

## **Biopreservation**

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**Biopreservation/biological preservation** is defined as preservation method to improve safety and stability of food products in a natural way by using desired' microorganisms and/or their metabolites without changing the sensory quality. **Protective or antagonistic** microorganisms that are added to food product only to inhibit pathogens and/or to extend shelf-life. **These cultures differ from starter cultures** in their functional objectives.



# Biopreservation can be applied in food products by two basic methods:

Adding crude, semi-purified or purified microbial metabolites

Adding pure and viable microorganisms

Use of microorganisms/their metabolites as food preservatives contribute to the hurdle approach in food preservation



### Antimicrobial Metabolites of Lactic Acid Bacteria

#### **Organic acids:**

- 1. Amount and type of acids produced during fermentation influence subsequent microbial activity in the fermented material.
- 2. Acetic acid is more antagonistic against yeasts.
- 3. Inhibitory effect of organic acids is mainly caused by undissociated form of the molecule, which diffuses across the cell membrane towards more alkaline cytosol and interferes with essential metabolic functions.
- 4. Toxic effects of lactic and acetic acid include reduction of intracellular pH and dissipation of membrane potential.

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#### Hydrogen peroxide:

Antimicrobial activity of hydrogen peroxide is attributed to its strong oxidizing effect on bacterial cell and destruction of basic molecular structures of cell proteins. In raw milk, hydrogen peroxide produced by lactic acid bacteria can, after being catalyzed by lactoperoxidase, oxidise endogenous thiocyanate.

 Oxidized intermediary products are toxic to different bacteria.

### **Carbon dioxide**

- Creates an anaerobic environment by replacing the existent molecular oxygen.
- Antifungal activity of CO2 is due to inhibition of enzymatic decarboxylation and accumulation in membrane lipid bilayer resulting in dysfunction in permeability.

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#### **Reuterin:**

- Reuterin is a pH neutral, water soluble, low molecular wt. substance, non-bacteriocin and resistant to nuclease, protease and lipolytic enzymes.
- Active over wide range of pH values and capable of inhibiting growth of wide spectrum of microorganisms, but labile to heat (100 C for 10 mins).
- Strong antimicrobial activity.
- Reuterin in range of 15-30 µg/ml effectively inhibit growth of Gram-positive and Gramnegative bacteria, and lower eukaryotic organisms including yeast, fungi and protozoa.

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#### **Bacteriocins:**

- Most studied are nisin, pediocin and sakacin.
- Nisin, produced by *Lc. lactis* subsp. *lactis*, is only bacteriocin that has practical application in food products.
- Mainly applied in prevention of late blowing of cheese by inhibiting outgrowth of *Clostridium* spores and in pasteurized cheese spreads to inhibit *Clostridium* and *Listeria*.
- Levels used in food products: 2.5-100 ppm.
- Pediocin PA-1 (also AcH) from *P. acidilactici* successfully control growth of *L. monocytogenes* in cottage cheese, half-and-half cream and cheese sauce, raw/fresh meat, cooked meat products and fermented meat products.
- Pediocins seems to be more effective in meat products than nisin but not approved for use.



#### **Fermentates of Selected Organisms**

- Microgard<sup>™</sup> products marketed by Danisco are fermentates of *Propionibacterium freudenreichii* subsp. *shermanii* that are commonly used commercially as biopreservatives in cottage cheese.
- 2. Their inhibitory activity is attributed to propionic acid, acetic acid and heat stable peptide.
- 3. Alta<sup>™</sup> and Perlac<sup>™</sup> are fermented wheybased products used as shelf-life extenders.



#### **Biopreservation by Means of Protective** Lactic Cultures

- Since lactic acid bacteria are commonly used as starter cultures in food fermentations, so use of bacteriocin producers as protective cultures.
- 2. Bioprotective culture may act as starter cultures in food fermentation process or they may protect foods without any detrimental organoleptic changes.
- 3. Natural bacteriocin producers, such as *Lactobacillus plantarum, Pediococcus acidilactici, Enterococcus faecalis and Enterococcus faecium* have been used as protective cultures in various products e.g. outgrowth of clostridia spores in cheese milk was completely prevented when nisin A producing strain was mixed at 10% rate with starter culture.



BS-10®Nisin producing L. lactis spp. Lactis, Chris HansenBIOPROFIT™L. rhamnosus LC705, BioGaiaBOVAMINE Meat Cultures™Texas Tech UniversityHOLDBAC™ DANISCOL. plantarum, L. rhamnosus, L. sakei, L. paracasei a

Propionibacterium freundenreichii spp. Shermanii,

#### Bacteriocin based bioprotective cultures

- 1. No health risks
  - · No toxin production
  - · No production of biogenic amines or other metabolites detrimental to health
  - · Non pathogenic
- 2. Bring about beneficial effects in product
  - · Adaptation to product/storage conditions (e.g. psychrotrophic, salt tolerant)
  - · Reliable/consistent protective activity
  - •Predictability of metabolic activity under given conditions (e.g. lactic acid/gas production)
  - · Competitiveness against endogeneous microbial flora
- 3. No negative sensory effects on product
  - · No slime/gas/acid formation
  - No discolouration
  - · No proteolytic or lipolytic activity
- 4. Function as indicator under abuse conditions

Desired properties of a protective LAB-culture



### Applications of Protective LAB in Different Food Products

Protective culture	Target organism	Food product	
Lactobacillus sakei TH1	L. monocytogenes	Cooked ham and servelat sausage	
Lactobacillus casei D6 Lactobacillus paracasei I5	L. monocytogenes	Frankfurters	
Lactobacillus alimentarius (FloraCarn L-2)	Spoilage flora	Cooked ham	type
Lactobacillus alimentarius (FloraCarn L-2)	L. monocytogenes	Ground beef	s of food
Lactobacillus alimentarius (FloraCarn L-2)	Ropy slime producing Lactobacillus sakei	Frankfurters	product
Lactobacillus alimentarius (FloraCarn L-2)	L. monocytogenes	Bacon cubes	S
Lactobacillus and Lactococcus strains	B.cereus, L. monocytogenes	Milk	
Lactobacillus reuteri BPL-36	Salmonella, S. aureus, L. monocytogenes, Spoilage flora (Pseudomonas spp), E. coli O157:H7	Milk, Cheese	

Studies on the effect of non-bacteriocinogenic protective cultures in different



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#### **Milk and Dairy Products**

- Cheese suffers from spoilage through *Clostridium* spp. (late blowing) and furthermore, susceptible to contamination with *L. monocytogenes*.
- 2. This latter problem arises mainly in cheeses in which the pH increases during ripening, such as Italian cheeses Taleggio, Gorgonzola and Mozarella.
- 3. Addition of this paired nisin producing starter system to make cheddar cheese increase shelf life of pasteurised processed cheese from 14-87 days at 22°C and control *L. monocytogenes, Cl. sporogenes* and *S. aureus*.

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#### **Vegetable Products**

- 1. Bacteriocinogenic LAB have potential for biopreservation of minimally processed vegetables and fermented vegetables.
- 2. In minimally processed vegetables such as prepackaged mixed salads and different types of sprouts, bacteriocinogenic LAB act on coliforms, enterococci and *L. monocytogenes*.
- 3. Bacteriocinogenic starter cultures is useful for fermentation of cabbage/olives to prevent spoilage.
- 4. Biocompetitive control or use of biocompetitive micro-organisms to inhibit mycotoxin forming molds can be obtained by (1) use of biocompetitive non-aflatoxinogenic molds or (2) use of antagonistic yeasts/bacteria.



#### Fish, fish products and seafood

- 1. Spoilage of fresh fish is generally caused by Gramnegative bacteria.
- 2. When vacuum packaged, spoilage of fresh fish, smoked fish and seafood is dominated by mainly Gram-positive bacteria and *L. monocytogenes* can cause problems.
- 3. Potential of *Carnobacterium* spp. to control *L. monocytogenes* in cold-smoked salmon, the divercin V41 producing *Carnobacterium* divergens V41, bacteriocinogenic *C.* maltaromaticum A9b and non-bacteriocinogenic *C.* maltaromaticum A10a have been used.



### **Food Preservation Through Fermentation**

- Fermentation: Bioconversion of organic substances by microorganisms and/or enzymes (complex proteins) of microbial, plant or animal origin.
- Fermented foods contribute to about onethird of the diet worldwide.
- During fermentation, breakdown of carbohydrates under limited supply of oxygen or under anaerobic conditions take place e.g. yeasts converts sugar to alcohol and CO2.
- Some aerobic, specific conversions may be referred as fermentation such as Acetobacter convert ethyl alcohol to acetic acid in presence of oxygen.

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- Lactic acid fermentation: sugar in foodstuff may be converted to lactic acid and other end products.
  In gassy fermentations, sugar molecules are altered
- to form acids, alcohols and carbon dioxide.
  Sodium chloride is useful in fermentation process of
  - foods by limiting growth of putrefactive organisms.
- Fermentation is applied in preservation of cereals, roots, tubers, fruit and vegetables, milk, meat, fish.
- Commercially produced fermented foods include dairy products (cheese, yoghurt, fermented milks), sausages and soy sauce.
- Foods fermented by LAB: Cucumbers, olives, cabbage, coffee, cherries, vanilla beans, meat, dairy
- Yeasts: Malt, grapes, wines, wines, bread
- Mold: Soybeans



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### Genetically Modified Organisms in Preservation

- 1. Microbial cultures can be genetically improved using both traditional and molecular approaches and improvement of bacteria, yeasts and molds is done.
- 2. Considered for commercial food applications include ability to produce antimicrobial compounds (e.g. bacteriocins, hydrogen peroxide) for inhibition of undesirable microorganisms.
- 3. In many developing countries, focus is also on degradation or inactivation of natural toxins (e.g. cyanogenic glucosides in cassava), mycotoxins (in cereal fermentations) and anti-nutritional factors (e.g. phytates).



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#### **Recombinant Antifreeze Protein**

- 1. Antifreeze proteins are potent cryogenic protection agents for cryopreservation of food and pharmaceutical materials.
- 2. Food-grade expression and fermentation system (antibioticfree) for production and secretion of high levels of rAFP was developed.
- 3. A novel recombinant type I antifreeze protein analogue (rAFP) was produced by *L. lactis*.
- 4. Lyophilized, crude rAFP produced by *L. lactis* was tested in frozen meat and frozen dough processing model. Frozen meat treated with antifreeze protein showed less drip loss, less protein loss, and high score on juiciness by sensory evaluation.
- 5. Frozen dough treated with rAFP showed better fermentation capacity than untreated frozen dough.

