4 Protection

Food Cultures with Protective Effect
NATURAL CARE

INDEX

4PROTECTION, THE NATURAL GUARD FOR YOUR PRODUCT IDENTITY

INTRODUCTION

WHAT IS 4PROTECTION LINE AND WHY USE IT

HOW 4PROTECTION LINE WORKS

4PROTECTION APPLICATIONS:

AYM – Anti Yeast and Moulds
AL – Anti Listeria monocytogenes
AC – Anti Clostridia
AOSM – Anti Other Spoilage Microorganisms

ABOUT SACCO
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 Food Cultures with Protective Effect 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.
Protection Food Cultures with Protective Effect add an extra hurdle to prevent the growth of unwanted microorganisms, protecting the quality and food safety and help reduce food waste.

WHAT IS 4PROTECTION LINE AND WHY USE IT

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

4Protection Food Cultures with Protective Effect have a multiple biological interaction with the food matrix and in some cases the wild biota in the food. In regards to the protective effect, three main mechanisms are involved:
- taking the physical space;
- fighting for nutrients;
- producing of inhibitory molecules, such as metabolites e.g., bacteriocins, organic acids, peptides.

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 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 to the characteristics of the products, the technological process and the activity needed from the use of our protective cultures.
The 4Protection Special Food Cultures Line helps to improve the products quality and the brand image, reducing non-compliant products, business costs and therefore food waste.

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

- **Anti Yeasts and Moulds** (AYM)
- **Anti Listeria monocytogenes** (AL)
- **Anti Clostridia** (AC)
- **Anti Other Spoilage Microorganisms** (AOSM)
**AYM – Anti yeast and moulds**

4Protection AYM has been designed to fight the most common problem of dairy producers, i.e. 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.

<table>
<thead>
<tr>
<th>Product</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPR A</td>
<td>Yogurt, fresh fermented products, fresh cheese, soft cheese, semi hard cheese and hard cheese</td>
</tr>
<tr>
<td>LR B</td>
<td>Yogurt, fresh fermented products, fresh cheese, soft cheese, semi hard cheese and hard cheese</td>
</tr>
<tr>
<td>LR4 PD</td>
<td>Yogurt, fresh fermented products, fresh cheese, soft cheese, semi hard cheese and hard cheese</td>
</tr>
<tr>
<td>CLP C</td>
<td>Fresh cheese, soft cheese, semi hard and hard cheese</td>
</tr>
</tbody>
</table>

**4Protection Anti Y&M efficacy on yogurt and fermented milks**

LPR A, LR B and LR4 PD show a strong efficacy inhibiting the development of yeast and moulds on yogurt and fermented milks guaranteeing the shelf life extension without the addition of preservatives nor negatively altering the organoleptic characteristics of your products (Fig.1).

![Figure 1. Sensory evaluation with 4Protection AYM cultures range](image-url)
The following examples show the anti yeast (Fig. 2-3) and moulds (Fig. 4-5) activity of 4Protection Special Food Cultures Line.

### 4Protection Anti Yeast effect

Figure 2. Growth of spoilage yeast during storage

Figure 3. Inhibition capability of AYM cultures

### 4Protection Anti Moulds effect

Figure 4. Protocol for anti mould activity in set yogurt

Figure 5. Shelf life extension against *Penicillium palitans*

- **4yo’ starter cultures** + 50 spores/spot *Penicillium palitans*
  - Storage at 10°C (50°F)
- **4yo’ starter cultures** + LR4 PD* + 50 spores/spot *Penicillium palitans*
  - Storage at 10°C (50°F)

*Inoculation level: 1 dose*
AL – Anti *Listeria monocytogenes*

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

<table>
<thead>
<tr>
<th>Product</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPAL</td>
<td>Soft cheese</td>
</tr>
<tr>
<td>CNBAL</td>
<td>Cheese ripened at low temperature and without sugar, like semi hard and hard cheese, gorgonzola, blue cheese</td>
</tr>
</tbody>
</table>

![Graph showing counts of Listeria monocytogenes in cheese](image)

Figure 6. 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

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

Figure 7 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.

**Figure 8. Evidence of bacteriocine production - (Halo size)**

**References:**

- Testing commercial biopreservative against spoilage microorganisms in MAP packed Ricotta fresca cheese – Spanu, Scarano, Piras, Spanu, Pala, Cacci, Lamon, Cassu, Ibbi, Nieddu, De Santis (Food microbiology, 2017)
- Triton X-114 phase partitioning for the isolation of a pediocin-like bacteriocin from *Carnobacterium divergens* – Métivier, Boyaval, Dufres, Dousset, Compont, 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, Trouille, Molli, Dousset, Boyaval, Marion (Applied and Environmental Microbiology, 1999)
- Enumeration of *Carnobacterium divergens* V41, *C. piscicola* V1 and *Lactobacillus brevis* LB62 by in situ hybridization-flow cytometry – Connil, Dousset, Otte, Pilet, Brueil, Montel (Letters in Applied Microbiology 1998)
- Divercin V41, a new bacteriocin with two disulfide bonds produced by *Carnobacterium divergens* V41: primary structure and genomic organization – Métivier, Pilet, Dousset, Sorokine, Angladem 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, Métivier, Sorokine, Anglade, Boyaval, Marion (Applied and Environmental Microbiology, 1996)
AC – Anti Clostridia

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

<table>
<thead>
<tr>
<th>Product</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC 4P1</td>
<td>Semi soft, semi hard and hard cheese</td>
</tr>
<tr>
<td>LCP 4P2</td>
<td>Smear ripened cheese (typical flavour)</td>
</tr>
<tr>
<td>MO N4P01</td>
<td>Semi soft, semi hard and hard cheese (nisin producer)</td>
</tr>
<tr>
<td>MO N4P02</td>
<td>Semi soft, semi hard and hard cheese (nisin producer)</td>
</tr>
<tr>
<td>MO L4P03</td>
<td>Semi soft, semi hard and hard cheese (non-nisin producer)</td>
</tr>
<tr>
<td>MO L4P04</td>
<td>Semi soft, semi hard and hard cheese (non-nisin producer)</td>
</tr>
<tr>
<td>DY 4P13</td>
<td>Semi soft and semi hard cheese</td>
</tr>
</tbody>
</table>

Clostridia control in semi-hard production using LC 4P1

- Control
- LC 4P1

![Clostridia control in semi-hard production using LC 4P1](image)

<table>
<thead>
<tr>
<th>N° spores/L</th>
<th>Raw milk (after end of ripening at 120 days 24h at 7°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control</td>
</tr>
<tr>
<td>500</td>
<td>LC 4P1</td>
</tr>
<tr>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>1500</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>2500</td>
<td></td>
</tr>
<tr>
<td>3000</td>
<td></td>
</tr>
<tr>
<td>3500</td>
<td></td>
</tr>
<tr>
<td>4000</td>
<td></td>
</tr>
</tbody>
</table>

![Image of samples](image)
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, Kooi, Møller, M. Petersen, Nielsen, Vogensen, Ardö (International dairy journal 2005).

Figure 10. Count of spores of Clostridium tyrobutyricum in raw milk, pressed curd and final whey with lysozyme (blue histogram) and LC 4P1 (green histogram).

Figure 11. Test result with AC on left and control on right.
AOSM – Anti Other Spoilage Microorganisms

Protection 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.

<table>
<thead>
<tr>
<th>Product</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR B</td>
<td>Raw or pasteurized milk</td>
</tr>
</tbody>
</table>
Mesophilic growth during milk storage

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

Acidification time

Figure 16. Acidification time is reduced with maturation of milk with LR B
Final product sensory

Effect of LR B - 4 production lots average data

Figure 18-19. Inhibition effect of LR B in a fresh cheese. Reduction of 2-3 log of contaminant.

References:
- Antimicrobial activity of Lactobacillus rhamnosus against Pseudomonas fluorescens and Pseudomonas putida from raw milk. – D’Amico de Alcântara, Bruzaroski, Luiz, Batista de Souza, Poli-Frederico, Fagnani, Walter de Santana (Journal of Food Processing and Preservation, 2019)
ABOUT SACCO

SACCO is an international company with family spirit that offers a large range of innovative products.

This includes starter cultures for food fermentation (in particular dairy) and nutritional supplements (probiotic cultures), as well as instruments for the improvement of food.

The sister company Caglificio Clerici has been an Italian leader in rennet production since 1872. Sacco furthermore acquired the Italian culture producer CSL in 2013.

The high quality of our products, the continuous innovation, the ability to work closely with our clients, and the focus on training and developing employees, are the pillars of Sacco.

In recent years the company has further invested extensively in R&D, including brand new facilities in 2018 and 2019, and has been a "pioneer" in areas such as protective cultures.

Sacco distributes its products in all key markets (110+ countries), and has ISO 22000 and FSSC 22000 accreditation and a GMP certified plant.

Sacco is a company of Sacco System, the biotech network applied in food, nutraceutical and pharmaceutical industry.

Find out more about our 4Protection Special Food Cultures range and customized solutions. Visit us at www.saccosystem.com or email us at info@saccosystem.com.
TRADITION, PASSION
INNOVATION