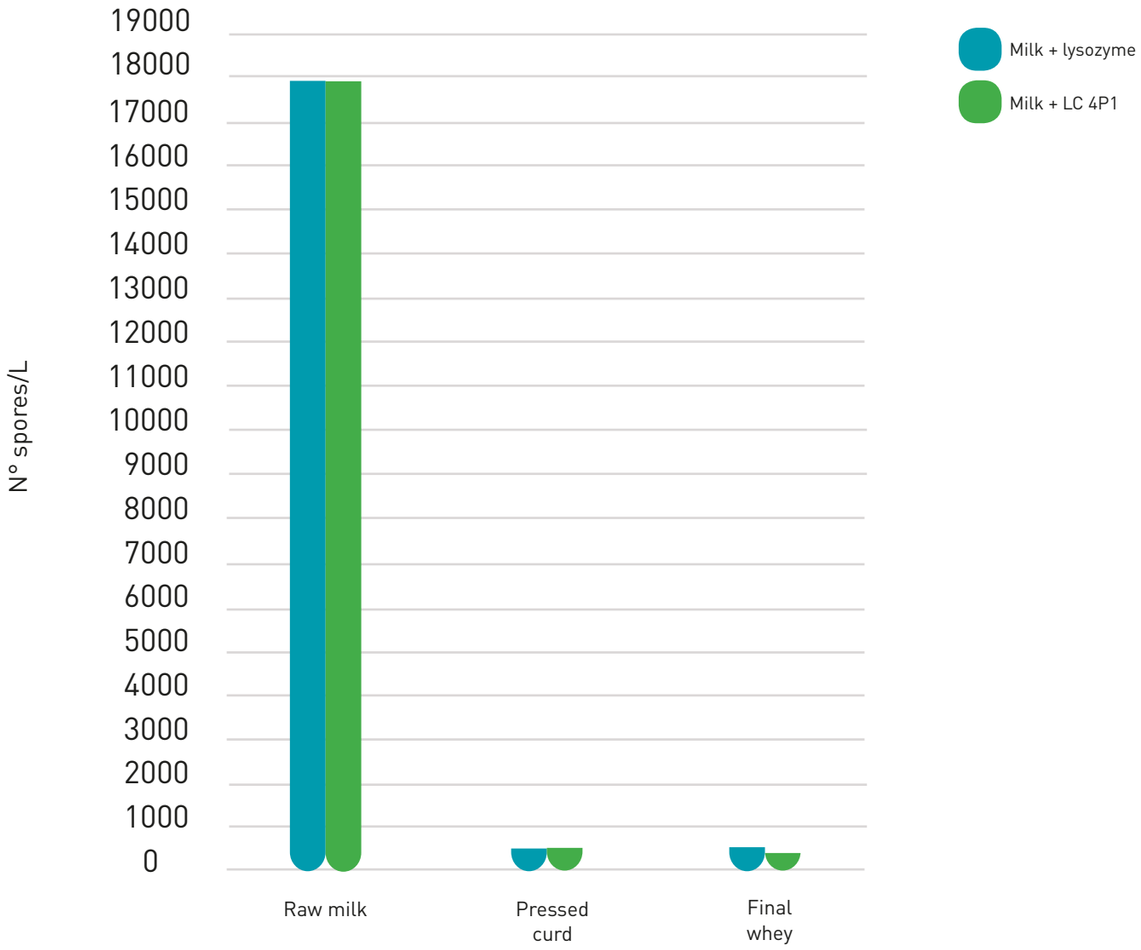
Clostridia control in semi-hard production using LC 4P1



LC 4P1 after 120 days

Control

Comparison with lysozyme



Count of spores of Clostridiumtyrobutirricum in row milk, pressed curd and final whey with lysozyme (blue histogram) and LC 4P1 (green histogram)

MILK + LC 4P1 | MILK

Test result with AC on left and control on right

Articles and studies:

- **Potential of anticlostridial *Lactobacillus* isolated from cheese to prevent blowing defects in semihard cheese** – Christiansen, Vogensen, Nielsen, Ardö (International journal of dairy Technology 2010).

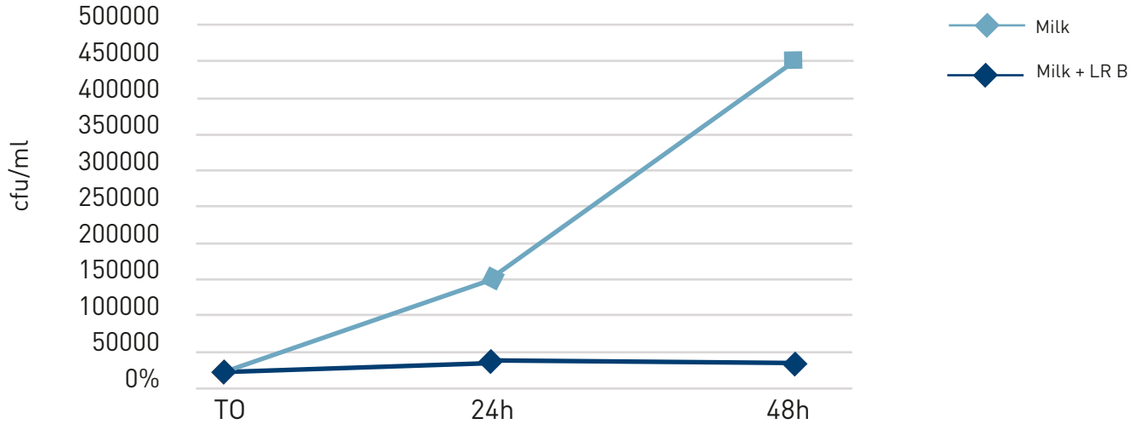
- **Anticlostridial activity of *Lactobacillus* isolated from semi-hard cheeses** – Christiansen, M.H. Petersen, Kosk, Møller, M. Petersen, Nielsen, Vogensen, Ardö (International dairy journal 2005)

AOSM – Anti Other Spoilage Microorganism

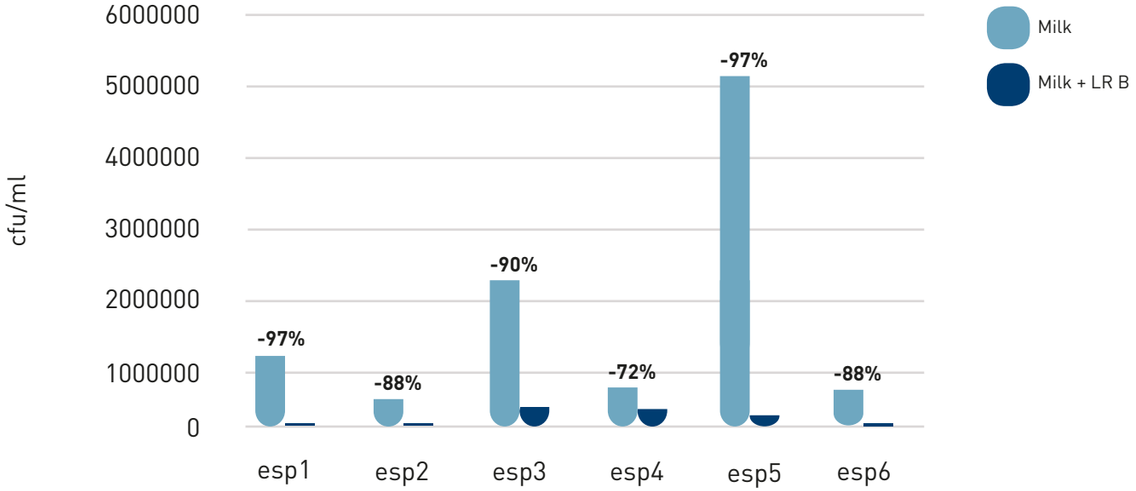
4Protection AOSM reduces the growth of unwanted indigenous microorganism present in milk or coming from the environment, thus improving the milk storage stability and quality, allowing for a standardization of the production process, in terms of acidification, yield and overall sensory.

Product	Applications
LR B	Raw or pasteurized milk

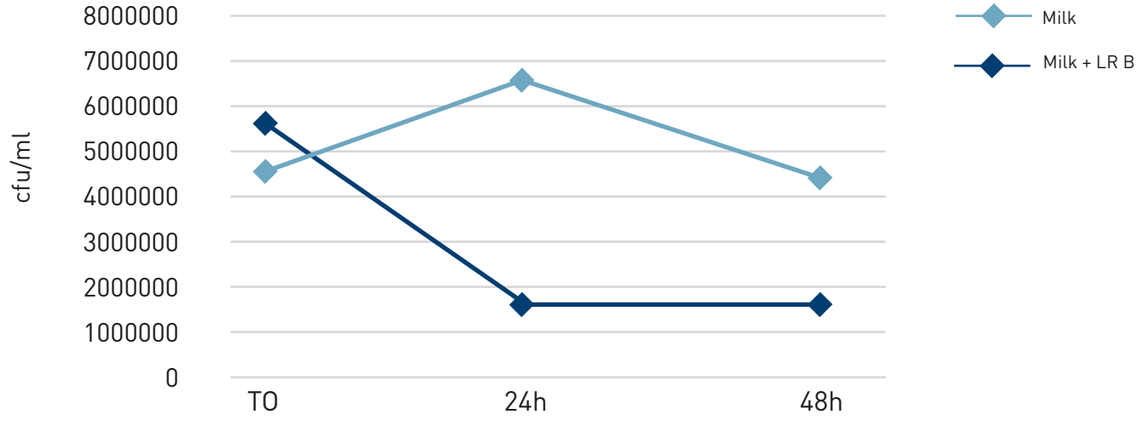
Psychrotrophic bacterial growth during milk storage



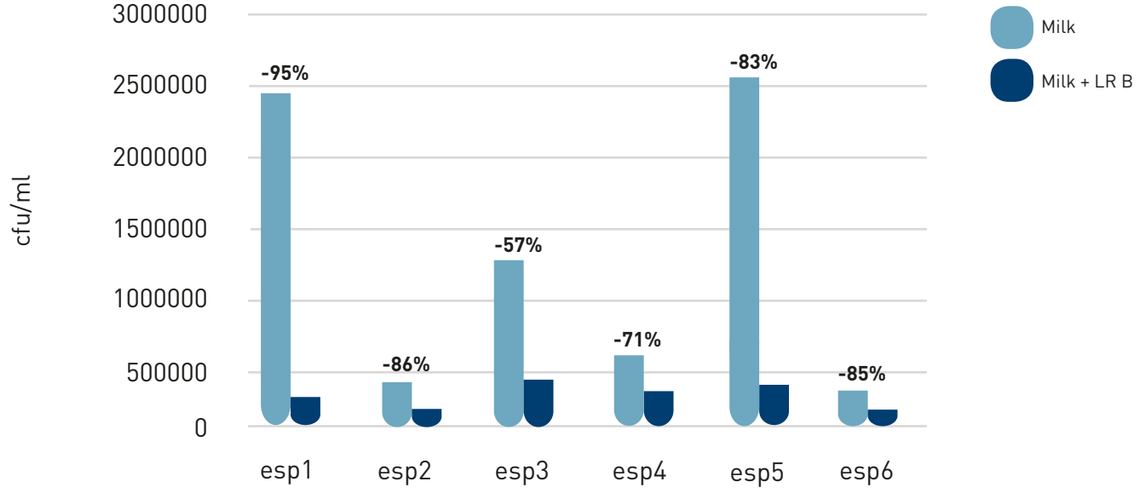
LR B effect on psychrotrophic bacteria during milk maturation (48h)



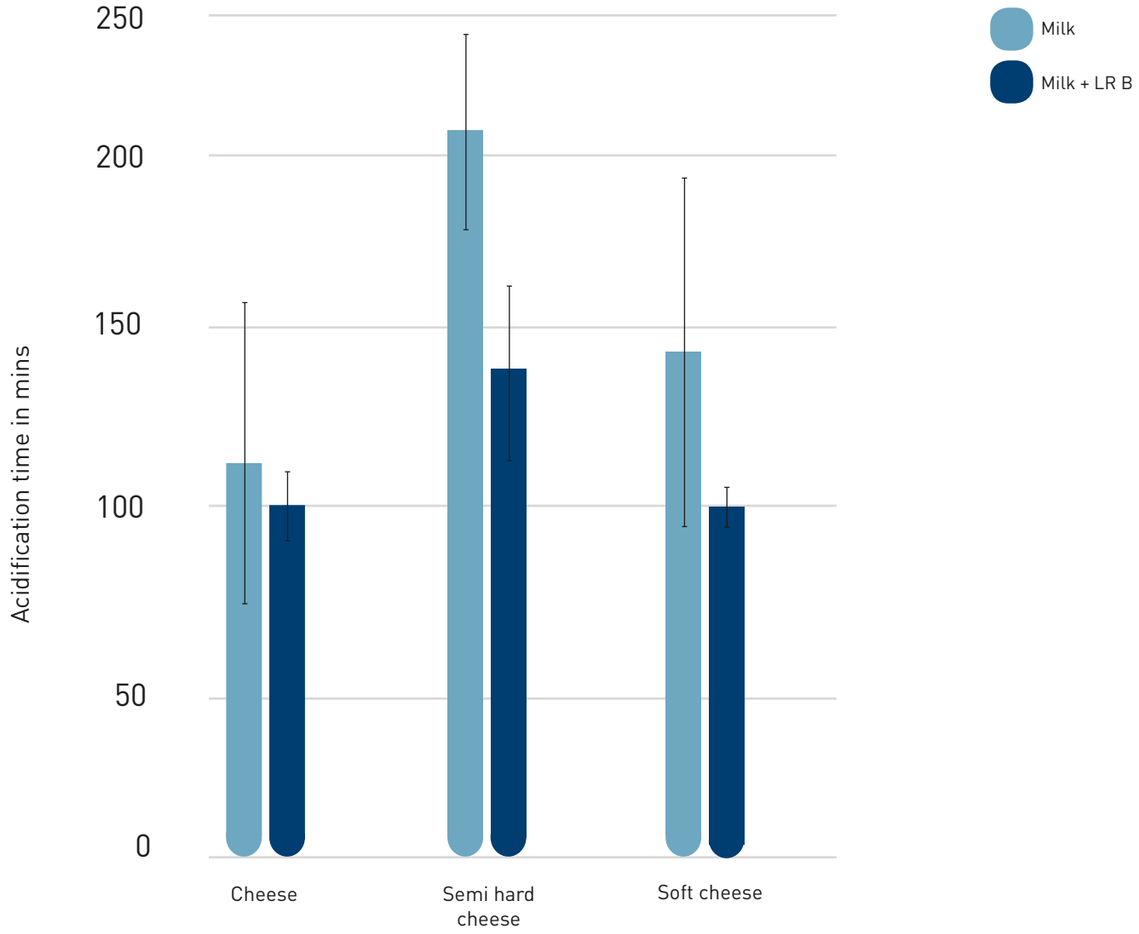
Mesophilic growth during milk storage



LR B effect on mesophilic bacteria during milk maturation (48h)

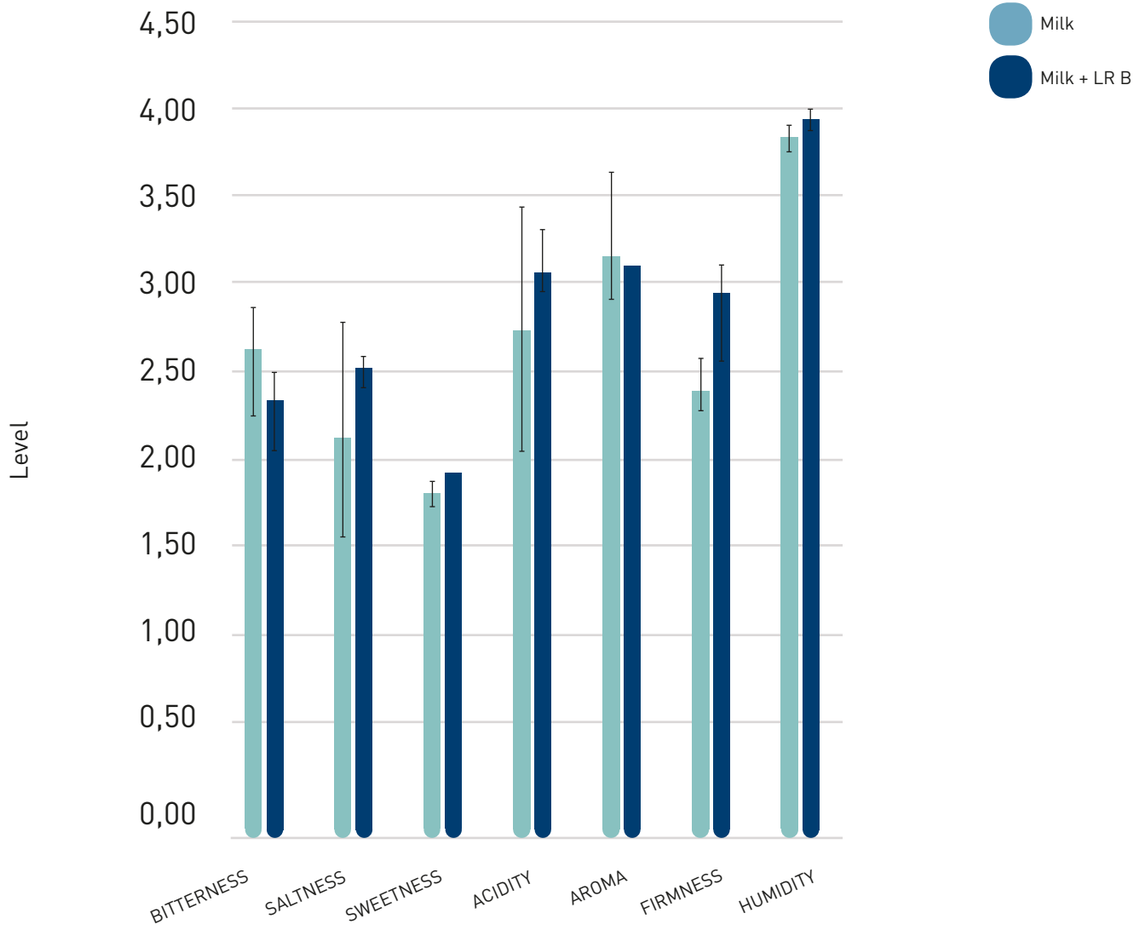


Acidification time

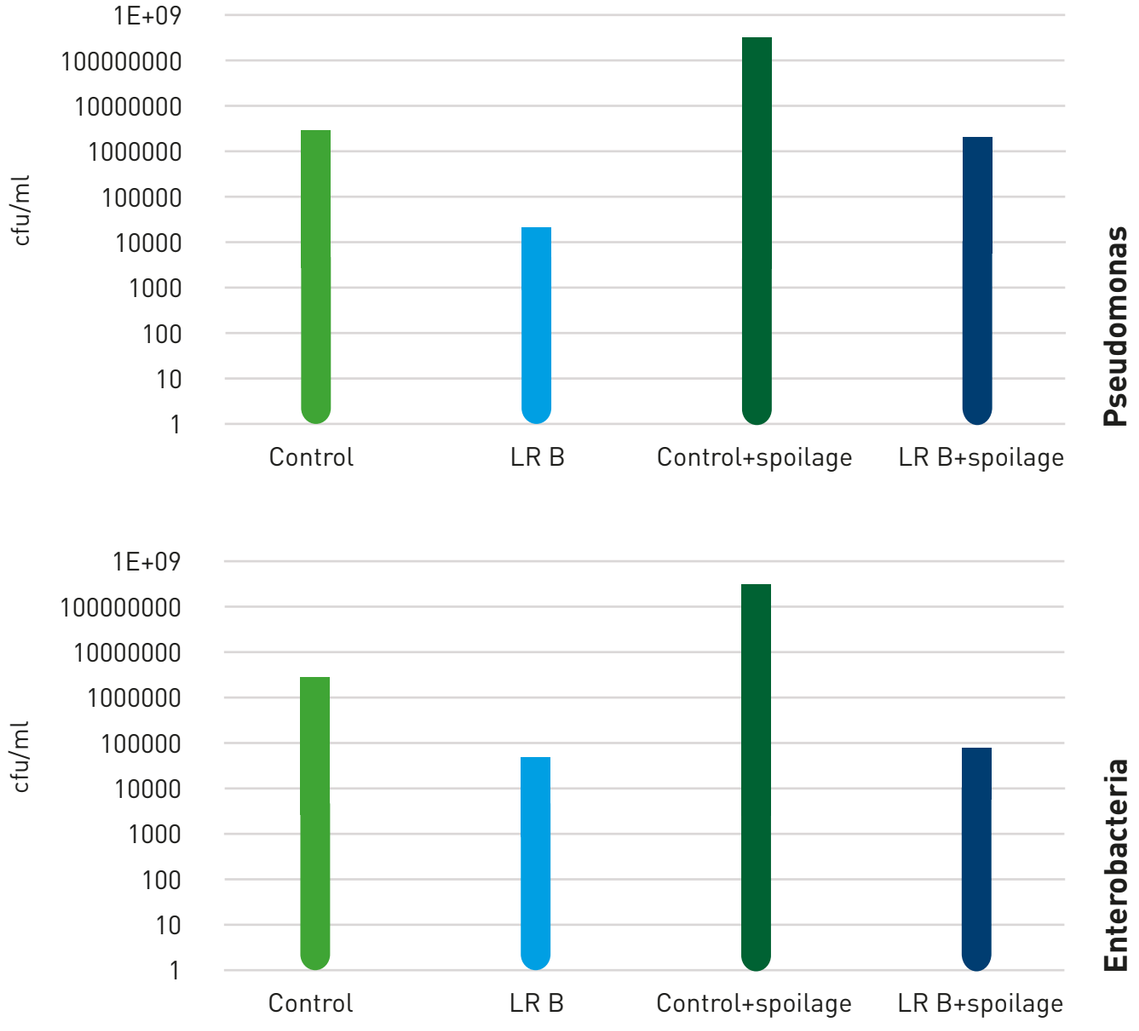


Acidification time is reduced with maturation of milk with LR B

Final product sensory



Effect of LR B - 4 production lots average data



Inhibition effect of LR B in a fresh cheese. Reduction of 2-3 log of contaminant

4PROTECTION LINE FOR MEAT

Product	Applications
Lyocarni BXH-69	Fresh meat, cooked and sliced products with nitrite salt added after cooking and cooling
Lyocarni BMX-37	Fresh meat, cooked and sliced products with nitrite salt added and with anti-listerial properties after cooking and cooling
Lyocarni BOM-13	Fresh meat products without nitrite salt added or on cooked and sliced meat products after cooking and cooling
Lyocarni BOX-74	Fresh meat products without nitrite salt added or on cooked and sliced meat products after cooking and cooling, and with anti-listerial properties
Lyoflora FP-18 **Lyoflora FP-50**	Fresh meat, cooked and sliced products after cooking and cooling only with anti-listerial properties

Contamination of meat products with *Listeria monocytogenes* is an increasing problem. Therefore Sacco has developed a product range of protective cultures. Protection with Sacco cultures for meat application can be achieved by competitive exclusion, most efficient against spoilage bacteria, bacteriocin production efficiently killing *Listeria monocytogenes* and a combination of both principles.

Action	Product
Competitive exclusion with *Lactobacillus sakei*	Lyocarni BOM 13 Lyocarni BXH-12 Lyocarni BXH-69
Bacteriocin producing *Carnobacterium* culture	Lyoflora FP-18 Lyoflora FP-50
Combination of both principles	Lyocarni BOX-74 Lyocarni BMX-37

Articles and studies:

Available on request

- Challenge test with Lyocarni BOX-74 used on cured, cold smoked filet (2015)
- Challenge test with Lyocarni BOX-74 used on emulsion sausage (2014)
- Challenge test with Lyocarni BOX-74 used on cooked ham (2014)
- Challenge test with Lyoflora FP-18 used on a meat emulsion sausage (2014)
- Application of bacteriocin producing lactobacilli for the control of *Listeria* in Italian salami – Andersen, Cislaghi, Coconcelli (2005)

4PROTECTION LINE FOR FISH

Ready-to-eat fish products and cold smoked salmon are products linked to listeriosis outbreaks.
The hurdles utilized in processing cold smoked salmon, such as salting, smoking and drying are not enough to ensure that *Listeria monocytogenes* can not develop.
Some lactic acid bacteria produce bacteriocins which are antimicrobial compounds to which *Listeria monocytogenes* is susceptible.
Consequently application of a starter culture producing bacteriocin will be an additional hurdle enhancing safety of food products.
Sacco has protective cultures for seafood application, Lyoflora FP-18 and Lyoflora FP-50 consist of *Carnobacterium* strains producing bacteriocins (see scientific documentation from Ifremer and Oniris).
The application could be done by injection, dipping, after dry salting or spraying.

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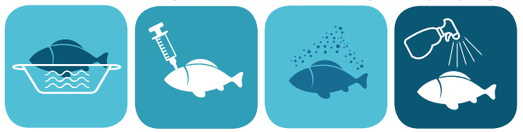
ABSTRACT

APPLICATION EXAMPLES



Articles and studies:

- **Challenge test: *Listeria monocytogenes* on salmon with protective culture (2016)**

- **Effect of inoculation of *Carnobacterium divergens* V41, a biopreservative strain against *Listeria monocytogenes* risk, on the microbiological, chemical and sensory quality of cold-smoked salmon** – Brillet, Pilet, Prévost, cardinal, Leroi (International Journal of Food Microbiology 2005)

- **Specific molecular detection of *Carnobacterium piscicola* SF668 in cold smoked salmon** – Pellé, Dousset, Prévost, Drider (Letters in Applied Microbiology 2005)

- **Biodiversity of *Listeria monocytogenes* sensitivity to bacteriocin-producing *Carnobacterium* strains and application in sterile cold smoked salmon** – Brillet, Pilet, Prévost, Boutterfroy, Leroi (Journal of Applied Microbiology 2004)

- **Evidence on inhibition of *Listeria monocytogenes* by divercin V41 action** - Richard, Brillet, Pilet, Prévost, Drider (Letters in Applied Microbiology 2003)

- **Production of biogenic amines and divercin V41 in cold smoked salmon inoculated with *Carnobacterium divergens* V41, and specific detection of this strain by multiplex-PCR** – Connil, Prévost, Dousset (Journal of Applied Microbiology 2002)

- **Inhibition of *Listeria monocytogenes* by *Carnobacterium* spp. Strains in simulated cold smoked fosh system stored at 4°C** – Duffes, Loeroi, Boyaval, Dousset (International Journal of Food Microbiology 1999)

ABSTRACT DAIRY
Anti *Listeria monocytogenes*

Letters in Applied Microbiology 2003, **36**, 288–292

Evidence on inhibition of *Listeria monocytogenes* by divercin V41 action

C. Richard, A. Brillet, M.F. Pilet, H. Prévost and D. Drider
Laboratoire de Microbiologie Alimentaire et Industrielle, ENTIAA, Rue de la Géraudière, Nantes cedex, France

2002/365: received 27 November 2002, revised 23 January 2003 and accepted 31 January 2003

ABSTRACT

C. RICHARD, A. BRILLET, M.F. PILET, H. PRÉVOST AND D. DRIDER. 2003.

Aims: The aim of this study was to investigate the role of divercin V41 in inhibition and prevention of *Listeria monocytogenes*.

Methods and Results: *Carnobacterium divergens* V41 deficient in bacteriocin production was isolated and characterized by enzyme-liked immunosorbent assay, multiplex polymerase chain reaction and bacteriocin diffusion test. *Carnobacterium divergens* V41 (divercin^{+}) and *Carnobacterium divergens* V41C9 (divercin^{-}) were grown in the presence of *L. monocytogenes* in smoked salmon model medium. *Carnobacterium divergens* V41, but not *C. divergens* V41C9, was able to inhibit growth of *L. monocytogenes*. The results indicate that inhibition of *L. monocytogenes* in the presence of *C. divergens* V41 is because of the production of divercin V41 and not to a nutritional advantage.

Conclusions: *Carnobacterium divergens* V41 may be a promising agent in food safety.

Significance and Impact of the Study: The study demonstrates a potential use of a bacteriocin producing lactic acid bacteria in the area food protection.

Keywords: *Carnobacterium divergens* V41, divercin V41, class IIa bacteriocin, anti-listerial activity, growth inhibition, food-borne pathogen.

ABSTRACT DAIRY
Anti *Listeria monocytogenes*

Letters in Applied Microbiology 2000, **30**, 42–46

Triton X-114 phase partitioning for the isolation of a pediocin-like bacteriocin from *Carnobacterium divergens*

A. Métivier[1,2], P. Boyaval[2], F. Duffes[2], X. Dousset[3], J.-P. Compoint[1] and D. Marion[1]

[1]I.NRA, Laboratoire de Biochimie et Technologie des Protéines, BP71627, Nantes, [2]INRA, Laboratoire de Recherches de Technologie Laitière, Rennes, and [3]ENITIAA, Laboratoire de Microbiologie, rue de la Géraudière, Nantes, France

2274/99: received 5 August 1999 and accepted 17 September 1999

A. METIVIER, P. BOYAVAL, F. DUFFES, CX. DOUSSET, J.-P. COMPOINT AND D. MARION. 2000.

A new procedure combining Triton X-114 phase partitioning and cation exchange chromatography was developed to purify a bacteriocin from a complex culture medium. This pediocin-like bacteriocin, secreted by *Carnobacterium divergens* and named divercin V41, was entirely recovered in the lower detergent-rich phase whereas all other substances (compounds from culture medium, bacterial metabolites) remained in the upper detergent-poor phase. Subsequent cation-exchange chromatography of the TX-114-rich phase allowed recovery of the pure active bacteriocin and also detergent removing. This new purification method is versatile, fast (only two steps) and can be carried out on whole broth.

ABSTRACT DAIRY
Anti *Listeria monocytogenes*

Delineation of Key Amino Acid Side Chains and Peptide Domains for Antimicrobial Properties of Divercin V41, a Pediocin-Like Bacteriocin Secreted by *Carnobacterium divergens* V41

PARWIN BHUGALOO-VIAL,[1,2] JEAN-PAUL DOULIEZ,[1] DANIEL MOLLÉ,[3] XAVIER DOUSSET,[2] PATRICK BOYAVAL,[3] AND DIDIER MARION[1]*

Unité de Biochimie et Technologie des Protéines, INRA, 44316 Nantes Cedex 03,[1] *Laboratoire de Microbiologie, ENITIAA, 44072 Nantes Cedex,*[2] *and Unité de Recherches de Technologie Laitière, INRA, 35042 Rennes Cedex,*[3] *France*

Received 5 August 1998/Accepted 12 April 1999

Divercin V41 (DV41) is a class IIa bacteriocin produced by *Carnobacterium divergens* V41. This antilisterial peptide is homologous to pediocin PA-1 and contains two disulfide bonds. To establish the structure-activity relationships of this specific family of bacteriocin, chemical modifications and enzymatic hydrolysis were performed on DV41. Alteration of the net charge of this cationic bacteriocin by succinylation and acetylation revealed that, in a certain range, the electrostatic interactions were surprisingly not necessary for the activity of DV41. Cleavage of DV41 by endoproteinase Asp-N released two fragments N1[1–17] and N2[18–43] corresponding to the conserved hydrophilic N-terminal and the variable hydrophobic C-terminal sequences, respectively. Inhibitory assays showed that only the C-terminal fragment was active, and after trypsin cleavage at Lys42 or disulfide reduction it lost its inhibitory activity. These results suggested that both hydrophobicity and folding imposed by the Cys25-Cys43 disulfide bond were essential for antilisterial activity of the C-terminal hydrophobic peptide. Chemical oxidation of tryptophan residues by N-bromosuccinimide demonstrated that these residues were crucial for inhibitory activity since modification of any one of them rendered DV41 inactive. On the contrary, only the modification of all the three tyrosine residues caused a total loss of antilisterial activity. These latter results strengthened previous results suggesting that the N-terminal domain containing the YGNGV consensus sequence was not involved in the binding of DV41 to a potential specific receptor on listerial cells.

ABSTRACT DAIRY
Anti *Listeria monocytogenes*

Microbiology (1998), **144**, 2837–2844 Printed in Great Britain

Divercin V41, a new bacteriocin with two disulphide bonds produced by *Carnobacterium divergens* V41: primary structure and genomic organization

Anita Métivier,[1,2] Marie-France Pilet,[1] Xavier Dousset,[1] Odile Sorokine,[3] Patricia Anglade,[4] Monique Zagorec,[4] Jean-Christophe Piard,[4] Didier Marion,[5] Yves Cenatiempo[2] and Christophe Fremaux[2,6]

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[5] INRA, Unité de Biochimie et Technologie des Protéines, 44316 Nantes cedex, France

Divercin V41 is a new bacteriocin produced by *Carnobacterium divergens* V41, a lactic acid bacterium isolated from fish viscera. The amino acid sequence of divercin V41 showed high homologies with pediocin PA-1 and enterocin A. Two disulphide bonds were present in the hydrophilic N-terminal domain and in the highly variable hydrophobic C-terminal domain, respectively. A DNA probe designed from the N-terminal sequence of the purified peptide was used to locate the structural gene of divercin V41. A 6 kb chromosomal fragment containing the divercin V41 structural gene (*dvnA*) was cloned and sequenced. The results indicate that divercin V41 is synthesized as a pre-bacteriocin of 66 amino acids. The 23-residue N-terminal extension is cleaved off to yield the mature 43-amino-acid divercin V41. In addition, the fragment encodes putative proteins commonly found within bacteriocin operons, including an ATP-dependent transporter, two immunity-like proteins and the two components of a lantibiotic-type signal-transducing system. The genetic organization of the fragment suggested important gene rearrangements.

Keywords: bacteriocin, *Carnobacterium*, lactic acid bacteria, anti-*Listeria*

ABSTRACT DAIRY
Anti *Listeria monocytogenes*

Letters in Applied Microbiology 1998, **27**, 302–306

Enumeration of *Carnobacterium divergens* V41, *Carnobacterium piscicola* V1 and *Lactobacillus brevis* LB62 by *in situ* hybridization–flow cytometry

N. Connil[1], X. Dousset[1], B. Onno[1], M.F. Pilet[1], M.F. Breuil[1] and M.C. Montel[2]

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1714/98: received 14 July 1998 and accepted 15 July 1998

N. CONNIL, X. DOUSSET, B. ONNO, M.F. PILET, M.F. BREUIL AND M.C. MONTEL. 1998. The specific detection and enumeration of *Lactobacillus brevis* LB62, *Carnobacterium divergens* V14 and *Carnobacterium piscicola* VI were studied by *in situ* hybridization–flow cytometry. The method was performed on the exponential growth phase with three probes targeting 16S rRNA labelled with fluorescein isothicyanate (FITC) : EUB338 probe universal for Eubacteria, Lb probe specific for *Lact. brevis* and Cb probe specific for the genus *Carnobacterium*. EUB338 was used to determine the permeabilization and hybridization conditions for the cells. The Lb probe gave no hybridization signal whereas the Cb probe allowed the detection and quantification by flow cytometry at 520 nm of the two *Carnobacterium* strains in pure culture or in mixtures with *Listeria innocua* F.

ABSTRACT DAIRY
Anti *Listeria monocytogenes*

Purification and Amino Acid Sequences of Piscicocins V1a and V1b, Two Class IIa Bacteriocins Secreted by *Carnobacterium piscicola* V1 That Display Significantly Different Levels of Specific Inhibitory Activity

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Received 27 February 1996/Accepted 17 September 1996

Two bacteriocins produced by *Carnobacterium piscicola* V1 were purified and characterized. Piscicocin V1a (molecular mass = 4,416 Da) and piscicocin V1b (molecular mass = 4,526 Da) are nonlantibiotic, small, heat-stable antibacterial peptides. Piscicocin V1b is identical to carnobacteriocin BM1, while piscicocin V1a is a new bacteriocin. Its complete sequence of 44 amino acid residues has been determined. Piscicocin V1a belongs to the class IIa bacteriocins having the consensus YGNGV motif. These peptides inhibit various gram-positive bacteria, including *Listeria monocytogenes*. Piscicocin V1a is approximately 100 times more active than piscicocin V1b against indicator strains. However, the antagonistic spectrum is the same for both piscicocins. Comparison of these results with the analysis of the amino acid sequence and secondary structure predictions suggests that (i) the conserved N-terminal conserved domain is involved in the receptor recognition and therefore in an "all-or-none" response against target bacterial cells and (ii) the C-terminal variable and hydrophobic domain determines membrane anchoring and therefore the intensity of the antagonist response.

ABSTRACT DAIRY
Anti Clostridia

doi: 10.1111/j.1471-0307.2010.00626.x

ORIGINAL RESEARCH

Potential of anticlostridial *Lactobacillus* isolated from cheese to prevent blowing defects in semihard cheese

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Five anticlostridial Lactobacillus *strains isolated from cheese were selected for a mixed adjunct culture. Cheese with the mixed adjunct culture (experimental) and without (control) was made in triplicate and ripened as vacuum-packed and surface-ripened cheese. Cheese gross composition was similar. Excessive gas formation occurred only in control cheeses. In contrast to control cheeses, the experimental cheeses were dominated by the added adjunct* Lactobacillus *strains (repetitive-PCR). Casein breakdown was not influenced, however, the total amount of amino acids and pH was slightly lower in the experimental cheeses. Anticlostridial nonstarter* Lactobacillus *strains have potential as protective adjunct cultures against blowing defects in cheese.*

Keywords Cheese, Blowing defects, *Clostridium*, *Lactobacillus*, Antimicrobial activity.

ABSTRACT DAIRY
Anti Clostridia

Anticlostridial activity of *Lactobacillus* isolated from semi-hard cheeses

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Received 15 April 2004; accepted 9 July 2004

Abstract

Non-starter lactic acid bacteria grow to high numbers in semi-hard cheeses during ripening, and may suppress harmful bacteria. In this study, about 400 *Lactobacillus* isolates from Danish semi-hard cheeses were identified to species level using internal transcribed spacer-polymerase chain reaction (ITS-PCR) analysis. The majority of isolates belonged to the *Lb. paracasei* complex and were classified into approximately 135 types using pulsed field gel electrophoresis (PFGE). *Lactobacillus* isolates representing all the different PFGE types were screened, using an agar well diffusion assay, for antimicrobial activity against 15 single-strain *Clostridium* cultures. Almost half of the isolates possessed anticlostridial activity, and 10% possessed a broad and consistent activity. Nine strains were further investigated for properties of importance for use as mixed cultures in cheese and silage. The results showed that anticlostridial non-starter *Lactobacillus* growing in high quality semi-hard cheeses could be useful as protective adjunct cultures against the growth of *Clostridium*.

Keywords: Cheese; *Lactobacillus paracasei*; Antimicrobial activity; *Clostridium*

ABSTRACT
MEAT

APPLICATION OF BACTERIOCIN PRODUCING LACTOBACILLI FOR THE CONTROL OF *LISTERIA* IN ITALIAN SALAMI

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Key Words: Fermented sausage, starter culture, acidification, staphylococci, LAB, bacteriocin, Listeria, PCR

Introduction

All over the world *Listeria* contamination is a potential hazard in fermented, dry sausages produced without heat treatment. As heat treatment alters the meat structure such a product is not perceived as a traditional fermented, dry sausage by consumers. Normally, if present, the level of *Listeria* in fermented, dry sausages is relatively low and should not cause health problems when fermented sausages are consumed. Nevertheless, regulation in food requirements, as safety criteria, calls for absence of *Listeria monocytogenes* in 25 g food, and consequently, efforts are accomplished to prevent *Listeria* being present in traditionally produced fermented sausages. Commercial bacteriocin producing lactic acid bacteria (LAB) have successfully been tested on applied *L. monocytogenes* in fermented sausages (Andersen, 1999) but few data on effect on indigenous *Listeria* with such LAB strains are available (Hugas et al, 2003).

Some of the characteristics of Italian salami are high final pH, moulded surface, and pronounced meaty flavour. It is well-known that staphylococci enhance the development of meaty flavour but also that they are inhibited by lowering in pH (Tjener, 2003). Therefore, an adequate anti-listerial LAB starter culture should not lower pH so much that it influences the development of required flavour compounds and the sensory assessment.

ABSTRACT FISH
Anti *Listeria monocytogenes*

Letters in Applied Microbiology 2005, **40,** 364–368 doi:10.1111/j.1472-765X.2005.01696.x

Specific molecular detection of *Carnobacterium piscicola* SF668 in cold smoked salmon

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2004/1095: received 20 September 2004, revised and accepted 15 December 2004

ABSTRACT

E. PELLÉ, X. DOUSSET, H. PRÉVOST AND D. DRIDER. 2005.

Aims: To establish a rapid and reliable multiplex PCR (mPCR)-based method allowing specific identification of *Carnobacterium piscicola* SF668 during storage of cold smoked salmon (CSS).

Methods and Results: CSS was inoculated with *C. piscicola* SF668 and stored at 4°C. Samples were withdrawn at regular time intervals and analysed by counting the number of viable cells. About 25–100% of colonies grown on Elliker plates were subjected to mPCR amplification. The results show that strains presumably identified as *C. piscicola* SF668 were predominant over the test period.

Conclusions: mPCR is a powerful tool to study competitiveness of *C. piscicola* SF668, which inhibits the growth of *Listeria monocytogenes*.

Significance and Impact of the Study: The present study demonstrates the importance of molecular methods in studying competitiveness of strains with potential food applications.

Keywords: *Carnobacterium piscicola* SF668, cold smoked salmon, lactic acid bacteria, multiplex PCR.

ABSTRACT FISH
Anti *Listeria monocytogenes*

Journal of Applied Microbiology 2004, **97,** 1029–1037 doi:10.1111/j.1365-2672.2004.02383.x

Biodiversity of *Listeria monocytogenes* sensitivity to bacteriocin-producing *Carnobacterium* strains and application in sterile cold-smoked salmon

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2003/1166: received 18 December 2003, revised 5 April 2004 and accepted 4 June 2004

ABSTRACT

A. BRILLET, M.-F. PILET, H. PREVOST, A. BOUTTEFROY AND F. LEROI. 2004.

Aims: The aim of this study was to demonstrate the inhibitory capacity of *Carnobacterium* strains against a collection of *Listeria monocytogenes* strains in cold-smoked salmon (CSS).

Methods and Results: Three bacteriocin-producing strains, *Carnobacterium divergens* V41, *C. piscicola* V1 and *C. piscicola* SF668, were screened for their antilisterial activity against a collection of 57 *L. monocytogenes* strains selected from the French smoked salmon industry, using an agar spot test. All the *Listeria* strains were inhibited but three different groups could be distinguished differing in sensitivity to the three *Carnobacterium* strains. However, *C. divergens* V41 always had the highest inhibitory effect. The antilisterial capacity was then tested in sterile CSS blocks co-inoculated with *Carnobacterium* spp. and mixtures of *L. monocytogenes* strains. *C. divergens* V41 was the most efficient strain, maintaining the level of *L. monocytogenes* at <50 CFU g^{-1} during the 4 weeks of vacuum storage at 4 and 8°C, whatever the sensitivity of the set of *L. monocytogenes* strains.

Conclusions: *C. divergens* V41 may be a good candidate for biopreservation in CSS.

Significance and Impact of the Study: A biopreservation strategy for CSS against the risk of *L. monocytogenes* was investigated using bacteriocin-producing lactic acid bacteria.

Keywords: bacteriocin, biopreservation, *Carnobacterium*, cold-smoked salmon, *Listeria monocytogenes*.

ABSTRACT FISH
Anti *Listeria monocytogenes*

Journal of Applied Microbiology 2002, **92**, 611–617

Production of biogenic amines and divercin V41 in cold smoked salmon inoculated with *Carnobacterium divergens* V41, and specific detection of this strain by multiplex-PCR

N. Connil, H. Prévost and X. Dousset
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2001/143: received 16 June 2001, revised 1 October 2001 and accepted 16 October 2001

N. CONNIL, H. PRÉVOST AND X. DOUSSET. 2002.

Aims: The objective of this study was to determine the technological behaviour (implantation and biogenic amines production) of *Carnobacterium divergens* V41, an anti-*Listeria* bacteriocin producer (divercin V41), after inoculation in cold smoked salmon (CSS).
Methods and Results: Implantation of the strain was followed by multiplex-PCR during 27 days of storage at 4°C, and biogenic amines were quantified by HPLC. It was found that the strain was able to develop quite well in CSS among lactic wild flora. Divercin V41 (400 AU ml^{-1}) was produced in CSS, and the biogenic amine content was not modified by inoculation of the bacteria.
Conclusions: *Carnobacterium divergens* V41 is a safe, interesting, bioprotective agent.
Significance and Impact of the Study: This strain could potentially be used for efficient prevention of *L. monocytogenes* growth in CSS.

ABSTRACT FISH
Anti *Listeria monocytogenes*

Inhibition of *Listeria monocytogenes* by *Carnobacterium* spp. strains in a simulated cold smoked fish system stored at 4°C

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Received 22 April 1998; received in revised form 18 August 1998; accepted 27 November 1998

Abstract

Preservation of smoked salmon from bacterial spoilage, and especially from *Listeria monocytogenes*, by bacteriocin producers is a promising challenge. Over a hundred lactic acid bacteria, isolated from commercial vacuum packaged cold smoked salmon, were screened for their antagonistic activity against *L. innocua*. Twenty-two strains were able to produce bacteriocin-like proteinaceous substances. These strains were characterized physiologically and biochemically as *Carnobacterium* strains. Three different groups were determined by pulsed-field gel electrophoresis after *Sma* I and *Apa* I DNA digestion. Peptidoglycan hydrolases patterns completed the characterization of these strains. All were confirmed as being *Carnobacterium piscicola*. Growth and bacteriocin production of three strains of each group and two well known bacteriocin producers (*C. divergens* V41 and *C. piscicola* V1) were tested in a simulated cold smoked fish system at 4°C. These strains were able to reach 10^8 cfu ml^{-1} in 21 days and to produce as much bacteriocin activities in the cold smoked fish system as in the rich media. *Carnobacterium divergens* V41 and *C. piscicola* V1 were the most effective strains in co-culture experiments, inhibiting *L. monocytogenes* as early as day 4, whereas *C. piscicola* SF668 inhibiting effect was observed at day 13. The potential for using such biopreservation treatments on whole smoked salmon is discussed.

Keywords: *Listeria monocytogenes*; *Carnobacterium*; Bacteriocin; Salmon

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