LACTIC ACID BACTERIA

Functionalities and Applications in Foods
History

✓ Louis Pasteur (1857)
   germ theory for fermentative changes

✓ Joseph Lister (1873)
   microbial nature of lactic acid fermentation
   "Bacterium lactis"
With the discovery of microorganisms in the 19th century, it became possible to improve the products and the fermentation processes by using isolated and well-characterized cultures.

However, it took many years before the food industry changed their procedures towards well characterized and defined starter cultures.
History

- Besides yeast and moulds, lactic acid bacteria are widely used in the production of fermented foods.

- Lactic acid bacteria constitute the majority of the volume and the value of the commercial starter cultures.

- The largest part of the lactic acid cultures market consists of cultures for the dairy industry.

- The size of this market is currently approximately US $300 million.
Properties

- Gram positive
- Catalase negative
- Non-sporing
- < 50% G+C
- Homofermentative
- Heterofermentative
- Microaerophilic
Genera

1. *Lactobacillus*
2. *Streptococcus*
3. *Lactococcus*
4. *Enterococcus*
5. *Leuconostoc*
6. *Pediococcus*
7. *Carbobacterium*
8. *Aerococcus*
9. *Tetragenococcus*
Sources

- Dairy products
- Fermented meats
- Fermented vegetables
- Sewage
- Plant material
- Respiratory track
- Intestinal track
- Genital track
Importance

- Food technology
- Feed technology
- Probiotic properties
Dairy Products
**Reticulum:** honeycomb-like interior surface, which helps to remove foreign matter from the food material

**Rumen:** the organ that allows the bacterial and chemical breakdown of fiber

**Omasum:** it grinds the food material and prepares the food material for chemical breakdown

**Abomasum:** very similar to the stomach of non-ruminants; this is where the majority of chemical breakdown of food material occurs
## Mean composition of bovine milk

<table>
<thead>
<tr>
<th></th>
<th>composition (%)</th>
<th>size (diameter, nm)</th>
<th>number / ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>86.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>4.1</td>
<td>2000-6000</td>
<td>$10^{10}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(globules)</td>
<td></td>
</tr>
<tr>
<td>Proteins</td>
<td>3.6</td>
<td>50-300 (micelles)</td>
<td>$10^{14}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-6 (molecules)</td>
<td>$10^{17}$</td>
</tr>
<tr>
<td>Lactose</td>
<td>5.0</td>
<td>0.5 (molecules)</td>
<td>$10^{19}$</td>
</tr>
<tr>
<td>Ash</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Dairy Products

✓ Fermented dairy products are known since ancient times

✓ Fermentation contributes to

  the preservation of vast quantities of nutritious foods
  without significant fuel requirements
  providing a wide diversity of
  flavors
  aromas
  textures, but also,
  enriches food with vitamins and essential amino acids
Dairy Products

✓ Lactic acid bacteria convert fermentable sugars mainly to lactic acid, and are thus responsible for processing and preserving milk

✓ Other anti-microbial factors associated with lactic acid bacteria,

i.e. carbon dioxide
hydrogen peroxide
ethanol
diacetyl
bacteriocins

are responsible for inhibition of food spoilage and pathogens
Cheese

✓ Cheese is one of the most diverse food groups

✓ Around 500 varieties are produced from cow's milk, while 500 are produced from sheep's and/or goat's milk

✓ Most cheeses are produced using as coagulant animal rennet

✓ Several cheeses in the Iberian Peninsula are produced with the use of plant rennet (an aqueous extract from the cardoon flowers; Cyanara cadunuculus)
Cheese

Smear surface-ripened

(Beaufort, Comte, Gruyere, Saint Paulin, Munster)

Mould surface-ripened

(Camembert, Brie, Roquefort, Gorgonzolla)

Swiss type

(Emmental, Gruyere, Beaufort, Graviera)
Cheese

Grana
(Parmigiano-Regiano, Grana Padano)

Pasta Filata
(Mozzarella, Provolone, Kashkaval, Kasseri)

White-brined
(Feta, Domiati, Beyaz peynir, Halloumi)

Whey cheeses
(Ricotta, Mizithra, Manouri, Anari, Karichee)
Fermented milks

They can be divided in 3 main classes based on the metabolic products

i) lactic fermentations (e.g. Yoghurt, Laban, Bulgarian buttermilk)

ii) yeast-lactic fermentations (Kefir, Koumiss)

iii) mould-lactic fermentations (Villi)

Closely related products are manufactured from fermented milks by

a) de-wheying to concentrate the product, which could resemble soft
   cheese (e.g. Labneh)

b) drying of cereal/fermented milk mixture (e.g. Kishk or Trahana)

c) freezing fermented milk to resemble ice cream
Biochemistry of the Fermentation Process

Biochemical pathways leading to the formation of flavor compounds

The gray surface indicated compounds with a flavor note
Carbohydrates' catabolism
The first essential step in milk fermentations is the catabolism of milk lactose by the lactic acid bacteria (LAB).

The main end product is lactic acid (>50% of sugar carbon).

Lactic acid bacteria as a group exhibit an enormous capacity to degrade different carbohydrates and related compounds.

LAB adapt to various conditions and change their metabolism accordingly, yielding thus significantly different end product patterns.
Biochemistry of the Fermentation Process

- Lactose is taken up via the PTS and enters the cytoplasm as lactose-P.

- Lactose-P is cleaved by P-β-gal to yield glucose and galactose-6-P, which are further catabolized.

- The enzyme systems of lactose PTS and P-β-gal are generally inducible, and repressed by glucose.

- An equally common way for LAB to metabolise lactose is by means of a permease and subsequent cleavage by β-gal to yield glucose and galactose, which may again enter the major pathways.
Biochemistry of the Fermentation Process

- **Citrate** is present in milk (9 mM) and can also serve as energy source for LAB.

- Next to lactose, citrate metabolism plays an important role in milk fermentations.

- The ability of LAB to metabolize citrate is invariably linked to endogenous plasmid that contains the gene encoding the transporter, which is responsible for citrate uptake from the medium.
Citrate catabolism

2X citrate → 2X oxaloacetate + 2X acetate

2X oxaloacetate → 2X CO₂

2X CO₂ → 2X pyruvate + 2X NADH

2X NADH → 2X lactate + 2X NAD
Citrate catabolism

1. **CH₃CO-CoA**
   - 2X acetyl-CoA
   - 2X ADP
   - 2X ATP

2. **CH₃COOH**
   - 2X acetate
   - 2X acetate

3. **CHO**
   - 2X acetaldehyde

4. **HCOOH**
   - 2X formate

5. **a-acetolactate**
   - CO₂

6. **diacetyl**
   - NADH
   - NAD

7. **2X CO₂**
   - 2X NADH
   - 2X NAD

8. **2X pyruvate**
   - CO₂

9. **2X NADH**
   - 2X NAD

10. **2X formate**
    - 2X NADH
    - 2X NAD

11. **2X acetaldehyde**
    - 2X NADH
    - 2X NAD

12. **2X ethanol**
    - 2X NADH
    - 2X NAD
Biochemistry of the Fermentation Process

- Products of citrate catabolism, such as diacetyl, acetaldehyde and acetoin, have distinct aroma properties and influence significantly the quality of fermented foods.

- For instance, diacetyl determines the aromatic properties of fresh cheese, fermented milk, cream and butter.

- The production of $\text{CO}_2$ can add to the texture of some fermented dairy products.

- Not all LAB are able to metabolize citrate.

- *Lactococcus lactis*, *Leuconostoc sp.* and *Enterococcus* have been studied.
Proteolysis
## Main milk proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>g/l</th>
<th>% of total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Caseins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>αs-caseins</td>
<td>15-19</td>
<td>42</td>
</tr>
<tr>
<td>αs1</td>
<td>12-15</td>
<td>34</td>
</tr>
<tr>
<td>αs2</td>
<td>3-4</td>
<td>8</td>
</tr>
<tr>
<td>β-casein</td>
<td>9-11</td>
<td>25</td>
</tr>
<tr>
<td>κ-casein</td>
<td>3-4</td>
<td>9</td>
</tr>
<tr>
<td>γ-casein</td>
<td>1-2</td>
<td>4</td>
</tr>
<tr>
<td><strong>Whey proteins</strong></td>
<td>5-7</td>
<td>20</td>
</tr>
<tr>
<td>β-lactoglobulin</td>
<td>2-4</td>
<td>9</td>
</tr>
<tr>
<td>α-lactalbumin</td>
<td>1-1.5</td>
<td>4</td>
</tr>
<tr>
<td>proteoses-peptones</td>
<td>0.6-1.8</td>
<td>4</td>
</tr>
<tr>
<td><strong>blood proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>serum albumin</td>
<td>0.1-0.4</td>
<td>1</td>
</tr>
<tr>
<td>immunoglobins</td>
<td>0.6-1.0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>
Biochemistry of the Fermentation Process

✓ Proteolysis is the most complex event during cheese ripening

✓ It is important for flavour and texture development

✓ The main proteolytic agents in cheese manufacture are
  - the rennet
  - the indigenous milk protease (plasmin) and
  - the proteolytic enzymes of the microflora
Biochemistry of the Fermentation Process

[Diagram of biochemical structures]

O-H
CH₂
H₂N—C—COOH
H

serine

Casein Micelle

Casein Submicelle

hydrophobic core
CMP 'fatty' layer
κ-casein enriched surface
Ca₆(PO₄)₃ cluster
Biochemistry of the Fermentation Process

ρεννίνη

\[
\begin{align*}
\text{2HN...} & \text{Phe105} - \text{Met106} \ldots \text{COOH} \\
\text{2HN...} & \text{Phe105} \\
\text{παρα-κ-καζείνη} & \text{γλυκομακροπεπτίδιο}
\end{align*}
\]
Biochemistry of the Fermentation Process

✓ **Gastric proteinases** from calves, kids and lambs have been used traditionally as *rennet*.

✓ **Plant proteinases** (extracts from dried flowers of *Cynara cardunculus*) have been employed successfully for many centuries in Portugal and in Spain.

✓ They are claimed to be *similar* to chymosin in terms of *specificity and kinetic parameters*, but they produce higher levels of the ripening index than commercial animal rennet.
Biochemistry of the Fermentation Process

- **Plasmin** is the **indigenous milk proteinase**, and it is a trypsin-like serine proteinase.

- Its complex consists of the active **enzyme** (plasmin), its **zymogen** (plasminogen), **plasminogen activators** and **enzyme inhibitors**.

- **Activators** are associated with the casein micelles, while the **inhibitors** are in the **serum phase**.

- Plasmin is **active** on all caseins, especially **αs2- and β-**, but it shows very low activity on **κ-casein**.
Biochemistry of the Fermentation Process

- **Plasmin** activity is cheese is clearly indicated by the formation of γ-caseins from β-casein during ripening.

- **Pasteurization increases plasmin activity in milk**, probably because of the inactivation of plasmin inhibitors and by increasing the rate of plasminogen activation.

- In **hard cooked cheeses** like Swiss type cheeses or the Italian “grana” cheeses, **plasmin is mainly responsible** for the primary hydrolysis of caseins, since **rennet is almost inactivated** during cooking.
Biochemistry of the Fermentation Process

✓ LAB have limited abilities to synthesize amino acids

✓ Milk contains insufficient amounts of free amino acids to sustain LAB growth

✓ Although weak proteolytic bacteria, LAB possess a complex proteolytic system capable of hydrolysing milk proteins to peptides and amino acids

✓ Their proteolytic system contributes to the degradation of milk protein and hence to the texture, taste and aroma of dairy fermented products
oligopeptide transport system

di/tripeptide transport system

amino acids transport system

large oligopeptides

peptidases

small oligopeptides

peptidases

di/tripeptides

peptidases

amino acids
Biochemistry of the Fermentation Process

- **Amino acids**
  - Decarboxylation → **CO₂** → **Amines**
  - Oxidative deamination → **NH₃**
  - Transamination

- **Alpha-ketoacids**
  - Degradation

- **Aldehydes**
  - Reduction → **Alcohols**
  - Oxidation → **Acids**

- **Phenols**
  - **Indoles**

- **Sulfur compounds**
**Enzymes involved**

*AT*  aminotransferase  
*GDH*  glutamate dehydrogenase  
*HADH*  hydroxy acid dehydrogenase  
*KADH*  keto acid dehydrogenase  
*KADC*  keto acid decarboxylase  
*AlcDH*  alcohol dehydrogenase  
*AldDH*  aldehyde dehydrogenase
## Compounds resulting from Phe, Leu and Met degradation

<table>
<thead>
<tr>
<th>Type</th>
<th>Formula</th>
<th>Phe</th>
<th>Leu</th>
<th>Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-keto acid</td>
<td>R-CH2-CO-COOH</td>
<td>Phenylpyruvate</td>
<td>a-ketoisocaproate</td>
<td>a-keto methylthiobutyrate</td>
</tr>
<tr>
<td>Hydroxy acid</td>
<td>R-CH2-CHO-COOH</td>
<td>Phenyllactate</td>
<td>Hydroxyisocaproate</td>
<td>Hydroxy methylthiobutyrate</td>
</tr>
<tr>
<td>Carboxylic acid</td>
<td>R-CH2-COOH</td>
<td>Phenylacetic acid</td>
<td>Isovaleric acid</td>
<td>3-Methylthiopropionic acid</td>
</tr>
<tr>
<td>Aldehyde</td>
<td>R-CH2-CHO</td>
<td>Phenylacetaldehyde</td>
<td>3-Methylbutanal or isovaleraldehyde</td>
<td>Methionol</td>
</tr>
<tr>
<td>Alcohol</td>
<td>R-CH2-CHOH</td>
<td>3-Methylbutanol</td>
<td>Methional</td>
<td></td>
</tr>
<tr>
<td>Other volatile S-compounds</td>
<td></td>
<td></td>
<td></td>
<td>Methanethiol, dimethyl disulfide, dimethyl trisulfide</td>
</tr>
</tbody>
</table>
**Butter**

- diacetyl
- butter flavor

**Parmesan**

- 3-methyl-butanal
- spicy cocoa flavor

**Emmental**

- isovalerate
- isobutyrate
- sweaty notes

- phenylacetate
- phenylacetaldehyde
- fruity notes
Biochemistry of the Fermentation Process

ARGININE  \[\rightarrow\]  PUTRESCINE
\[H_2N-\text{CH}-(\text{CH}_2)_6-\text{CH}_2NH_2\]

ORNITHINE

PUTRESCINE  \[\rightarrow\]  SPERMIDINE
\[H_2N-\text{CH}-(\text{CH}_2)_4-\text{CH}-(\text{CH}_2)_4-\text{CH}-(\text{CH}_2)_6-\text{CH}_2NH_2\]

DSAM  \[\rightarrow\]  MTA

SPERMIDINE  \[\rightarrow\]  SPERMINE
\[H_2N-\text{CH}-(\text{CH}_2)_4-\text{CH}-(\text{CH}_2)_4-\text{CH}-(\text{CH}_2)_6-\text{CH}_2NH_2\]

LYSINE  \[\rightarrow\]  CADAVERINE
\[H_2N-\text{CH}-(\text{CH}_2)_2-\text{CH}-(\text{CH}_2)_6-\text{CH}_2NH_2\]

HISTIDINE  \[\rightarrow\]  HISTAMINE
\[\text{N}-(\text{CH}_2)_2-\text{NH}_2\]

TYROSINE  \[\rightarrow\]  TYRAMINE
\[\text{HO-CH}-(\text{CH}_2)_2-\text{NH}_2\]

TRYPTOPHAN  \[\rightarrow\]  TRYPTAMINE
\[\text{(CH}_2)_2-\text{NH}_2\]

Biogenic amines
**Biochemistry of the Fermentation Process**

- **Bioactive peptides** present in the amino acid sequence of milk proteins especially from β-casein (β-casomorphins).

- Although other animal as well as plant proteins contain potential bioactive sequences, milk proteins are currently the main source of a range of biologically active peptides.

- The structures of biologically active sequences were obtained from *in vitro* enzymatically and/or by *in vivo gastrointestinal digest* of the appropriate precursor proteins.
### Some known casomorphins

<table>
<thead>
<tr>
<th><strong>β-Casomorphin 1-3</strong></th>
<th>H-Tyr-Pro-Phe-OH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-Casomorphin 1-4</strong></td>
<td>H-Tyr-Pro-Phe-Pro-OH</td>
</tr>
<tr>
<td><strong>β-Casomorphin 1-4, amide</strong></td>
<td>H-Tyr-Pro-Phe-Pro-NH2</td>
</tr>
<tr>
<td><strong>β-Casomorphin 5</strong></td>
<td>H-Tyr-Pro-Phe-Pro-Gly-OH</td>
</tr>
<tr>
<td><strong>β-Casomorphin 7</strong></td>
<td>H-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-OH</td>
</tr>
<tr>
<td><strong>β-Casomorphin 8</strong></td>
<td>H-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-OH</td>
</tr>
</tbody>
</table>
Biochemistry of the Fermentation Process

✓ **Bioactive peptides** are potential **modulators of various regulatory processes** in human body

- **Opioid peptides** are

  opioid receptor ligands,

  which can modulate absorption processes in the intestinal tract

- **Angiotensin-I-converting enzyme (ACE)-inhibitory peptides** are

  hemodynamic regulators and exert an antihypertensive effect
Biochemistry of the Fermentation Process

- **Immunomodulating casein peptides**
  
  stimulate the activities of cells of the immune system

- **Antimicrobial peptides**
  
  kill sensitive microorganisms

- **Antithrombotic peptides**
  
  inhibit aggregation of platelets
  
  caseinophosphopeptides may function as carriers
  
  for different minerals, especially Ca
Lipolysis
Biochemistry of the Fermentation Process

The main lipolytic agents in cheese include the

- indigenous milk lipoprotein lipase

- lipases and esterases produced by

  the starter and non-starter bacteria

and depending on the cheese variety

- enzyme preparations added during manufacturing
Biochemistry of the Fermentation Process

- Milk lipase causes significant lipolysis in raw milk cheese

- It is inactivated during pasteurisation at 76°C for 10 sec

- It is selective for fatty acids at the sn-3 position, where the most of the butyric acid in milk fat is esterified

- In most cheese varieties relatively little lipolysis occurs, with the exception of mould-ripened cheeses
LAB are generally considered as low lipolytic.

LAB lipolytic system has not been extensively studied compared to the proteolytic system, although there are several reports on lipases and esterases of LAB.

Vital information on their cellular location of all these enzymes, their specificity as well as their overall role during ripening remains to be determined.
Bacteriocins
Biochemistry of the Fermentation Process

The **bacteriocins** produced by LAB are antimicrobial peptides

- ribosomally synthesised
- small
- cationic
- amphiphilic (rather hydrophobic)

which vary in

- spectrum of activity
- molecular structure
- molecular mass
- thermostability
- pH range of activity
Biochemistry of the Fermentation Process

**Class I bacteriocins** = lantibiotics

- small, cationic, hydrophobic, and heat-stable peptides
- contain unusual amino acids (lanthionine and/or 3-methyl-lanthionine)

**Class Ia lantibiotics**

- cationic and hydrophobic peptides
- with flexible, linear structure
- act by forming pores in target membranes
- (e.g. nisin produced by several strains of *Lactococcus lactis*)

**Class Ib lantibiotics**

- rigid globular peptides with no net charge or a net negative charge
- (e.g. mersacidin produced by *Bacillus subtilis*)
Schematic representation of the structure of the lantibiotic nisin A
Dehydrations of serines and threonines yield 2,3-didehydroalanines (Dha) and 2,3-didehydrobutyrines (Dhb), respectively.

The formation of thioether bonds between cysteines and dehydrated residues results in lanthionine (Lan: Ala-S-Ala) and 3-methyllanthionine (MeLan: Abu-S-Ala, in which Abu is an aminobutyric acid).

Prepeptide maturation and primary structure of lantibiotics.
Gene clusters involved in production of, and immunity to, lantibiotics
Schematic representation of nisin biosynthesis and regulation in *Lactococcus lactis*
Hijacking of lipid II for pore formation

(a) The structure of lipid II is composed of an N-acetylglucosamine-β-1,4-N-acetylmuramic acid disaccharide (GlcNAc–MurNAc), attached by a pyrophosphate to a membrane anchor of 11 isoprene units (undecaprenylpyrophosphate). A pentapeptide is attached to the muramic acid. Transpeptidase and transglycosylase enzymes cross-link the disaccharide and pentapeptide groups of multiple lipid II molecules to generate the peptidoglycan.

(b) The amino-terminus of nisin binds lipid II while the carboxy-terminus inserts into the bacterial membrane. Four lipid II and eight nisin molecules have been shown to comprise a stable pore, although the arrangement of the molecules within each pore is currently unknown.

(c) The NMR solution structure of the 1:1 complex of nisin and a lipid II derivative in DMSO. Nisin is illustrated in red, the prenyl chain of lipid II is depicted in white, the phosphate atoms of the pyrophosphate group are shown in orange, and the muramic acid is portrayed in blue. The N-acetylglucosamine (GlcNAc) is shown in magenta and the pentapeptide chain is presented in yellow.
Biochemistry of the Fermentation Process

Class II bacteriocins

small, cationic, hydrophobic, heat-stable peptides

that are not post-translationally modified

Class IIa pediocin-like bacteriocins with strong antilisterial effect

Class IIb two-peptide bacteriocins

Class IIc bacteriocins that do not belong to the other subgroups

Class III bacteriocins

large, hydrophilic, heat-labile proteins
**Macedocin, the lantibiotic produced by** *S. macedonicus*

<table>
<thead>
<tr>
<th>Leader peptide</th>
<th>propeptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>macedocin</td>
<td>-MEKETTIIESIQEVSLLELDQIIGA --GKNGVFKTISHECHLNTWAFLATCCS</td>
</tr>
<tr>
<td>Streptococcin A-M49</td>
<td>-MTKEHEINIQSIEVSLEELDQIIGA --GKNGVFKTISHECHLNTWAFLATCCS</td>
</tr>
<tr>
<td>Streptococcin A-FF22</td>
<td>-MEKNNEVINSIQEVSLLELDQIIGA --GKNGVFKTISHECHLNTWAFLATCCS</td>
</tr>
<tr>
<td>Lacticin 481</td>
<td>--MKEQNSFNQLEVSLEDLILGA --KGSSGVIHTISCECNNSWQFVFTCCS</td>
</tr>
<tr>
<td>Butyrivibriocin OR79</td>
<td>---MNKEEALNALTNPIDEKELEQILGG ---GNGVIKTISHECMNWFQVFTCCS</td>
</tr>
<tr>
<td>Variacin</td>
<td>------MTNAPQALDEVTDAELDAILG-- --GGSVPIHTSHECMNWFQVFTCCS</td>
</tr>
<tr>
<td>Mutacin II</td>
<td>MNKLNSNAVVSLNEVSDSELDTILGG NRWWQGVPTVSVECRMSWQHVFTCC-</td>
</tr>
</tbody>
</table>

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**mac cluster**

**Regulation** | **Biosynthetic** | **Immunity**

**tnpA partial**

**scn cluster**
Macedocin, the lantibiotic produced by *S. macedonicus*

**Induction Factor**

of macedocin biosynthesis

Macedocin is produced only when *S. macedonicus* is grown in milk!

Peptides of low molecular mass corresponding to fragments of

- $\beta$-lactoglobulin
- $\alpha_{S1}$-casein
- $\beta$-casein

**Inhibitory spectrum**

of macedocin
# Inhibitory spectrum of macedocin

<table>
<thead>
<tr>
<th>Indicator organism</th>
<th>Macedocin activity (mm inhibition zone)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. agalactiae</em> LMG 14694T</td>
<td>8</td>
</tr>
<tr>
<td><em>S. anginosus</em> LMG 14502T</td>
<td>9</td>
</tr>
<tr>
<td><em>S. bovis</em> LMG 8518T</td>
<td>7</td>
</tr>
<tr>
<td><em>S. equinus</em> LMG 14897T</td>
<td>10</td>
</tr>
<tr>
<td><em>S. gordonii</em> LMG 14518T</td>
<td>10</td>
</tr>
<tr>
<td><em>S. mutans</em> LMG 14558T</td>
<td>0</td>
</tr>
<tr>
<td><em>S. oralis</em> LMG 14532T</td>
<td>10</td>
</tr>
<tr>
<td><em>S. pneumoniae</em> LMG 14545T</td>
<td>13</td>
</tr>
<tr>
<td><em>S. pyogenes</em> LMG 21599T</td>
<td>13</td>
</tr>
<tr>
<td><em>S. salivarius</em> LMG 11489T</td>
<td>7</td>
</tr>
<tr>
<td><em>S. sobrinus</em> LMG 14641T</td>
<td>0</td>
</tr>
<tr>
<td><em>S. thermophilus</em> LMG 6896T</td>
<td>15</td>
</tr>
<tr>
<td><em>Lactococcus lactis</em> ACA-DC 6890T</td>
<td>15</td>
</tr>
</tbody>
</table>
**S. macedonicus in Kasseri cheese**

**Enumeration of**
**S. macedonicus**
on M17 agar,
supplemented with
25 μg/ml streptomycin,
throughout ripening of Cheese C

Species-specific PCR of *S. macedonicus* colonies isolated throughout ripening of Cheese C

1: B1 (unripened Baski) 2: B2 (ripened Baski)
3: K0 (Kasseri d0) 4: K7 (Kasseri d7)
5: K15 (Kasseri d15) 6: K30 (Kasseri d30)
7: K60 (Kasseri d60) 8: K90 (Kasseri d90)
9: positive control 10: blank
Macedocin detection in Kasseri cheese during ripening

Cheese samples heated at 80°C for 10 min

Cheese extracts

1: B1  2: B2  3: K0  4: K7
5: K15  6: K30  7: K60  8: K90

In the center the negative control (Cheese A)
Inhibition of *Clostridium tyrobutyricum* by *S. macedonicus ACA-DC 198* under conditions simulating Kasseri cheese production

*Clostridium* Spores

*Clostridium* Vegetative cells
Other fermented foods
Lactic acid bacteria in sourdough

Inhibition of *Bacillus cereus* in bread prepared from sourdough containing *Lactobacillus sanfranciscensis*
Lactic acid bacteria in wine

\[ \text{Malic acid} \rightarrow Oenococcus oeni \rightarrow \text{Lactic acid} \]

The malolactic fermentation in wine

- Decreases the acidity of the wine and
- Influences the flavor of wine
Lactic acid bacteria in beer

Effect of *L. plantarum* on *Fusarium* during malting of barley

Effect of *L. plantarum* on mash filterability