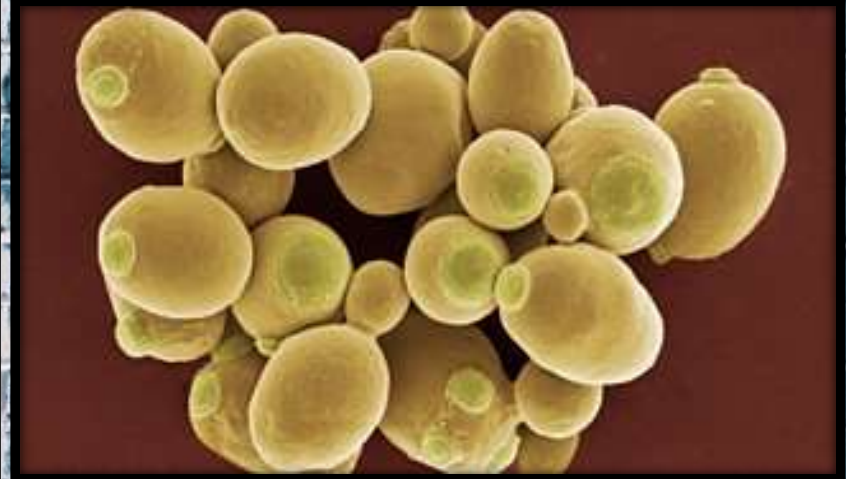


A petri dish containing a dark blue agar medium with several distinct mold colonies. The colonies vary in color, including bright yellow, vibrant green, and pale white. Some colonies are circular and well-defined, while others are more irregular and fuzzy. The background is black, making the colors of the mold stand out.

**DIVERSITY AND CONTROL
OF SPOILAGE FUNGI IN
DAIRY PRODUCTS**

Introduction

Fungi



Yeast



Mold

Estimated that 5% - 10% of the world's food production is lost due to fungal deterioration

**Dairy
Product are
less
susceptible to
fungal
spoilage**



Heat treated milk

Keep in refrigerator

Fermented products

Acidic pH



Undesirable Species

Penicilium spp.

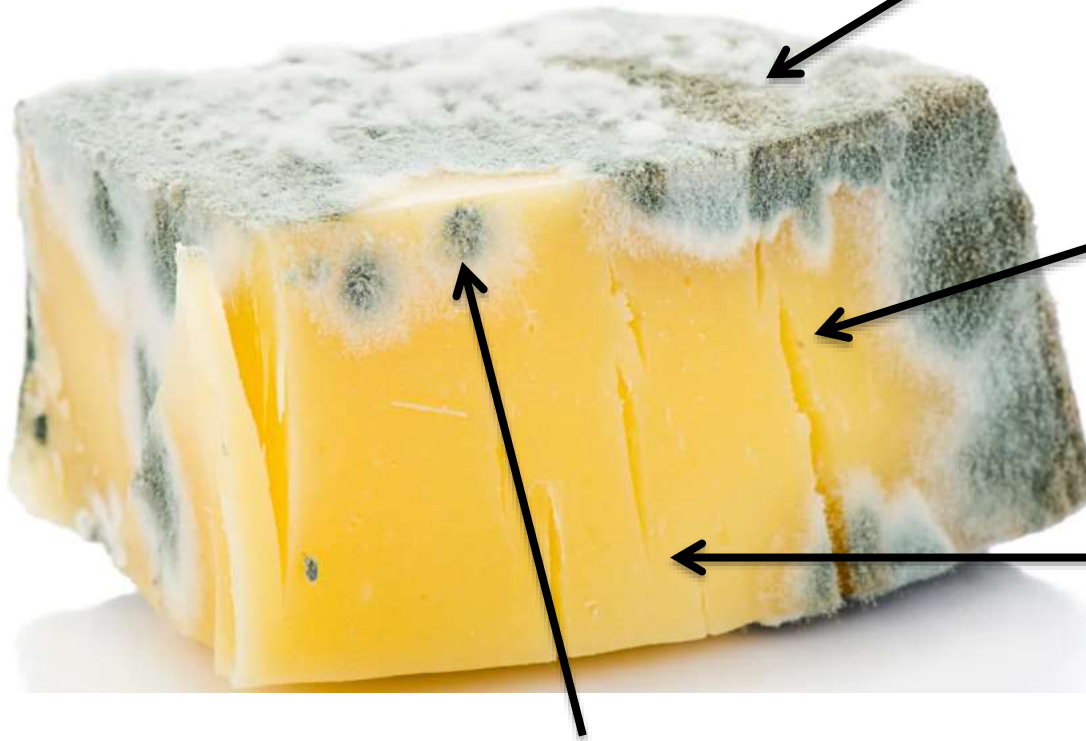
Aspergillus spp.

Visible growth of fungus at product surface

Off odour and flavors

Visible changes in colour and texture

Mycotoxins



**The Stable and
milking parlor
environment**

**Customer's improper
handling and care**

Teat surface

**Sources of Fungal
Contamination**

Airborne Fungi

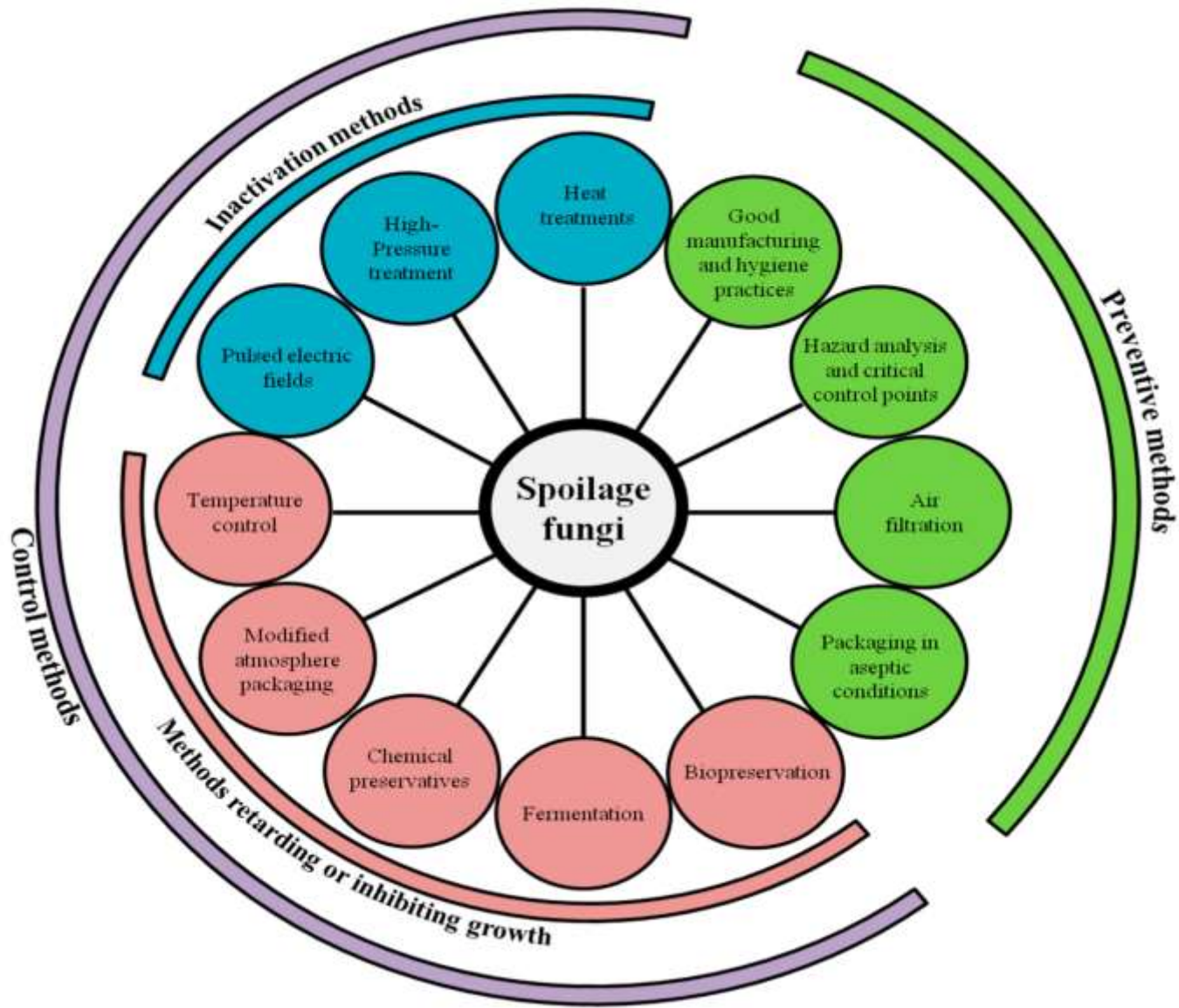
**Packaging
Materials**

Cheese Rind

**Contaminated
surface, equipment,
or ingredients**



**Prevention
and Control
of Fungal
Spoilage in
Dairy Product**



Preventive and control method used in dairy products

Preventive Methods

Good Manufacturing and Distribution Practice

Two essential points:

- i. Selecting good quality raw materials and monitoring the manufacturing process to prevent cross contamination
- ii. Avoid or retard microbial growth

This code relies on the use of GHP, HACCP and other Prerequisite Programs (Total Quality Management, TQM)

Preventive Methods

Air Filtration and Decontamination System

- Effective air-filtration system to reduce spore counts into the air where the product is vulnerable.
 - Carefully examine the direction of the air flows and the location of outlets.
 - Control of air pressurization to prevent air flow from dirtiest to cleanest zones
 - Cleanroom technology
-
- Air decontamination
 - Example: Alcohol, aldehydes, chlorine based agents.
 - Combination of disinfectants

Control Methods

Inactivation Treatment

Temperature Control

**Modified Atmosphere
Packaging(MAP)**

Chemical Preservatives



Inactivation Treatment

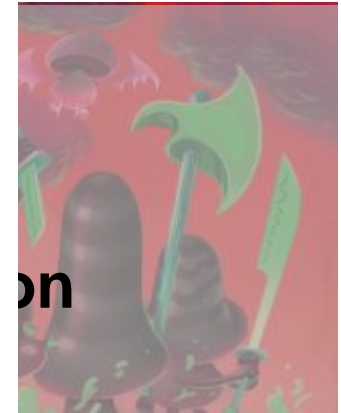
- Heat Treatment
- High Pressure Treatment
- Pulsed Electric Field





HEAT RESISTANCE

ment



lis, N.
laromyces



High Pressure Treatments

- **Inactivate yeast cell and mold spores**
- **Extend product shelf life**
- **No significant changes in protein and lipid structure to the products**

Meets consumer's expectations for more "natural" and less heavily processed food products

Pulsed Electric Fields

Efficient non-thermal food processing technique using short, high voltage pulses

Used for microbial inactivation
include yeast and mold



Temperature Control

Low Temperature

Minimize the growth in raw milk until processing and to extend the shelf life of non-sterile dairy food.

Psychrotrophic fungi have the ability to grow at refrigerated temperature

Beside storage temperature,

The spoiler **initial population** and **the time** required to form thallus on the products surface will also determine the fungi spoilage susceptibility

Modified Atmosphere Packaging

Replacement of air by a defined gas mixture

Control fungal spoilage and extend dairy food shelf life

Example:

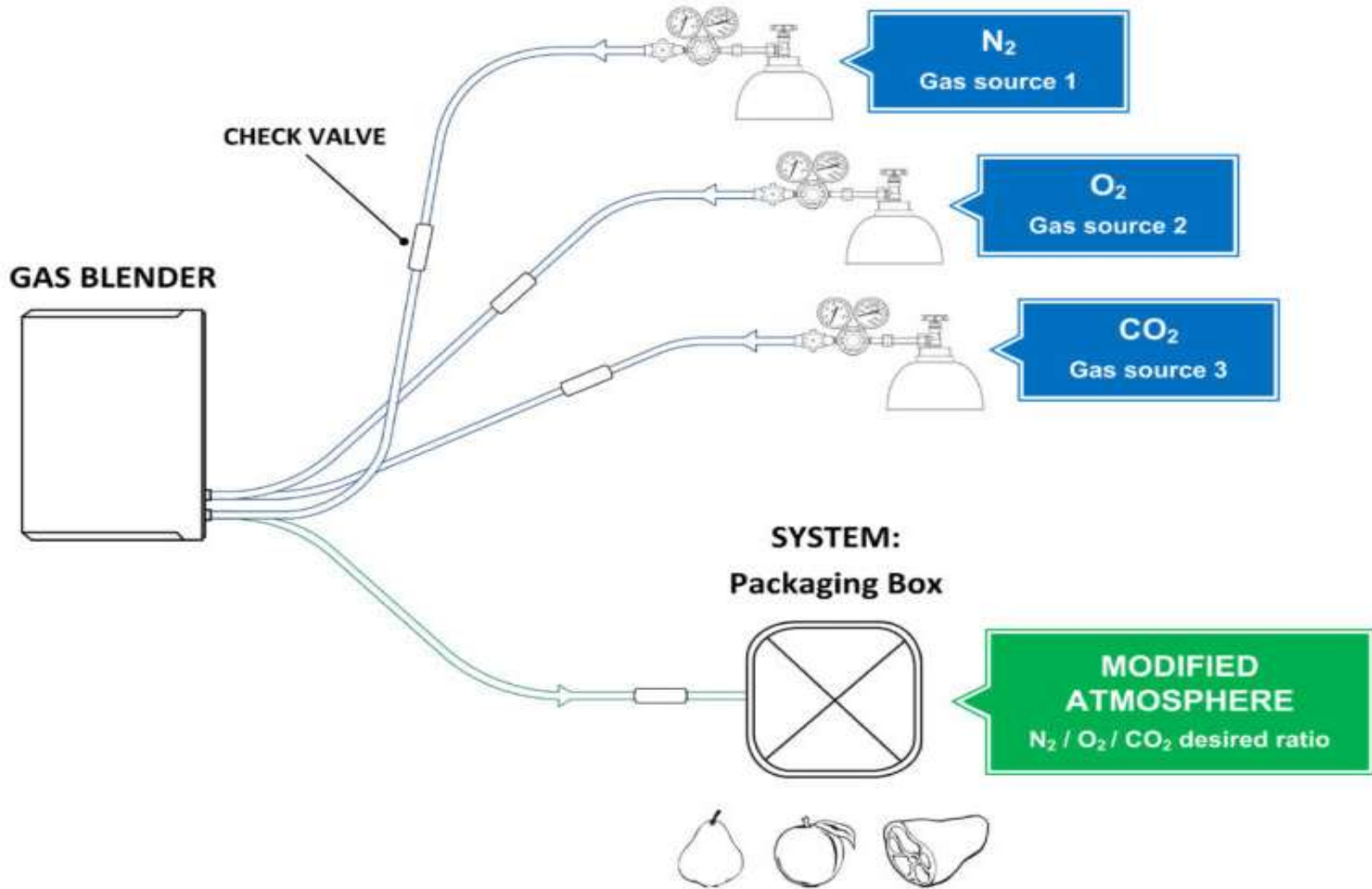
Lowering Oxygen Level

- **Inhibit partially the growth of strict aerobic fungi**
- **Slow down facultative anaerobes growth**

Increasing CO₂ Level

- **Antimicrobial Effect**
- **Inhibit fungal growth**
- **Decrease in pH. Why?**

Experimental Set-up



Picture show the Experimental Set Up for MAP

Chemical Preservatives

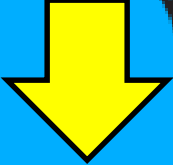
Preservatives include weak organic acids (sorbic acid, benzoic acid) and salts (potassium sorbate, sodium benzoate)

Sorbic and **Benzoic acid** have an optimal inhibitory activity at pH 4.5-5.5



The uncharged, undissociated acid form can diffuse freely across the cytoplasmic membrane and enter into the cell

SO, how



Once inside the cell, the acid will dissociate and release charged anions and protons

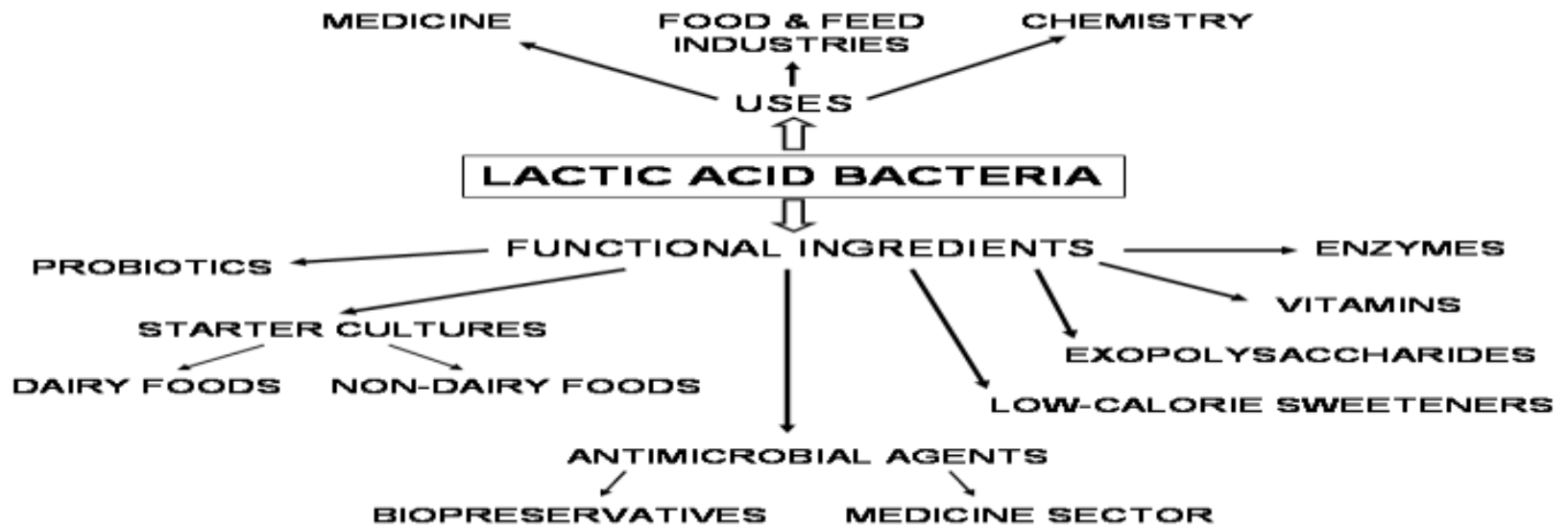


Anions and protons accumulation into the cell is responsible for intracellular pH decrease and may cause membrane disruption which finally lead to cell death.

Fermentation

The oldest preservation methods

Microbial groups involved in dairy product manufacturing may suppress or retard the growth of spoilage fungi





Stability

Safety

Compatibility

Biopreservation

Effectivity

Antimicrobial
Activity

Spoilage
Potential

Lactic Acid Bacteria

Shelf-life

Stater Culture

Antimicrobial Activity

Qualified Presumption Of Safety(QPS) status

Absence of Biogenic Amino Acid Production

Recognised as Safe (GRAS)

Improvement of Nutritional Properties



Main Properties expected from
Antifungal LAB and PAB

No impact on Starter Culture's
functionalities

No modification of Organoleptic
Properties

Antifungal activity

An easy propagation at high
populations and resistance to
lyophilisation or freezing

An activity at the lowest possible
inoculum to reduce the cost
associated with their use

Antifungal Bioprotective Cultures

- ❖ Commercially Produced Antifungal Bioprotective Cultures are:
 - **HoldbacYM-B** (DuPont Danisco)(mixed culture of *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii* sub sp. *shermanii*)
 - **FreshQ® series** (Dupont)(single *L. rhamnosus* culture)
 - **Befresh™ AF** (Handary)(mixture of *Lactobacillus paracasei* and *P. freudenreichii* sub sp. *shermanii*)



Screening For Antifungal Strains

- Culture of *Lactobacillus* species in Man, Rogosa, and Sharpe (MRS) media to detect antifungal activities (154 strains) against *R. mucilaginosa* and to a lower extent against *Aspergillus tamari*, *Candida krusei*, and *K. marxianus*.
- Plate raw milk samples on semi-selective media for LAB to develop an antifungal isolate collection, and using the agar-overlay method to systematically screen colonies for their antifungal activity against four spoilage fungi.

Antifungal Activities Against Fungi

➤ Bacterial species that have shown the antifungal activity:

- *Lactobacillus*
(*L. plantarum*, *L. casei*, *L. paracasei*, *L. brevis*)
- *Propionibacterium*,
- *P. freudenreichii*
- *Lactococcus*
- *Pediococcus*
- *Weissella*
- *Leuconostoc*

Major
Species

To a smaller extent, most
frequently cited genera

➤ Target fungal species:

- *Penicillium*, *Candida*, *Kluyveromyces*, *Rhodotorula* spp.,
Debaryomyces hansenii and *Yarrowia lipolytica*

Factors That Affecting the Antifungal Activity

❖ Intrinsic factors:

- Medium composition in terms of macro- and micronutrients
- pH
- a_w
- Eh
- Food structure

❖ Extrinsic factors:

- Temperature
- Composition of the surrounding atmosphere
- Humidity

Antifungal Activity Test

Testing of efficiency in real products using challenge and durability-tests and against one or several fungal targets.

Delavenne Method

The antifungal activity of *Lactobacillus harbinensis* KV931Np against six fungal targets in yoghurt

Schwenninger and Meile Method

The antifungal activity of three mixed cultures of *Lactobacillus paracasei* subsp. *paracasei* SM20, SM29, or SM63 and *Propionibacterium jensenii* SM11, against *Candida pulcherrima*, *C. magnoliae*, *C. parapsilosis*, and *Zygosaccharomyces bailii* in yoghurt and on cheese surfaces.

Aunsbjerg Method

The antifungal activity of *Lactobacillus paracasei* in chemically defined medium and in yoghurt against *P. solitum* and *Penicillium* sp.

Fermentates

- Fermentates, which are fermented dairy ingredients produced from milk, containing antifungal metabolites.
- MicroGARD (DuPont Danisco) and DuraFresh (Kerry) are two currently available commercial fermentates
- The use of this fermentate in cottage cheese partially inhibited *K. marxianus* and *P. expansum* and thus extended its shelf-life. It may also be used in sour cream, yoghurt, and dairy desserts.



Review

1. What is fungi?
2. Sources of contamination
3. Preventive and Control of Spoilage in Dairy Products
 - Inactivation Treatments
 - Temperature Controls
 - MAP
 - Chemical Preservatives
 - Fermentation
4. Bioprotective Cultures

Thank You
