Meat starter cultures

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Dr. Till Albrecht
History of starter cultures

1866 - PASTEUR discovered micro-organisms as source for fermentation processes

1892 - Chr. Hansen starts selling the first commercial starter cultures for the dairy industry

1935/1940 - JENSEN and PADDOCK established the usage of lactic acid bacteria for the ripening of fermented sausage in the USA
History of starter cultures

1955 - Dissertation of NIINIVAARA „Über den Einfluss von Bakterienkulturen auf die Reifung und Umrötung der Rohwurst“ is commonly accepted as birth of defined starter cultures for meat fermentation

1966 - NURMI develops the first mixture of lactic acid bacteria and micrococci as a starter preparation

1972 - First International Symposium of Starter Cultures in Helsinki helps to get starters accepted by the butchers and the meat industry
Definition of starter culture

Starter cultures are preparations of live microorganisms or their resting forms, whose metabolic activity has desired effects in the fermentation substrate, the food.

The preparations may contain unavoidable residues from the culture substrate and additives that support the vitality and technological functionality of the microorganisms (such as antifreeze or antioxidant compounds).

- Single-strain cultures: contain one strain of a species;
- Multi-strain cultures: contain more than one strain of a single species;
- Multi-strain mixed cultures: contain different strains from different species.

Source: Senate Commission on Food Safety of the DFG, 2010.
Microbial ecology of meat fermentation

**Endogenous factors**

**Meat**
- type / quality (e.g. beef, pork)
- fat content
- reduction rate

**Casing**
- Size

**Curing agent**
- (salt, nitrate, nitrite)

**Spices**

**Other ingredients**
- ascorbic acid
- sodium glutamate
- glucono δ-lactone
- sugar

**Meat, spice and environmental biota**

**Exogenous factors**

**Temperature**

**Relative humidity**

**Oxygen (airflow)**

**Smoke**

**Fermentation time**

**Starter culture**

**Starter organisms**

**Implicit factors, e.g. bacteriocins**

**Contaminating biota**

**Product quality**
- Color
- Flavor
- Texture
- Shelf life
Advantages of use of starter cultures

- Reduction of hygienic risks
- Ensuring constant high product quality
- Control of development of color and flavor
- Control of fermentation time
- Prevention of fault fermentation

→ Reduction of costs by shortening fermentation times and assures production of products of high safety and sensory quality
Key components of R&D activities in the starter culture business

• Screening for strains with desired properties
  - microbiology (metabolism, performance, etc.)
  - meat technology (acidification, development of flavor and color, etc.)
  - safety (bacteriocins, antagonistic principles, etc.)

• Safety assessment of strains
  - identity
  - possible pathogenicity
  - acquired antibiotic resistances

• Check for producibility of strains
  - yield (fermentation, freeze-drying)
  - stability
  - functionality
# Antagonistically acting metabolites and substances of lactic acid bacteria

<table>
<thead>
<tr>
<th>Metabolite / substance</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organic acids</strong></td>
<td></td>
</tr>
<tr>
<td>Lactic acid, acetic acid, propionic acid, formic acid, benzoic acid</td>
<td>Partly in use as additive</td>
</tr>
<tr>
<td><strong>Phenyllactate</strong></td>
<td>Antifungal</td>
</tr>
<tr>
<td><strong>Other metabolites</strong></td>
<td></td>
</tr>
<tr>
<td>Reuterin (3-Hydroxypropionaldehyde)</td>
<td>Bacteria, yeasts, moulds, protozoa</td>
</tr>
<tr>
<td>Diacetylene</td>
<td>In use as flavoring agent</td>
</tr>
<tr>
<td><strong>3-Hydroxy fatty acids</strong></td>
<td>Antifungal</td>
</tr>
<tr>
<td><strong>Other antagonistic substances</strong></td>
<td></td>
</tr>
<tr>
<td>Reutericyclin</td>
<td>Tetramic acid</td>
</tr>
<tr>
<td><strong>Cyclic dipeptide</strong></td>
<td>Antifungal</td>
</tr>
<tr>
<td><strong>Bacteriocin</strong></td>
<td></td>
</tr>
<tr>
<td>Nisin</td>
<td>In use as additive</td>
</tr>
<tr>
<td>Others bacteriocins</td>
<td>Lantibiotics, Class II and cyclic bacteriocins</td>
</tr>
</tbody>
</table>
Origin of starter organism for food fermentation

Indigenous flora of traditional fermented food

Own strain collection

Public strain collections

Screening of hundreds of strains is required!
Requirements to starter organisms

Production of the culture

- Optimal performance in an inexpensive artificial medium
- High cell densities ($10^{10} - 10^{11}$ CFU/ml)
- High survival rate during lyophilization
- High storage stability

Fermenter at BITEC
Production of starter cultures

Preserve → Preculture → Main culture

Separation

Packaging → Blending → Grinding → Lyophilization
Requirements to starter organisms

Production of the culture

- Optimal performance in an inexpensive artificial medium
- High cell densities \((10^{10} - 10^{11} \text{ CFU/ml})\)
- High survival rate during lyophilization
- High storage stability

Food fermentation

- Optimal performance (high competitiveness) in a complex food matrix, where ecological conditions are constantly changing
- Short lag phase (fast pH drop)
- Tolerance against prevailing ecological conditions (low pH)
- Expression of properties contributing to product quality
## Prevailing ecological conditions

<table>
<thead>
<tr>
<th>Factor</th>
<th>Fermenter</th>
<th>Sausage meat</th>
<th>Raw fermented sausage</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.8 – 6.5</td>
<td>5.6 – 5.9</td>
<td>4.8 – 5.3 (5.8)</td>
</tr>
<tr>
<td>Temperature</td>
<td>25 – 37°C</td>
<td>0 bis 2°C</td>
<td>RT</td>
</tr>
<tr>
<td>Water activity ($a_W$)</td>
<td>0.99</td>
<td>0.96 – 0.97</td>
<td>0.85 – 0.93 (0.95)</td>
</tr>
<tr>
<td>Salt content</td>
<td>&lt; 0.1%</td>
<td>2.6 – 3.0%</td>
<td>&gt; 2.6 – 3.0%</td>
</tr>
<tr>
<td>Sugar content</td>
<td>2.0 – 3.0%</td>
<td>0.2 – 0.7%</td>
<td>0%</td>
</tr>
<tr>
<td>Nitrite (NPS)</td>
<td>0</td>
<td>130 – 150 ppm</td>
<td>&lt; 150 ppm</td>
</tr>
<tr>
<td>Redoxpotential</td>
<td>adjusted</td>
<td>high</td>
<td>low (high at surface)</td>
</tr>
</tbody>
</table>
### Effect of starter cultures on the spontaneous flora

<table>
<thead>
<tr>
<th>Day</th>
<th>Cell number of the lactic acid bacteria (cfu / g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture 1</td>
</tr>
<tr>
<td>1</td>
<td>$1,0 \times 10^9$</td>
</tr>
<tr>
<td>7</td>
<td>$1,5 \times 10^9$</td>
</tr>
<tr>
<td>42</td>
<td>$9,1 \times 10^8$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>Cell count of the spontaneous flora of lactic acid bacteria (cfu / g)</th>
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<tr>
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<td>n. d.</td>
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<td>7</td>
<td>n. d.</td>
</tr>
<tr>
<td>42</td>
<td>n. d.</td>
</tr>
</tbody>
</table>
Technologically relevant properties of starter organisms

- Relationship to oxygen
- Type of fermentation
- Salt tolerance
- Nitrite tolerance
- pH tolerance
- Temperature range
- Spectrum of fermentable sugars
- Physiological enzyme activities
- Properties involved in the safety assessment
Potential of lactic acid bacteria from meat to form hydrogen peroxide and to exhibit catalase activity

<table>
<thead>
<tr>
<th>Species</th>
<th>Formation of $\text{H}_2\text{O}_2$</th>
<th>Presence of the activity of catalase (heme-dependent)</th>
<th>Presence of the activity of pseudocatalase (Mn-dependent)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus curvatus</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Lactobacillus sakei</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Pediococcus pentosaceus</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Pediococcus acidilactici</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

$$2 \text{H}_2\text{O}_2 \leftrightarrow \text{O}_2 + 2 \text{H}_2\text{O}$$
Technologically relevant properties of starter organisms

- Relationship to oxygen
- **Type of fermentation**
- Salt tolerance
- Nitrite tolerance
- pH tolerance
- Temperature range
- Spectrum of fermentable sugars
- Physiological enzyme activities
- Properties involved in the safety assessment
Fermentation type

**Heterofermentative lactic acid fermentation**

- Glucose
  - Fructose-1-6-bP
    - [Aldolase]
      - 2 Triose-3-P
        - 2 ADP
        - 2 ATP
        - 2 Pyruvate
        - 2 Lactate
  - Glucose-6-P
    - 2 NAD^+ (oxidation)
    - 2 NADH (reduction)
    - Xylulose 5-P + CO₂
      - [Phosphoketolase]
        - Triose-3-P
        - Acetyl-P
      - Xylulose 5-P
        - Acetyl-P
        - Triose-3-P
        - 2 ADP
        - 2 ATP
        - Pyruvate
        - Acetate
        - Lactate

**Gluconate metabolizing:**
- *L. sakei* strains: positive or (+)
- *L. curvatus* strains: negative

**Gluconic acid**
- GdL

**Homofermentative lactic acid fermentation**

- Glucose
  - Fructose-1-6-bP
    - [Aldolase]
      - 2 Triose-3-P
        - 2 ADP
        - 2 ATP
        - 2 Pyruvate
        - 2 Lactate
  - Glucose-6-P
    - 2 NAD^+ (oxidation)
    - 2 NADH (reduction)
    - Xylulose 5-P + CO₂
      - [Phosphoketolase]
        - Triose-3-P
        - Acetyl-P
      - Xylulose 5-P
        - Acetyl-P
        - Triose-3-P
        - 2 ADP
        - 2 ATP
        - Pyruvate
        - Acetate
        - Lactate

**L(+) : D(-) lactic acid**
- 50 : 50
- right-handed / left-handed
- physiological / non-physiological
Technologically relevant properties of starter organisms

- Relationship to oxygen
- Type of fermentation
- **Salt tolerance**
- **Nitrite tolerance**
- pH tolerance
- Temperature range
- Spectrum of fermentable sugars
- Physiological enzyme activities
- Properties involved in the safety assessment
Salt tolerance and/or nitrite tolerance

- Water activity is the ratio of the water vapor pressure \((p)\) above the food and the water vapor pressure above pure water \((p_0)\):
  \[
a_w = \frac{p}{p_0}
\]

<table>
<thead>
<tr>
<th></th>
<th>Water content</th>
<th>[salt]</th>
<th>(a_w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork meat S II</td>
<td>73%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back bacon S VIII</td>
<td>8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formula with 30% fat and 3% salt</td>
<td>53%</td>
<td>5.2%</td>
<td>0.97</td>
</tr>
<tr>
<td>Drying of 15%</td>
<td></td>
<td>7.1%</td>
<td>0.95</td>
</tr>
<tr>
<td>Drying of 30%</td>
<td></td>
<td>11.3%</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Raw sausage \(a_w < 0.91\) – Termination of bacterial growth and metabolism!
Enzymes are still active – proteolysis / lipolysis are further running!

Nitrite tolerance of our starter organism meet the salt tolerance.
Technologically relevant properties of starter organisms

- Relationship to oxygen
- Type of fermentation
- Salt tolerance
- Nitrite tolerance
- pH tolerance
- Temperature range
- Spectrum of fermentable sugars
- Physiological enzyme activities
- Properties involved in the safety assessment
Effect of the salt concentration on the kinetics of pH during raw sausage fermentation
(0.4 % dextrose, 24°C)

**Nitrate**

**Nitrite**
Effect of the fat content on the kinetics of pH during raw sausage fermentation
(0.4% dextrose, nitrite curing salt, 24°C)

The fat content corresponds to the content of added fat. The absolute value is 8% higher.

L. sakei from LS-25
Technologically relevant properties of starter organisms

- Relationship to oxygen
- Type of fermentation
- Salt tolerance
- Nitrite tolerance
- pH tolerance
- Temperature range
- Spectrum of fermentable sugars
- Physiological enzyme activities
- Properties involved in the safety assessment
Effect of the temperature on the kinetics of pH during raw sausage fermentation (0.4% dextrose, nitrite curing salt)
Effect of the temperature on the kinetics of pH during raw sausage fermentation (0.4% dextrose, nitrate)
Technologically relevant properties of starter organisms

- Relationship to oxygen
- Type of fermentation
- Salt tolerance
- Nitrite tolerance
- pH tolerance
- Temperature range
- Spectrum of fermentable sugars
- Physiological enzyme activities
- Properties involved in the safety assessment
Effect of different sugars on the kinetics of pH during raw sausage fermentation
(0.4% dextrose, nitrite curing salt, 24°C)

L. curvatus from ADVANCE RD-1
Effect of the dextrose concentration on the kinetics of pH during raw sausage fermentation
(0.4% dextrose, nitrite curing salt, 24°C)

[L. curvatus from ADVANCE RD-1]
Technologically relevant properties of starter organisms

- Relationship to oxygen
- Type of fermentation
- Salt tolerance
- Nitrite tolerance
- pH tolerance
- Temperature range
- Spectrum of fermentable sugars
- Physiological enzyme activities
- Properties involved in the safety assessment
Nitric oxide formation during curing

**pH drop**

- Lactic acid bacteria → Lactic acid
- GdL + H₂O → Gluconic acid

**Redox system**

\[ 2 \text{HNO}_2 + \text{C}_6\text{H}_8\text{O}_6 \rightarrow 2 \text{NO} + 2 \text{H}_2\text{O} + \text{C}_6\text{H}_6\text{O}_6 \]

- Ascorbic acid
- Dehydro ascorbic acid

**Enzymatic redox systems of meat**

- Cystein cystin system
  \[ 2 \text{NO}_2^- + 2 \text{R-SH} + 2 \text{H}_3\text{O}^+ \rightarrow 2 \text{NO} + \text{R-S-S-R} + 2 \text{H}_2\text{O} \]

- Ferro cytochrome C system
  \[ \text{NO}_2^- + \text{Cyt-c (red)} \rightarrow \text{NO-Cyt-c (ox)} \]
Flavor formation in meat fermentation

**Taste**
- Meat enzymes
- Starter culture
- Meat proteins
  - Cathepsin D
  - Calpain
- Peptides
- Carbohydrate
- Lactate
- Acetate
- Ethanol
- Acetoin
- Diacetyl
- Amino acids

**Aroma**
- Meat enzymes
- Staphylococci
- Fat
- Lipase
- Oxidative degradation
- Free fatty acids
- Fatty acids
  - Alkane
  - Alkene
  - Alcohols
  - Aldehydes
  - Ketone
  - Acids
- Amino acids
  - Leu
  - Ile
  - Val

- Key aroma compounds of fermented sausages:
  - 3-Methylbutanal (Leu)
  - 2-Methylbutanal (Ile)
  - 2-Methylpropanal (Val)

- Mainly peptidases of *Staphylococcus* spec.
- Exo-peptidases of *Lactobacillus* spec.
Technologically relevant properties of starter organisms

- Relationship to oxygen
- Type of fermentation
- Salt tolerance
- Nitrite tolerance
- pH tolerance
- Temperature range
- Spectrum of fermentable sugars
- Physiological enzyme activities
- Properties involved in the safety assessment
The Qualified Presumption of Safety (QPS) system

Four pillars

• Identity
• Body of knowledge
• Safety concerns
  - possible pathogenicity
  - acquired antibiotic resistances
• End use

Source:
# Species in FRUTAROM’s meat starter cultures

<table>
<thead>
<tr>
<th>Organism</th>
<th>Function</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactic acid bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus sakei</em></td>
<td>Acidification</td>
<td>Preservation</td>
</tr>
<tr>
<td><em>Lactobacillus curvatus</em></td>
<td>Possibly, formation of bacteriocins</td>
<td>Contribution to the formation of flavor, texture and red color</td>
</tr>
<tr>
<td><em>Lactobacillus paracasei</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pediococcus acidilactici</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pediococcus pentosaceus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Catalase-positive cocci</strong></td>
<td>Nitrate reduction</td>
<td>Formation and/or stabilization of flavor and red color</td>
</tr>
<tr>
<td><em>Staphylococcus carnosus</em></td>
<td>Proteolysis and lipolysis</td>
<td></td>
</tr>
<tr>
<td><em>subsp. utilis</em></td>
<td>Cleavage of $\text{H}_2\text{O}_2$ (catalase)</td>
<td></td>
</tr>
<tr>
<td><em>Kocuria salsicia</em></td>
<td>Reduction of redox potential</td>
<td></td>
</tr>
<tr>
<td><strong>Molds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Penicillium nalgiovense</em></td>
<td>Proteolysis and Lipolysis</td>
<td>Formation of flavor</td>
</tr>
<tr>
<td><em>Penicillium candidum</em></td>
<td>Growth on the surface</td>
<td>Prevention of growth of undesired organisms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protection against water loss, oxygen and light</td>
</tr>
</tbody>
</table>
### Starter cultures for slow raw sausage fermentation

<table>
<thead>
<tr>
<th>Culture</th>
<th>Species</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>LK-30</td>
<td><em>L. sakei, S. carnosus, K. salsicia</em></td>
<td>Harmonic pH-drop, highly competitive</td>
</tr>
<tr>
<td>LK-30 plus</td>
<td><em>L. sakei, L. paracasei, S. carnosus, K. salsicia</em></td>
<td>Milder than LK-30</td>
</tr>
<tr>
<td>LKB-5</td>
<td><em>L. sakei, S. carnosus, K. salsicia</em></td>
<td>Harmonizes and assures acidification process</td>
</tr>
<tr>
<td>LS-1</td>
<td><em>L. curvatus, S. carnosus, K. salsicia</em></td>
<td>Evenly pH-drop, highly competitive</td>
</tr>
</tbody>
</table>
Kinetics of pH of starter cultures for slow fermentation

0.4 % dextrose and 2.8 % nitrite curing salt.
Temperature: 21°C
### Starter cultures for fast raw sausage fermentation

<table>
<thead>
<tr>
<th>Culture</th>
<th>Species</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS-25</td>
<td><em>L. sakei, S. carnosus</em></td>
<td>fast pH drop, highly competitive</td>
</tr>
<tr>
<td>LS-25 plus</td>
<td><em>L. sakei, L. paracasei, S. carnosus</em></td>
<td>milder than LS-25</td>
</tr>
<tr>
<td>LS-3</td>
<td><em>L. curvatus, S. carnosus</em></td>
<td>fast pH drop, highly competitive</td>
</tr>
<tr>
<td>CONDI rasant</td>
<td><em>P. pentosaceus, S. carnosus</em></td>
<td>Fast pH drop, suitable for high temperatures</td>
</tr>
<tr>
<td>LSBA-15</td>
<td><em>L. sakei, S. carnosus, K. salsicia</em></td>
<td>Bacteriocin producer</td>
</tr>
<tr>
<td>ADVANCE LD-20</td>
<td><em>L. sakei, S. carnosus</em></td>
<td>Very fast pH drop, Extra mild Highly competitive</td>
</tr>
</tbody>
</table>
Kinetics of pH of starter cultures for fast fermentation

0.4 % dextrose and 2.8 % nitrite curing salt.
Temperature: 24°C
BITEC ADVANCE LD-20 (launch: 2012)

Composition

Multi-strain mixed culture

Lactobacillus sakei
Staphylococcus carnosus

Properties

• Mild acid taste
• Fast acidification
• Good development and stabilisation of the color
• Highly competitive

Application

• Sliceable, spreadable, and fresh fermented sausages
• Fermentation with nitrite curing salt
• Fermentation nitrate and salt
Examples of pH drops in different fermenting sausages produced with ADVANCE LD-20

![Graph showing pH drops over time for different types of sausages produced with ADVANCE LD-20. The graph includes lines for Beef, meager (5 g/kg dextrose), Beef, rich (5 g/kg dextrose), Pork, meager (4 g/kg dextrose), Pork, rich (4 g/kg dextrose), and Pork, meager (6 g/kg dextrose).]
Kinetics of pH of BITEC ADVANCE LD-20
(pork, 24 °C, nitrite curing salt)
Thank you!