American Cheese Society 2012

REX C INFANGER

AUGUST 2012 CULTURE 201:ADJUNCT,PROTECTIVE, AND PROBIOTIC CULTURES -CHEESE ENHANCEMENT THROUGH MICROBIAL INTERVENTION
PROTECTIVE, ADJUNCT AND PROBIOTIC CULTURES

August 2012

Today's Challenges in the Food Industry

The journey towards 2050

Consumer trends

Food Safety
Pathogen scare
Taste & freshness
Chemical scare

Health & Wellness
Functional foods
Natural/organic
Low salt/sugar

Sustainability
Product origin
Production methods
Recyclability & waste reduction

Rise in **natural, environmentally neutral** technologies and increased attention on **food waste**

Regulatory
Tighter regulations on health claims & microorganisms impact

Agricultural changes
New food pathogens
Animal husbandry

Demographics
Children, Women, Elderly
Emerging countries

Social and political trends

Challenges

Foodborne illness (USA): 76 million sick, 325,000 hospitalized, 5,000 die per year.

Approximately 25% of the world's food supply is discarded every year due to microbial spoilage.

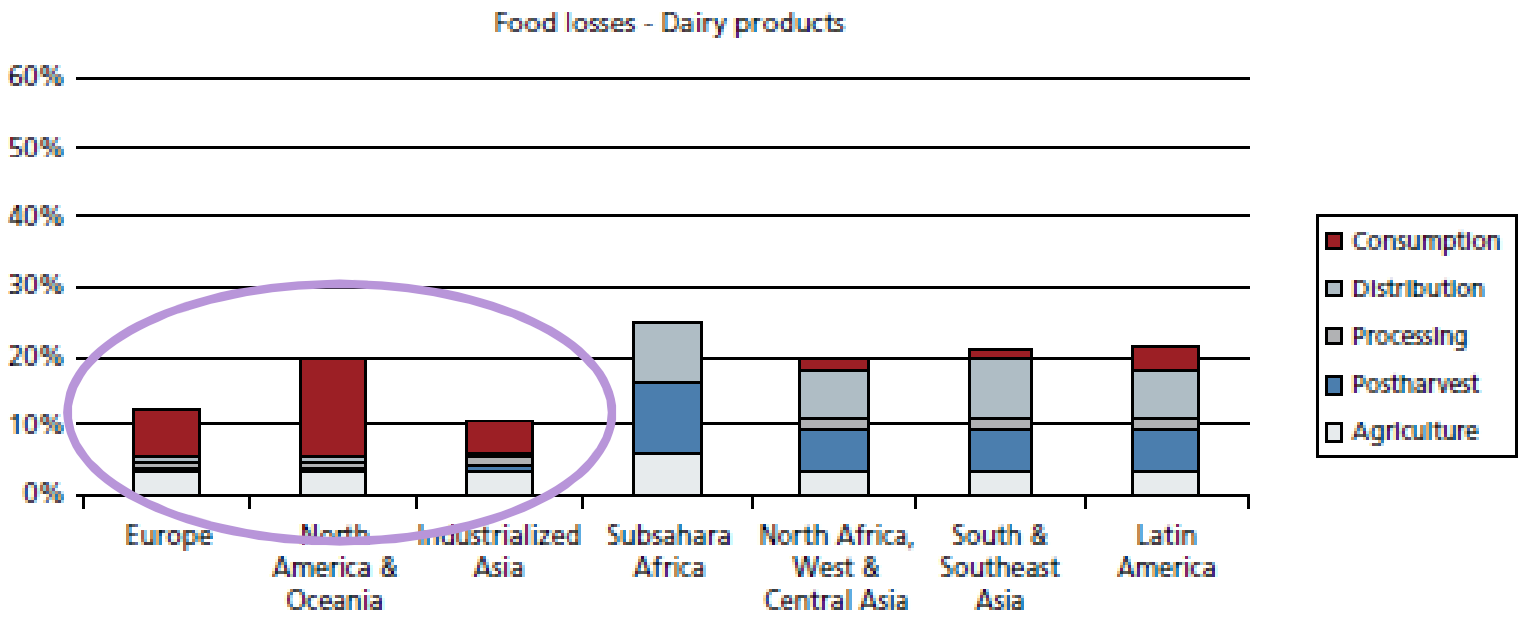
Huge economic impact.

The food industry desires alternatives to chemical preservatives.

Challenge: improve microbial safety and quality of the food supply.

Figures about Dairy Waste

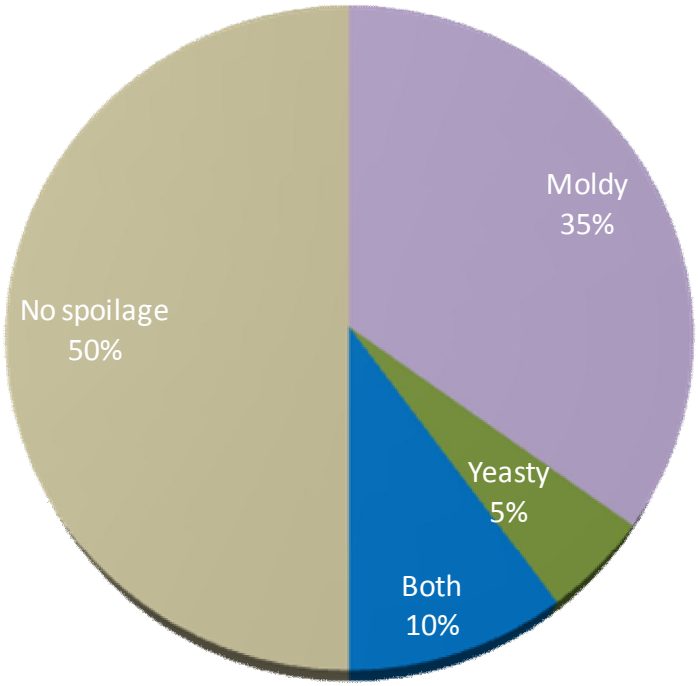
40 to 65% of dairy waste is generated by households in industrialized regions



Source: Global food losses and food waste - FAO 2011

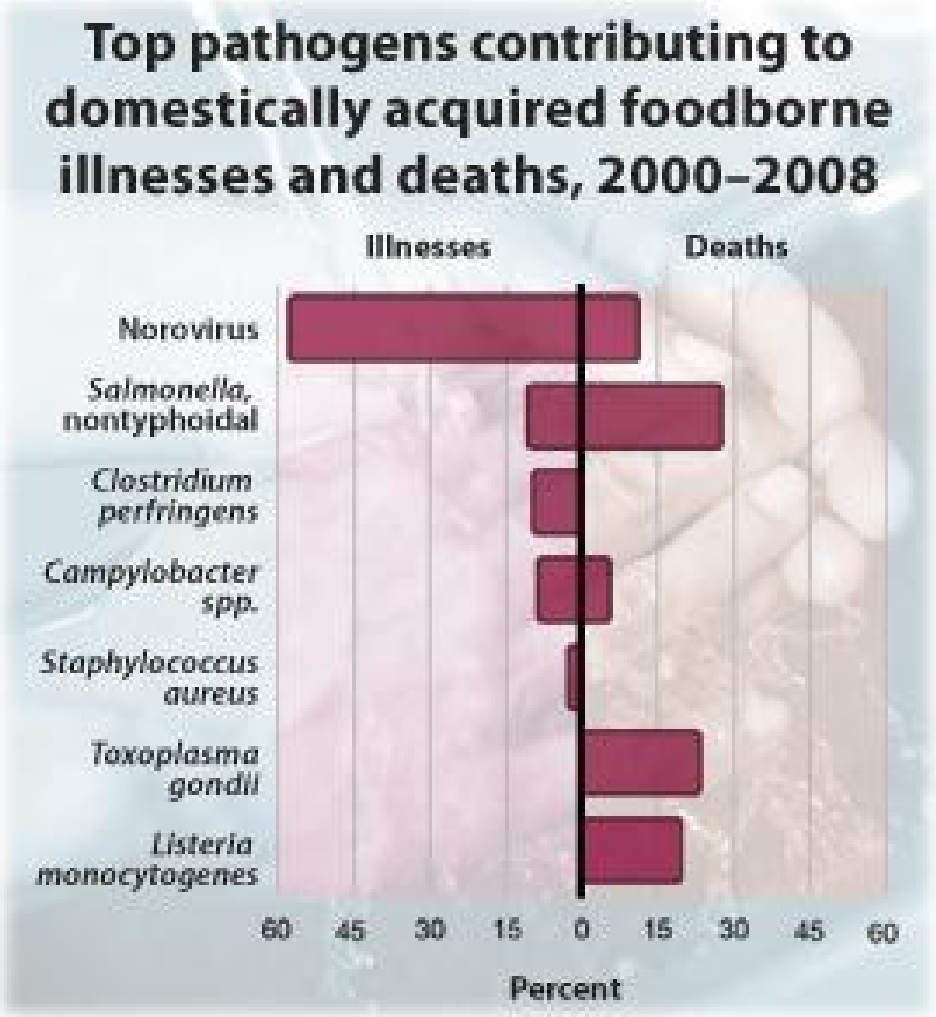
Dairy Waste - Consumer Insight

Half of the consumers declare they experienced spoilage in their dairy products at home



Source: Danisco global survey 2011
Base: 426 respondents, informed consumers

Increased Scrutiny of Food Borne Diseases



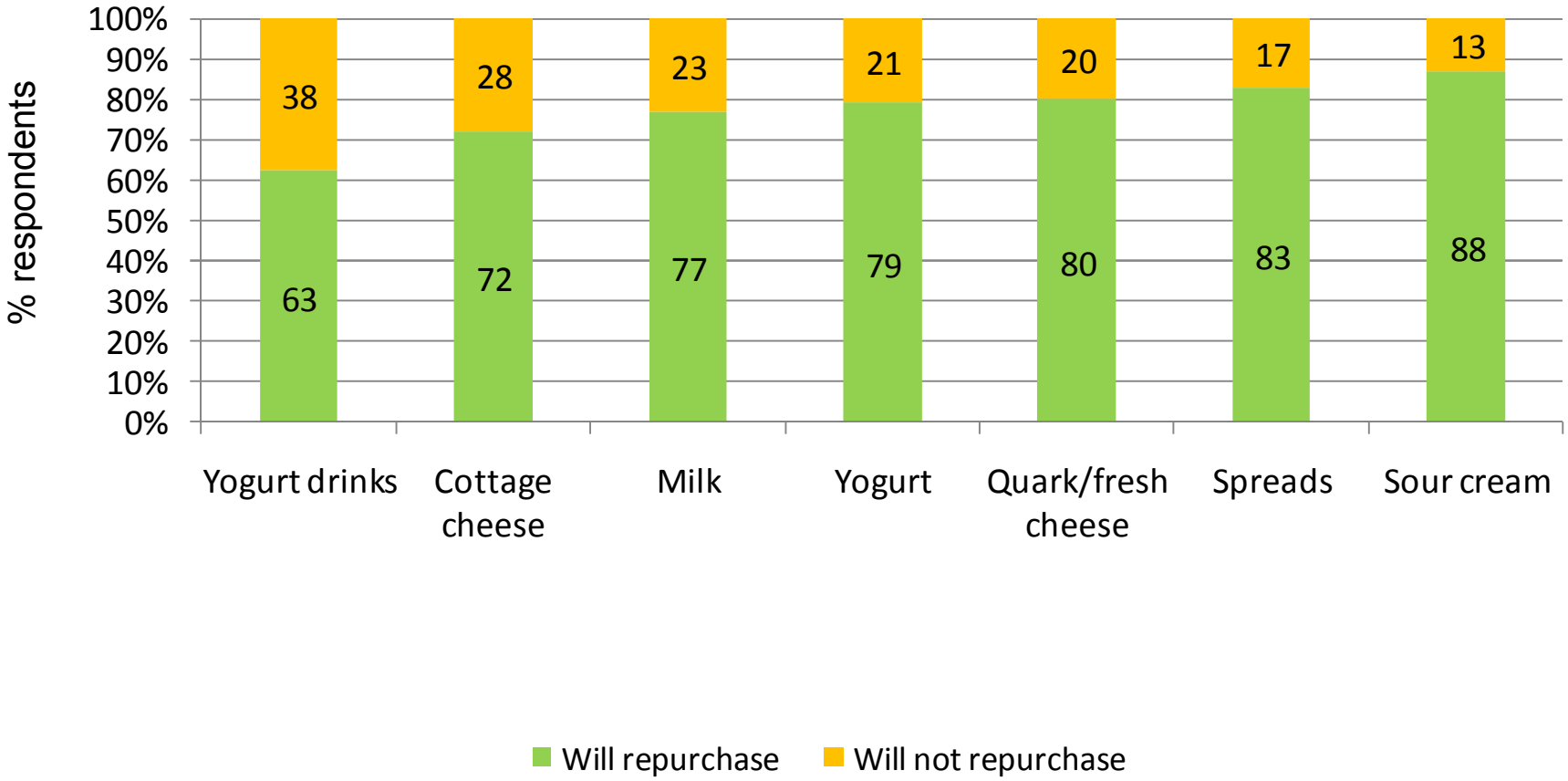
1 in 6 Americans (or 48 million people) get sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases.

and

Emergence of new and more virulent strains : Salmonella heidelberg, E.coli O104:H4.

Dairy Waste - Consumer Insight

Impact on repurchase rate



Source: Danisco survey 2011

Food Protection How do we Define It

FOOD PROTECTION

FOOD PRESERVATION

Maintenance of organoleptic quality of food by preventing chemical (e.g. oxidation) and microbial spoilage

Reduction of food waste

FOOD SAFETY

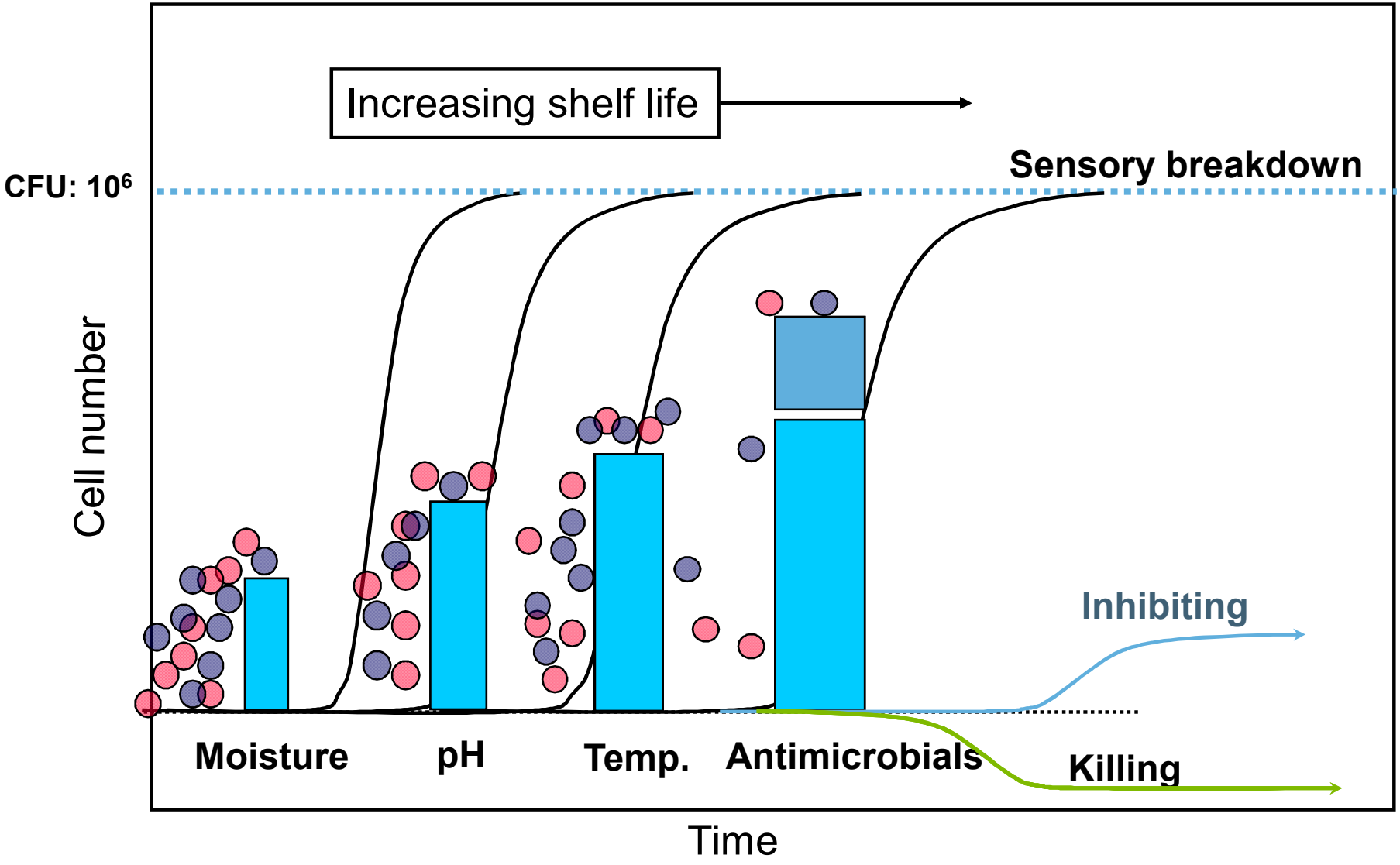
Minimizing of pathogen risks caused by bacterial infections throughout shelf life

Protection of human health

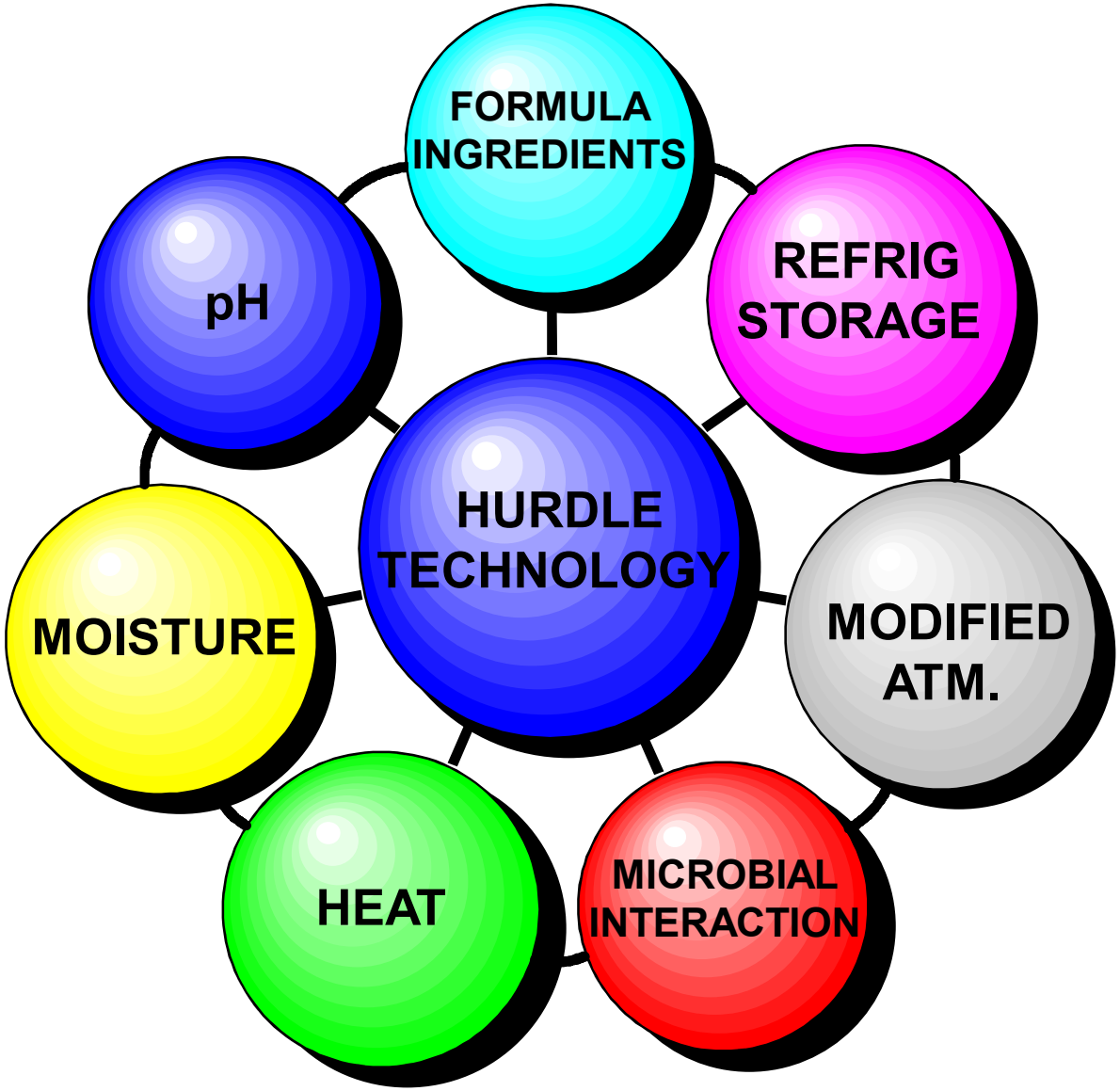
HOLDBAC® YM

Food Preservation

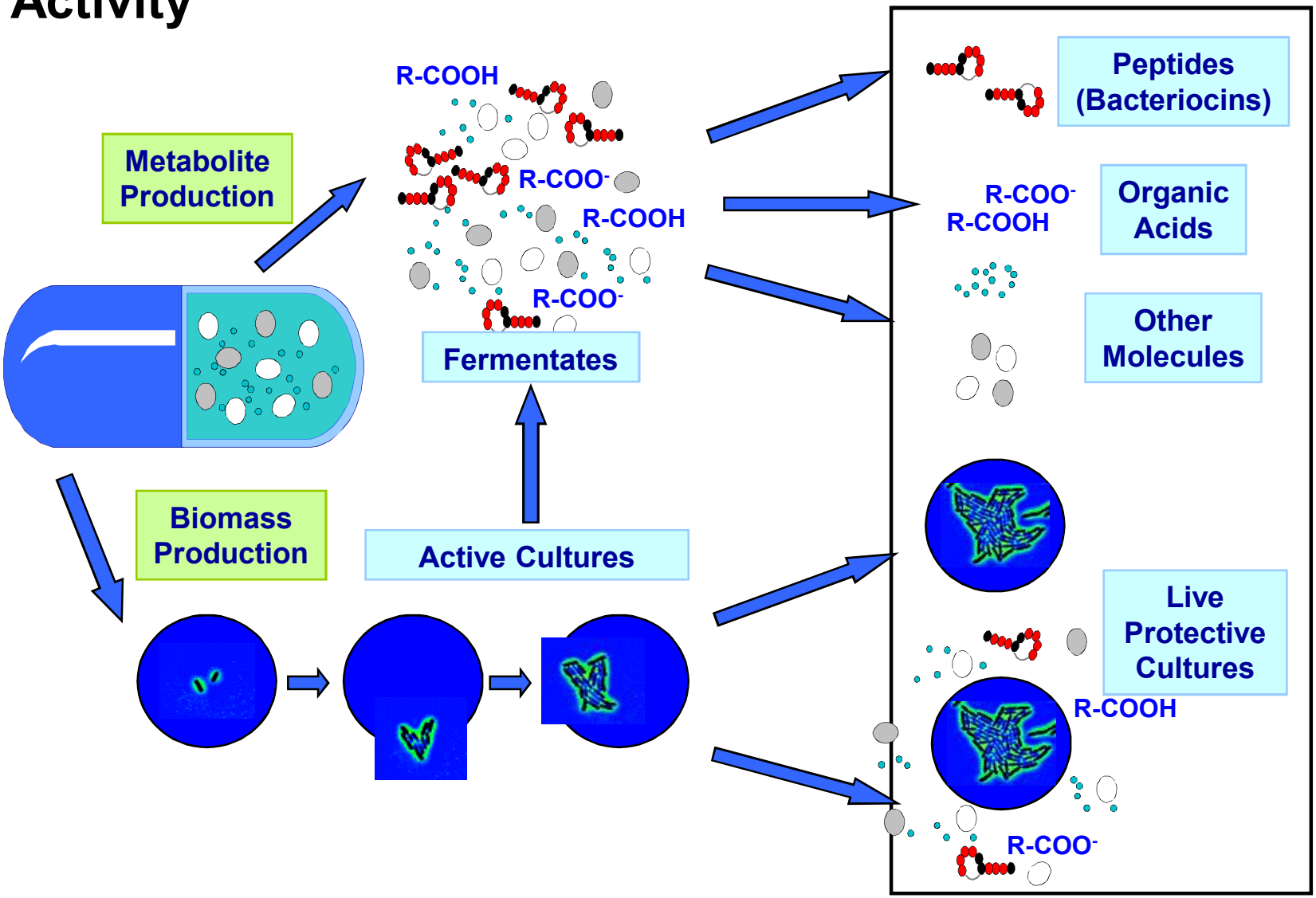
Multiple hurdles to achieve spoilage control



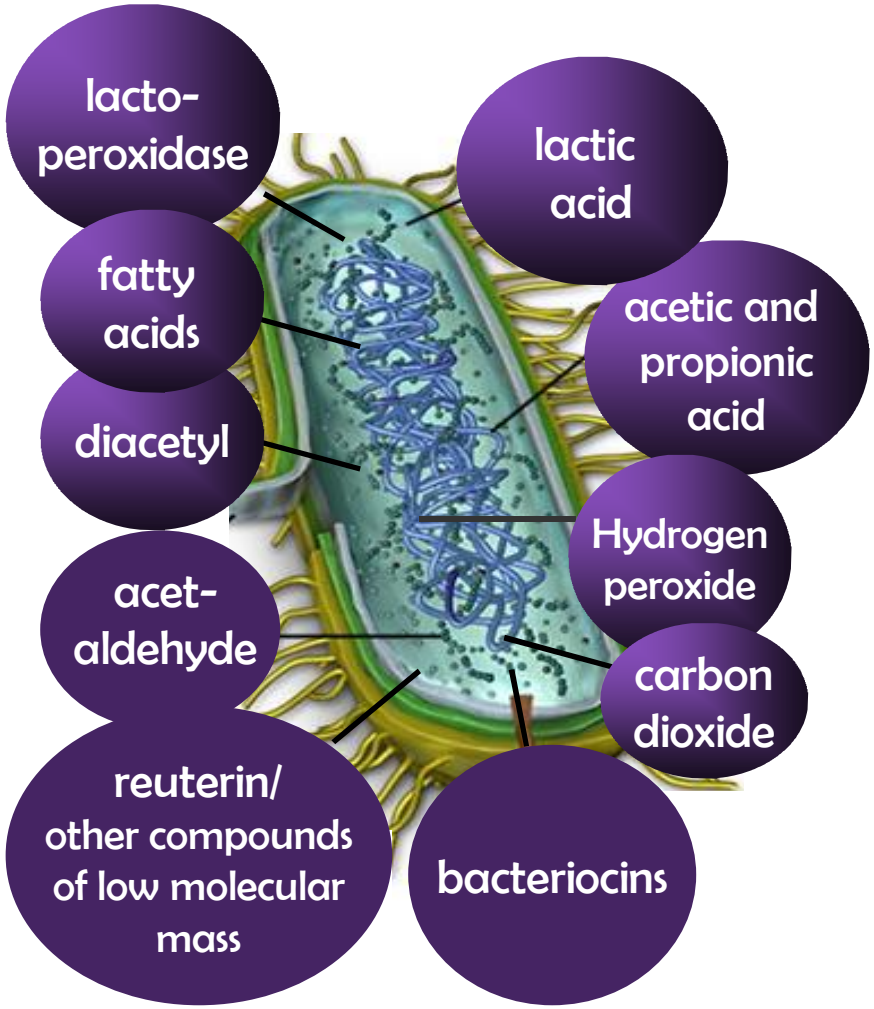
Factors Influencing Growth



Live Cultures: Mechanisms of Activity

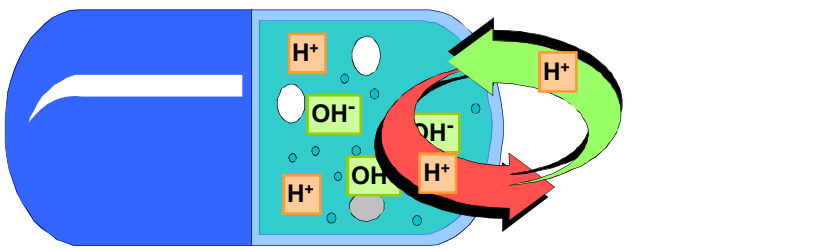


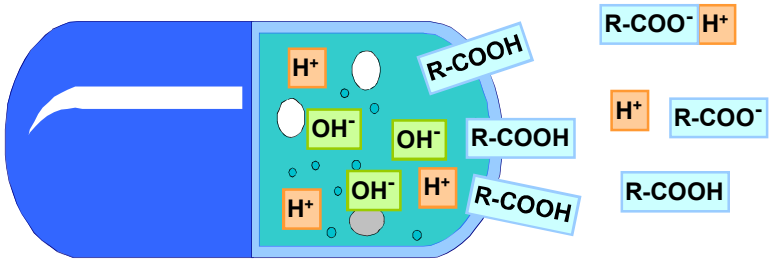
Microbial Fermentation

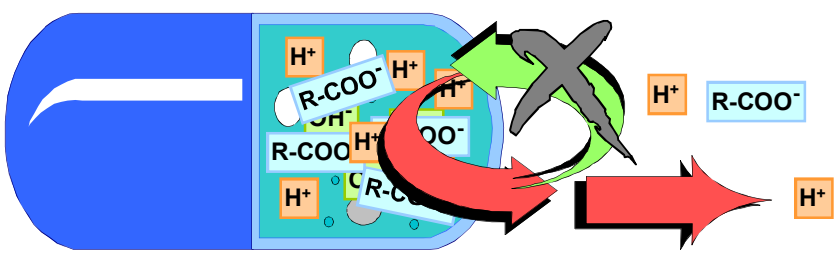


These substances are naturally occurring antimicrobials which by themselves or in combination are highly effective against spoilage and pathogenic microorganisms.

Mode of Action: Organic Acids







Normal Cell State:

- $\Delta pH_{in\text{-}out}$ = Molecular Transport
- $\Delta H^{+}_{in\text{-}out}$ = Energy Generation

Organic Acid Addition:

1. Lipophilic Properties
   ➔ Cellular Membrane Penetration

2. Proton Delivery
   - No $\Delta pH_{in\text{-}out}$ = Reduced Transport
   - No $\Delta H^{+}_{in\text{-}out}$ = No Energy Generation
     ⇨ Starvation

No Growth
=
BACTERIOSTATIC FUNCTIONALITY

Mode of Action: Organic Acids

	Mol wt.	pKa	Percentage undissociated at pH: 5.0	6.0	7.0
Acetic acid	60.1	4.76	35	5.2	0.55
Propionic acid	70.1	4.87	43	6.9	0.74
Lactic acid	90.1	2.74	0.5	0.06	0.01

- ✓ "Antimicrobial activity": acetic ≥ propionic > lactic

Potential Advantages of Protective Cultures

Shelf life protection against normal yeast and mold spoilage

Protection against mold spoilage due to temperature abuse

Longer fermentation time may enhance protection

Reduced fat content may enhance protection – Sour Cream

Protection against different mold species at relatively high contamination levels

Little or no effect on flavor

No adverse effect on pH during storage

Protection against gas formation caused by heterofermentative lactobacilli – Fresh Cheese

Functions of Cultures

Acid Production

- Fast Acid Strains will coagulate milk in 18 hours at 69.8° F

Flavor

- Acid production of lactic oxaloacetic, actetic, pryruvic, and proprionic
- Glycolitic pathway produces diacetyl
- Proteolytic pathway produces ammonia, acetaldehyde, ketones and esters
- Lipolytic pathway produces free fatty acids

Syneresis (Moisture Loss)

Protein Agglomeration (Curd Formation)

Gas Production (Eye Formation & Flavor)

Culture Growth Stages

Lag Phase

Growth Phase

Logarithmic Phase

Stationary Phase

Death

Mesophilic Cultures (52-104 ^{o} F)

Lactococcus lactis ssp. Cremoris (Streptococcus)

Lactococcus lactis ssp. lactis

Lactococcus lactis ssp. lactis,diacetylactis (taxonomically not recognized)

Leuconostoc mesenteroides ssp. cremoris (homofermentative acid production)

Thermophilic Cultures (86-122° F)

Streptococcus thermophilus

Lactobacillus delbrueckii ssp. bulgaricus

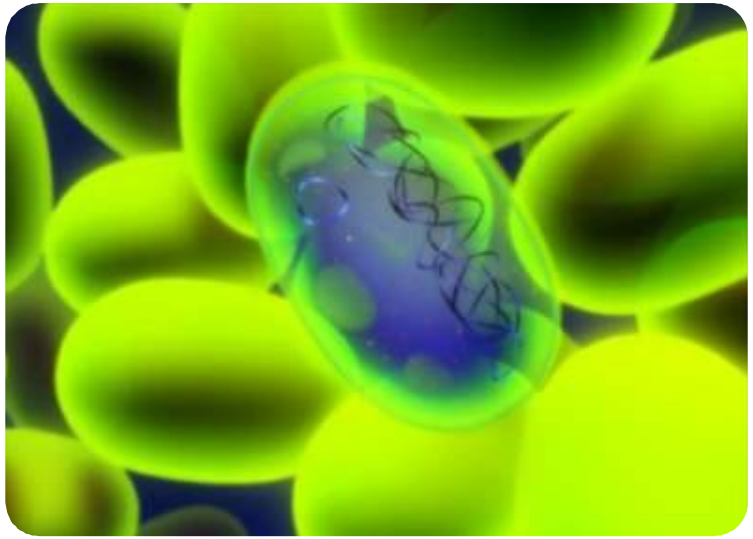
Lactobacillus helveticus

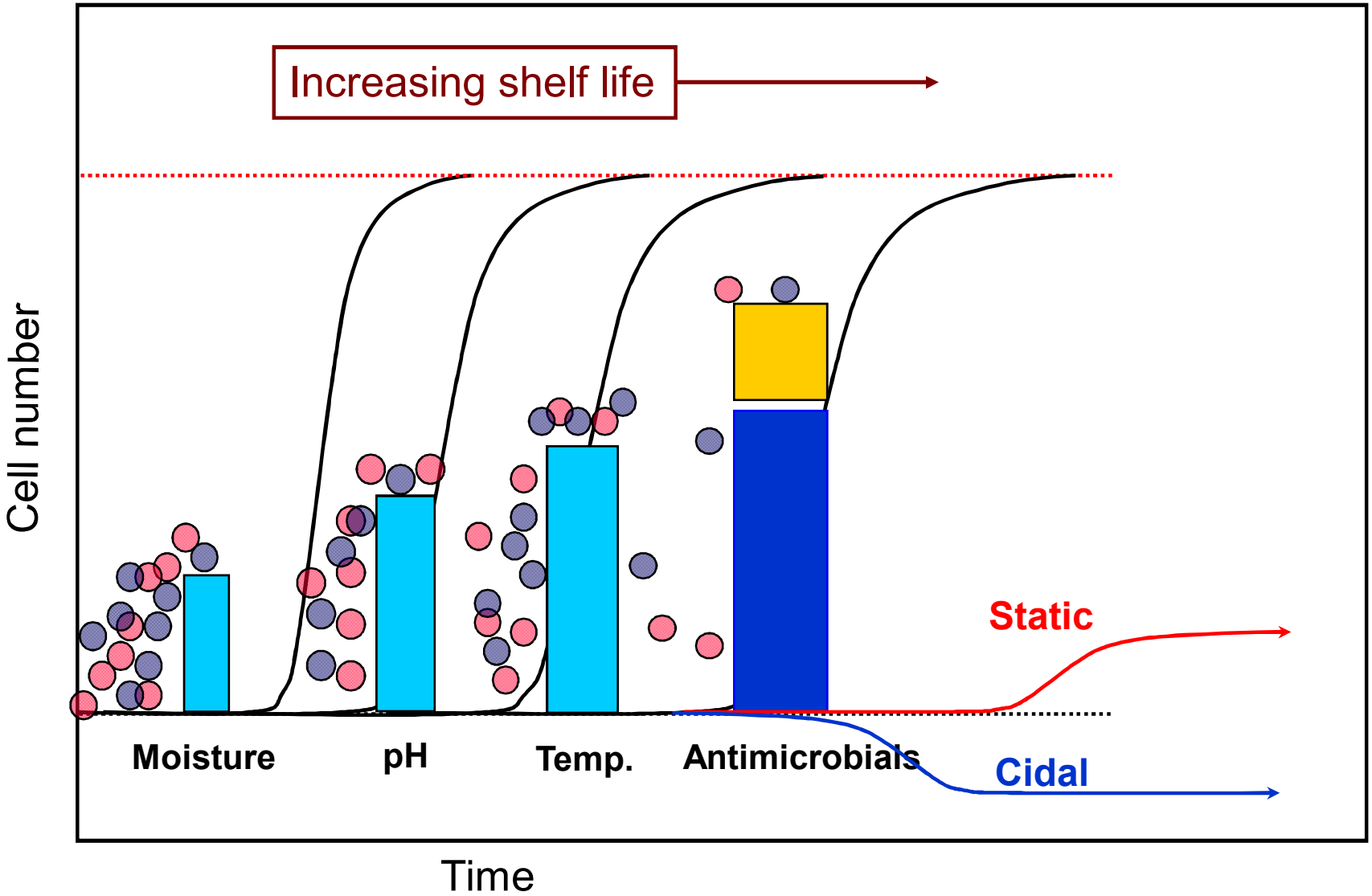
Propionibacterium shemanii

What are protective cultures? Definition

Protective cultures are:

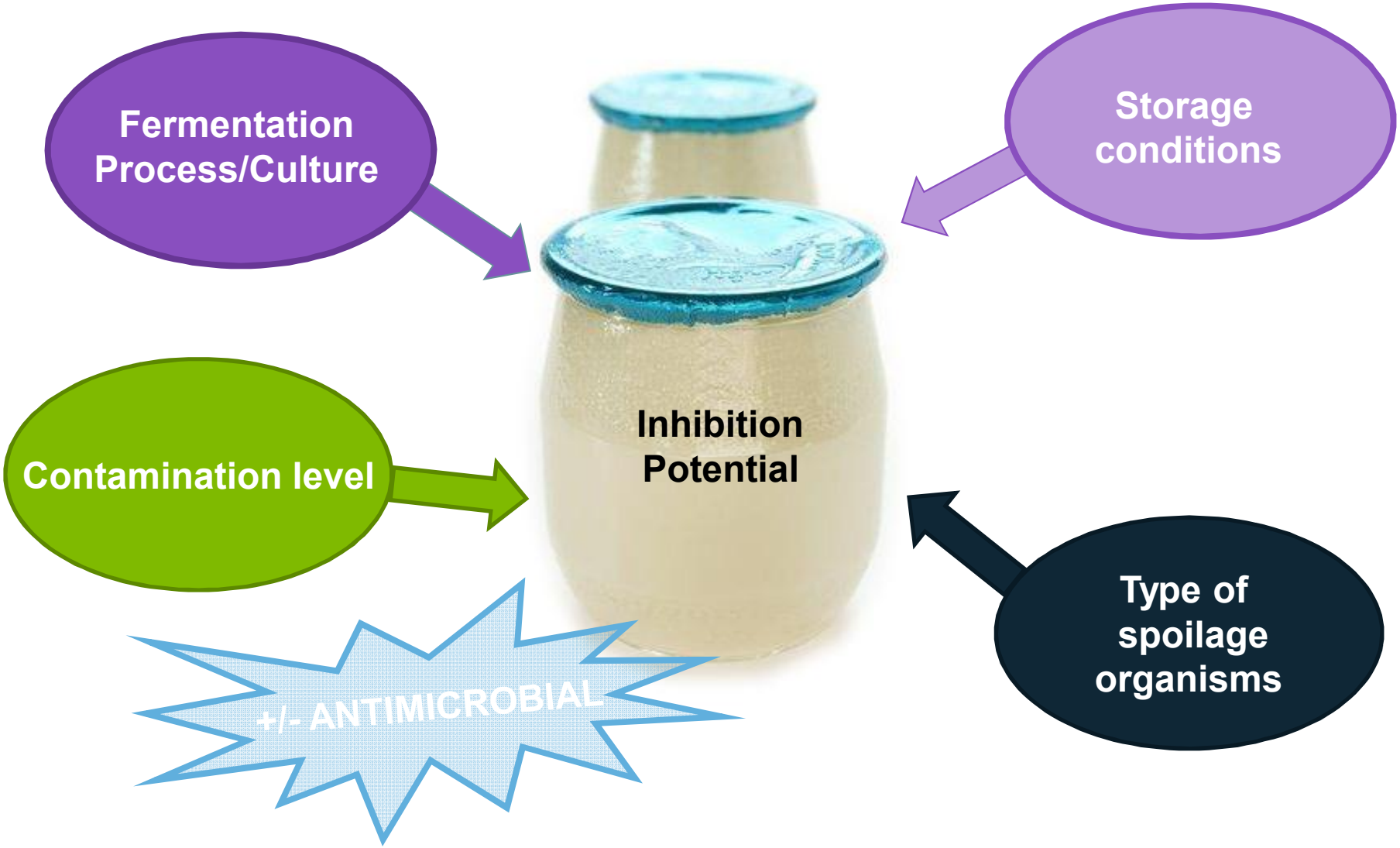
- Bacterial species from food – GRAS status
- Specifically selected for ability to control unwanted microorganisms
- Selected for no adverse effect on sensory quality
- Used as viable (live) cultures



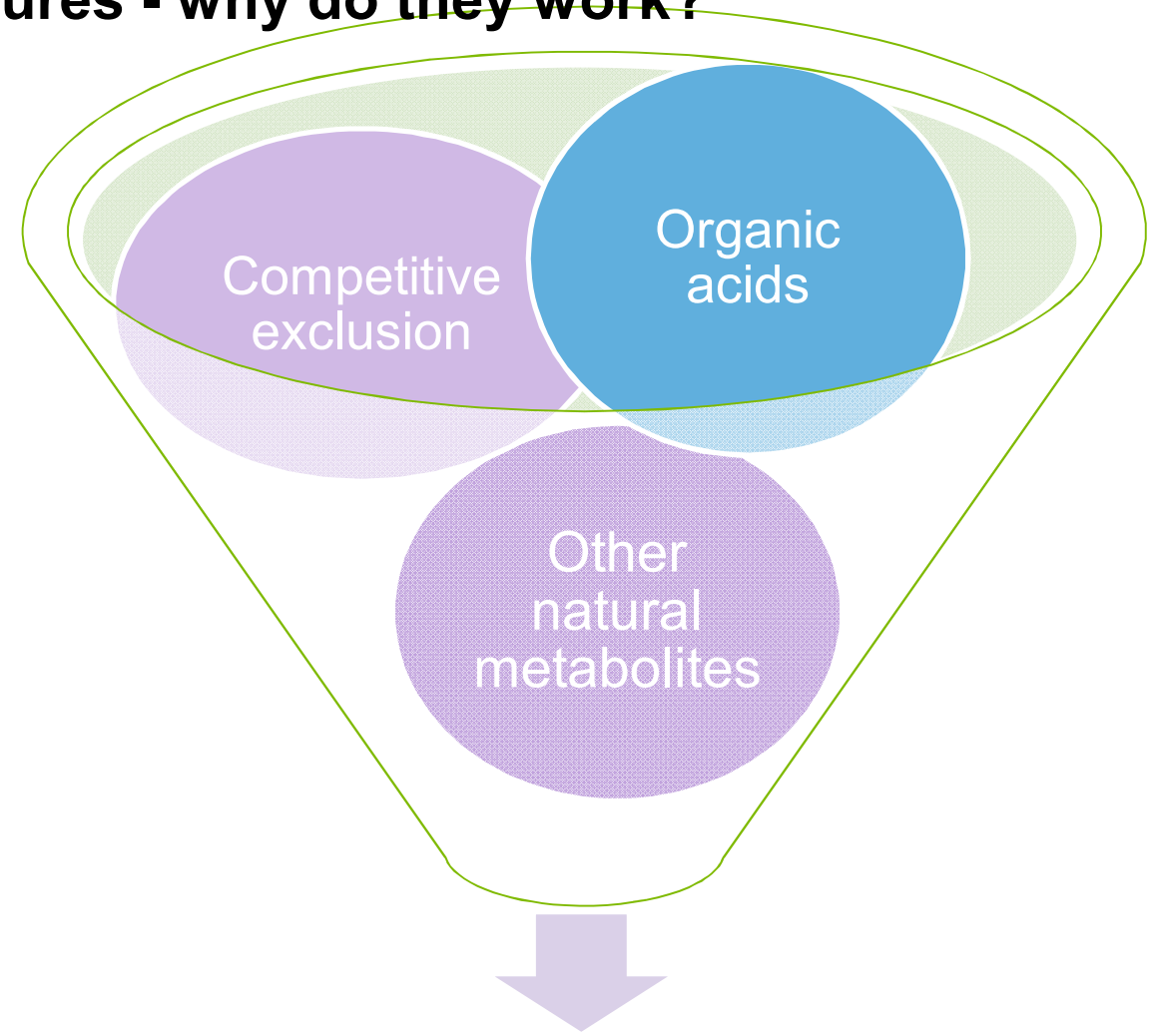

Increasing shelf life
Cell number
Static
Cidal
Moisture
pH
Temp.
Antimicrobials
Time

Food Preservation

Multiple parameters influence inhibition



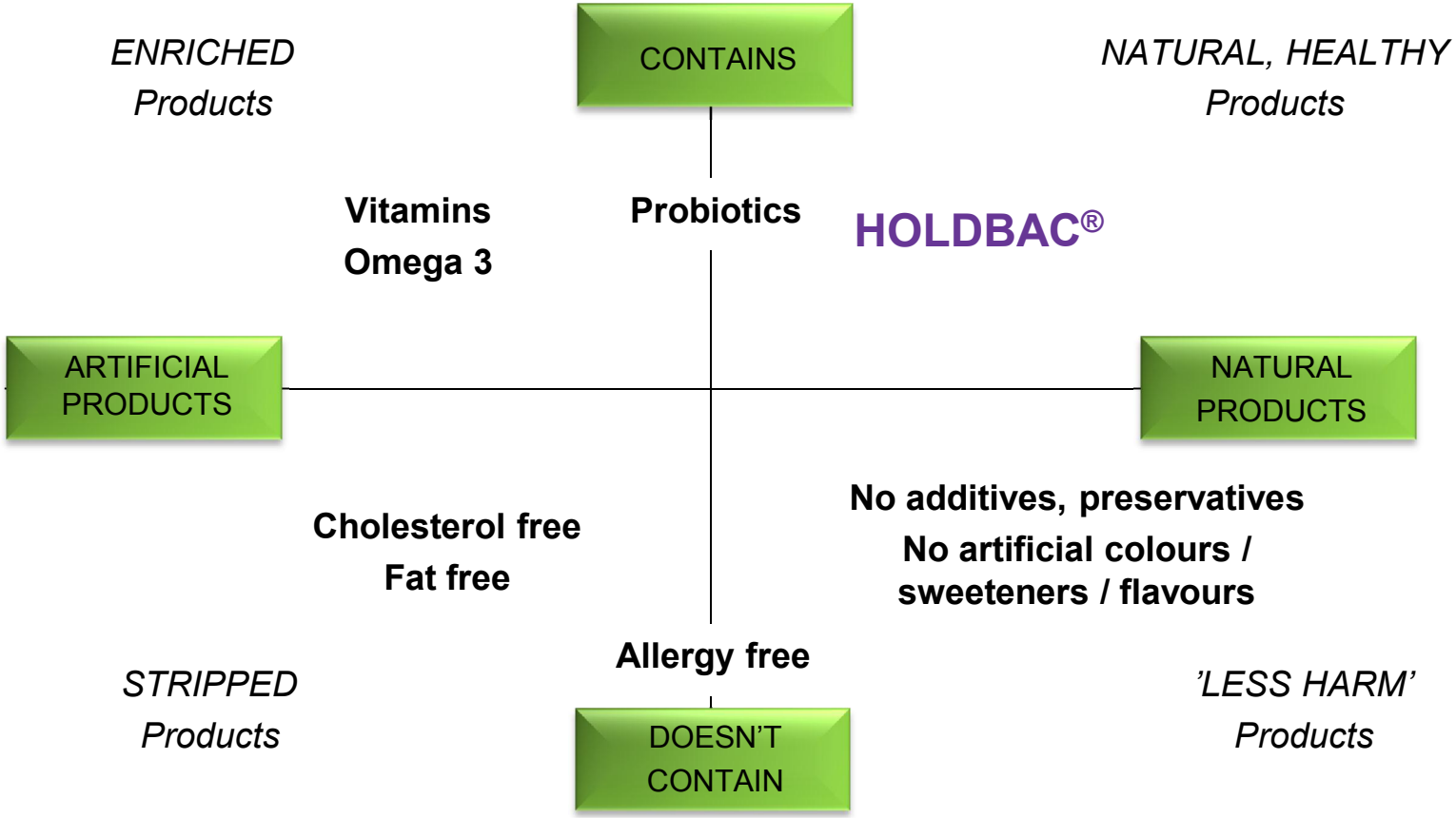
Protective cultures - why do they work?



Protective cultures

HOLDBAC® Cultures for an All-Natural Positioning

The Perception from Consumers



Source: Lindberg survey- Focus groups with 91 end-consumers – Nov 2010/Jan 2011

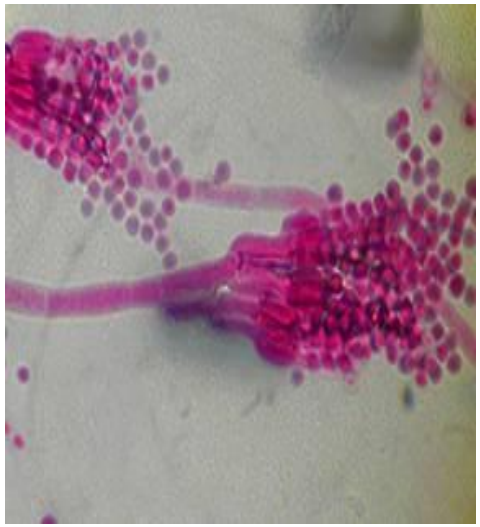
HOLDBAC® Cultures

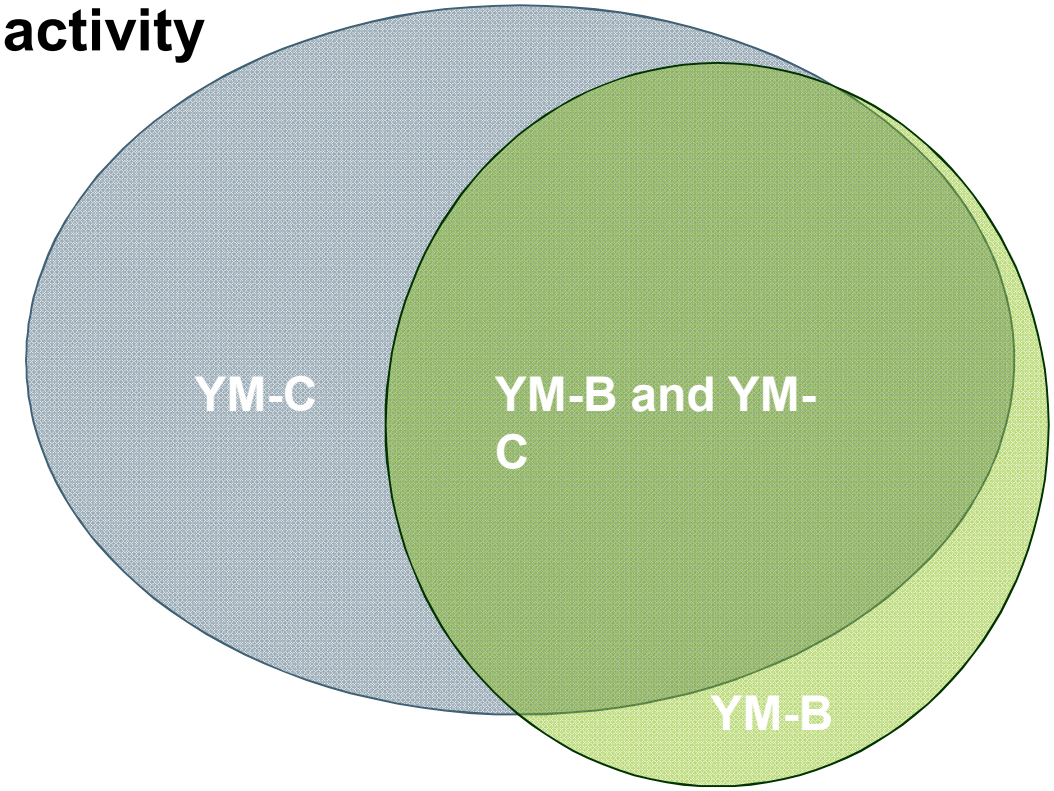
A natural way to protect dairy foods

- Patented live cultures
- Unique properties - control the growth of unwanted microorganisms throughout shelf life
- Isolated from food –GRAS organisms
- No additional labelling requirements in cultured products.

Products	Composition
HOLDBAC® YM-B FRO & LYO	*Lb. rhamnosus* *P. freudenreichii subsp. shermanii*
HOLDBAC® YM-C FRO & LYO	*Lb. paracasei* *P.freudenreichii subsp. shermanii*

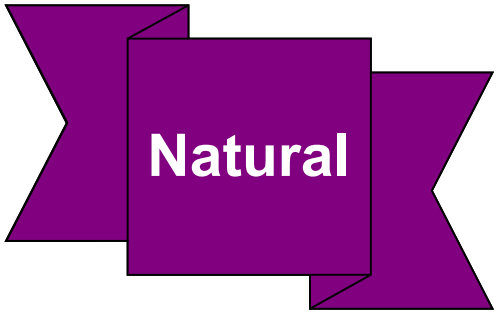
HOLDBAC® YM Scope of activity



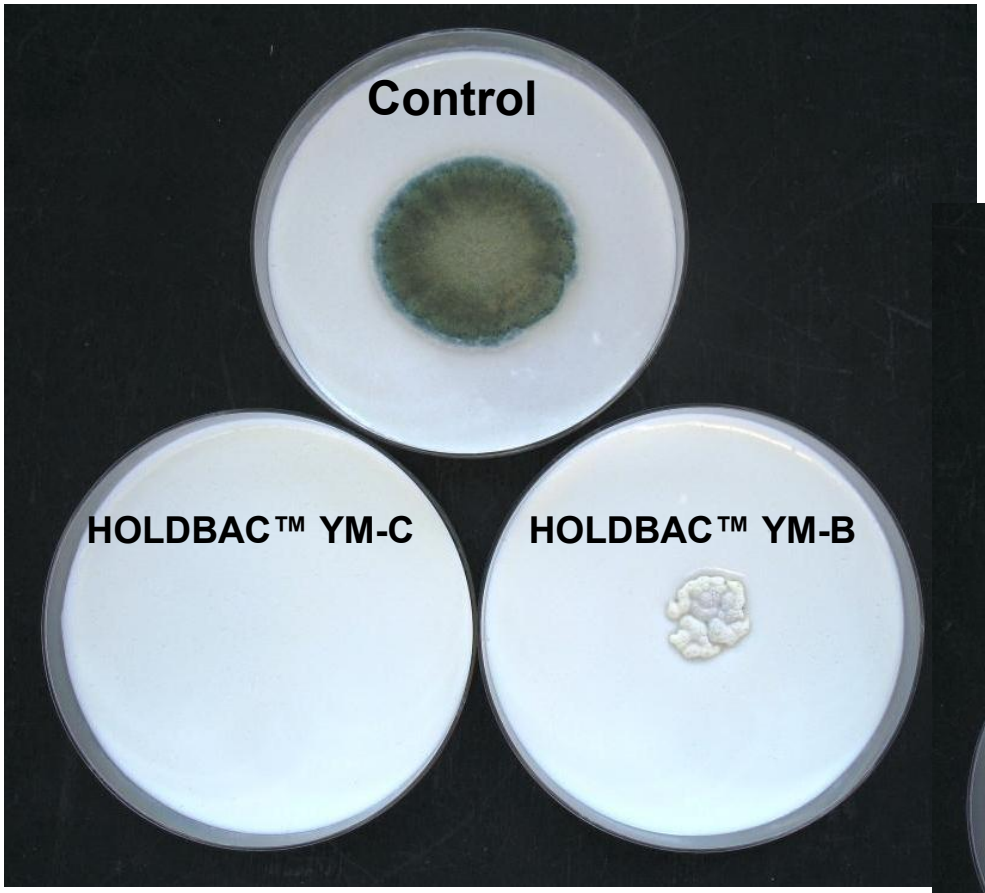


CANNOT turn bad quality raw ingredients into good products

CANNOT replace Good Manufacturing Practice!

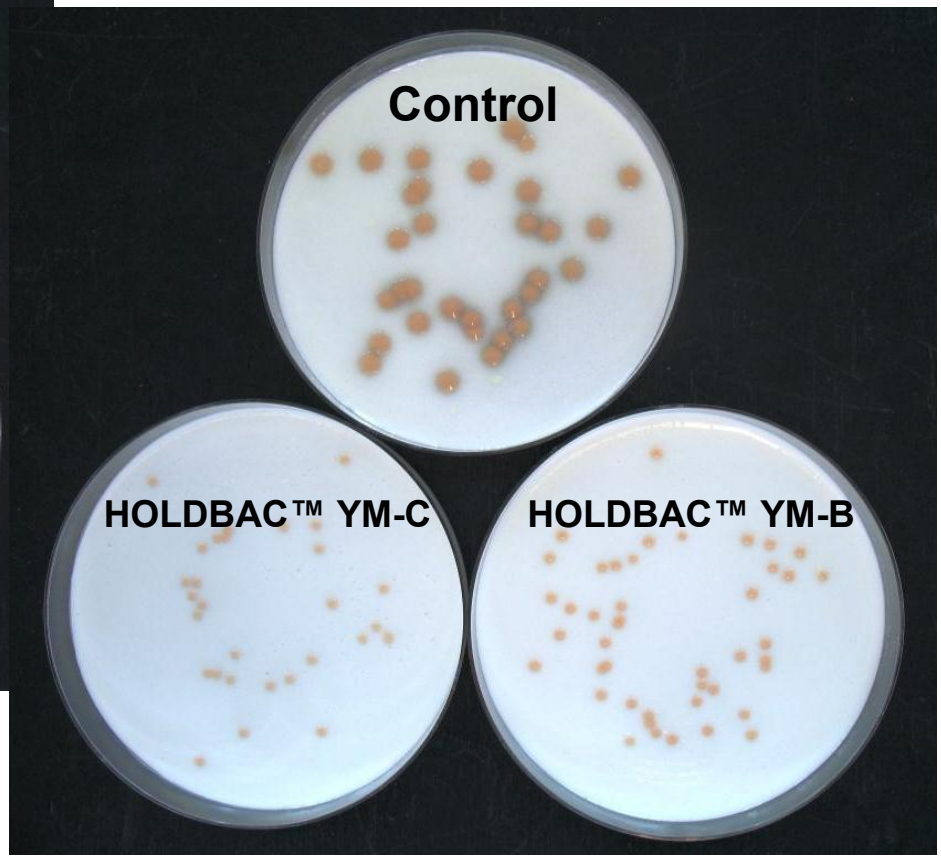


HOLDBAC® YM – do they actually work?



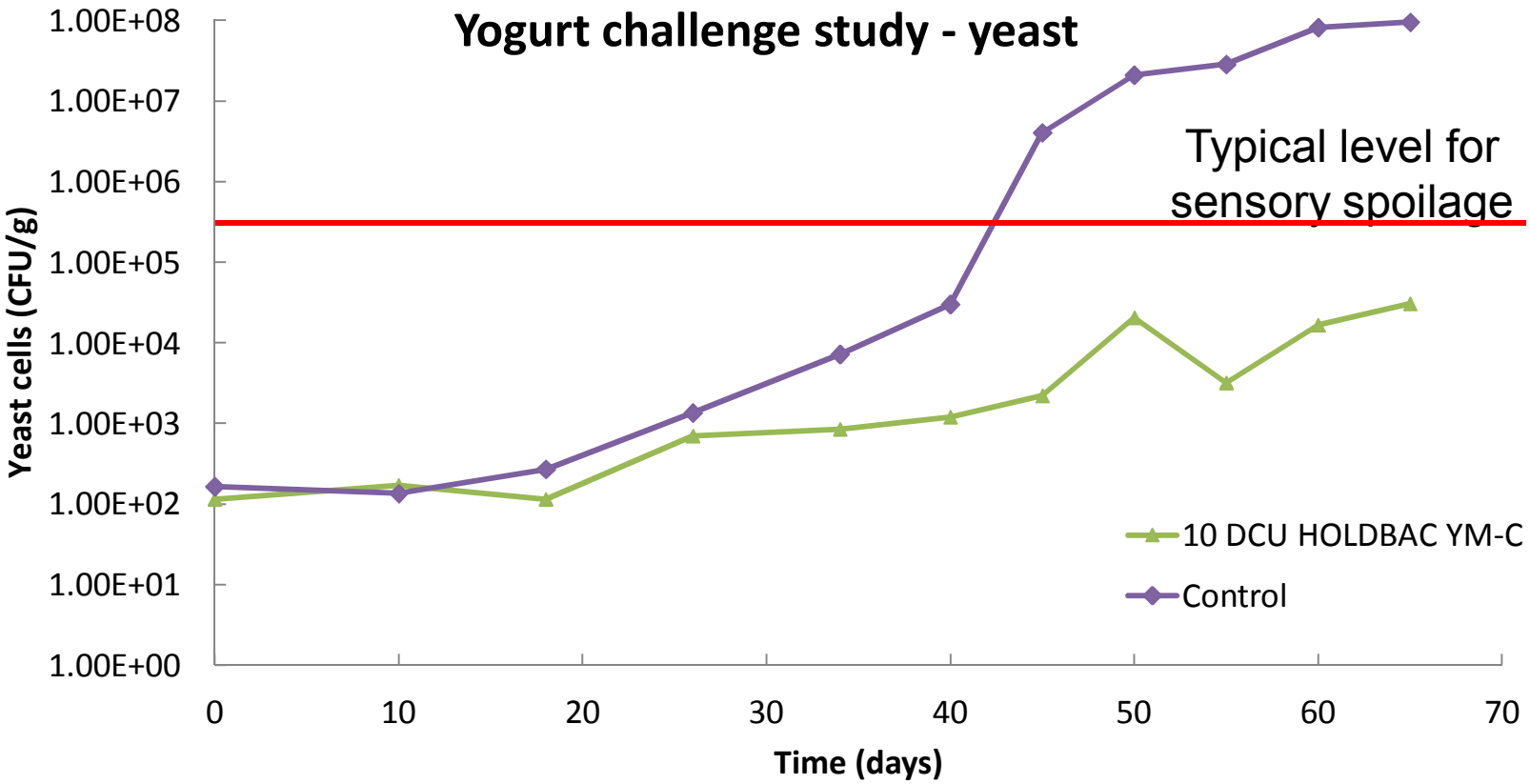
Mold

Yeast



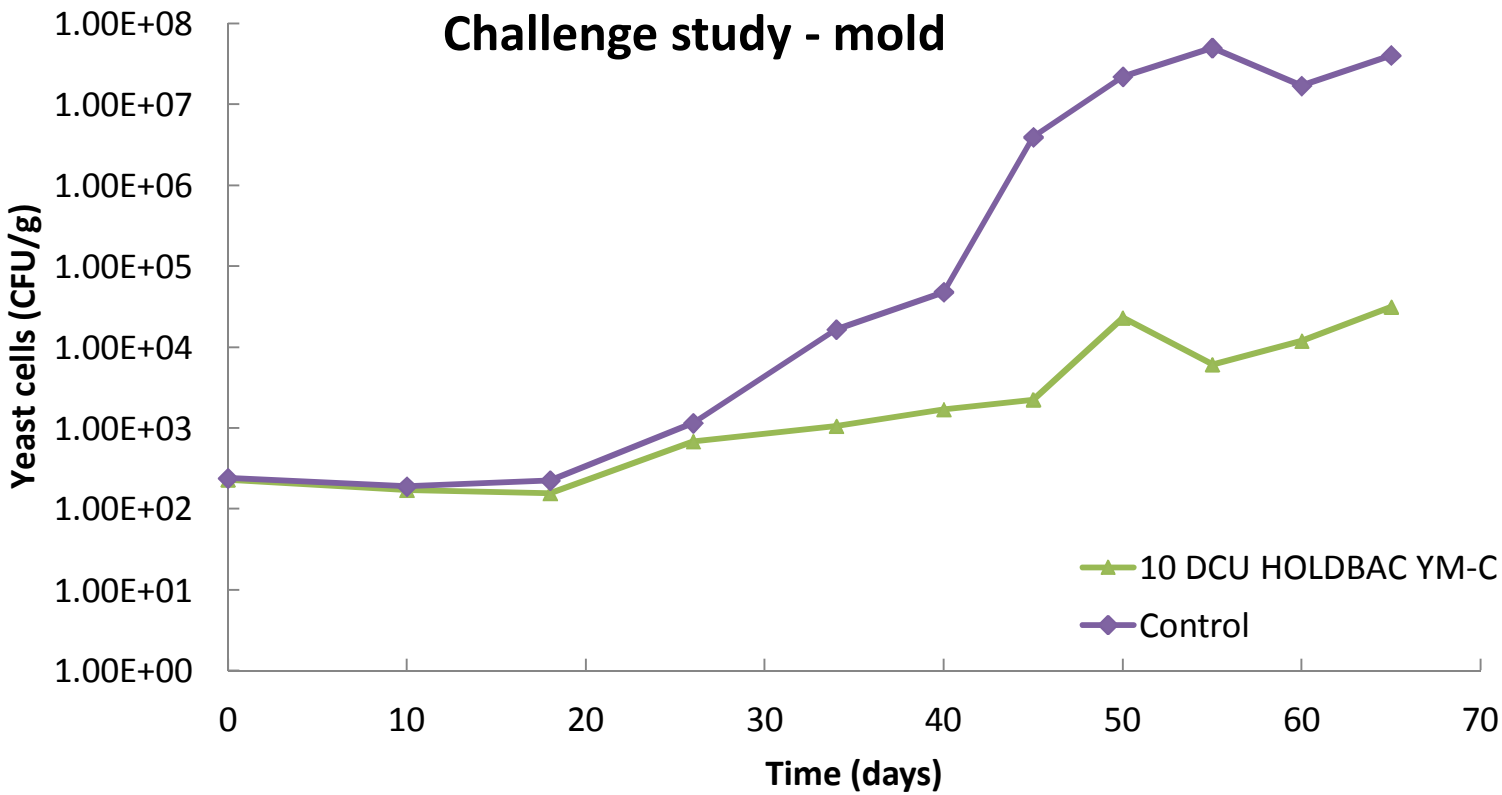
HOLDBAC™ YM dosage: 10 DCU / 100 liter milk

Reducing waste and returns
- extend shelf-life/minimize fluctuations



Dosage	HOLDBAC™ YM-C added at 10 DCU / 100 l milk
Mould challenge	Pool of four yeast strains at 100 CFU / ml
Test conditions	Stored at 10°C for 65 days

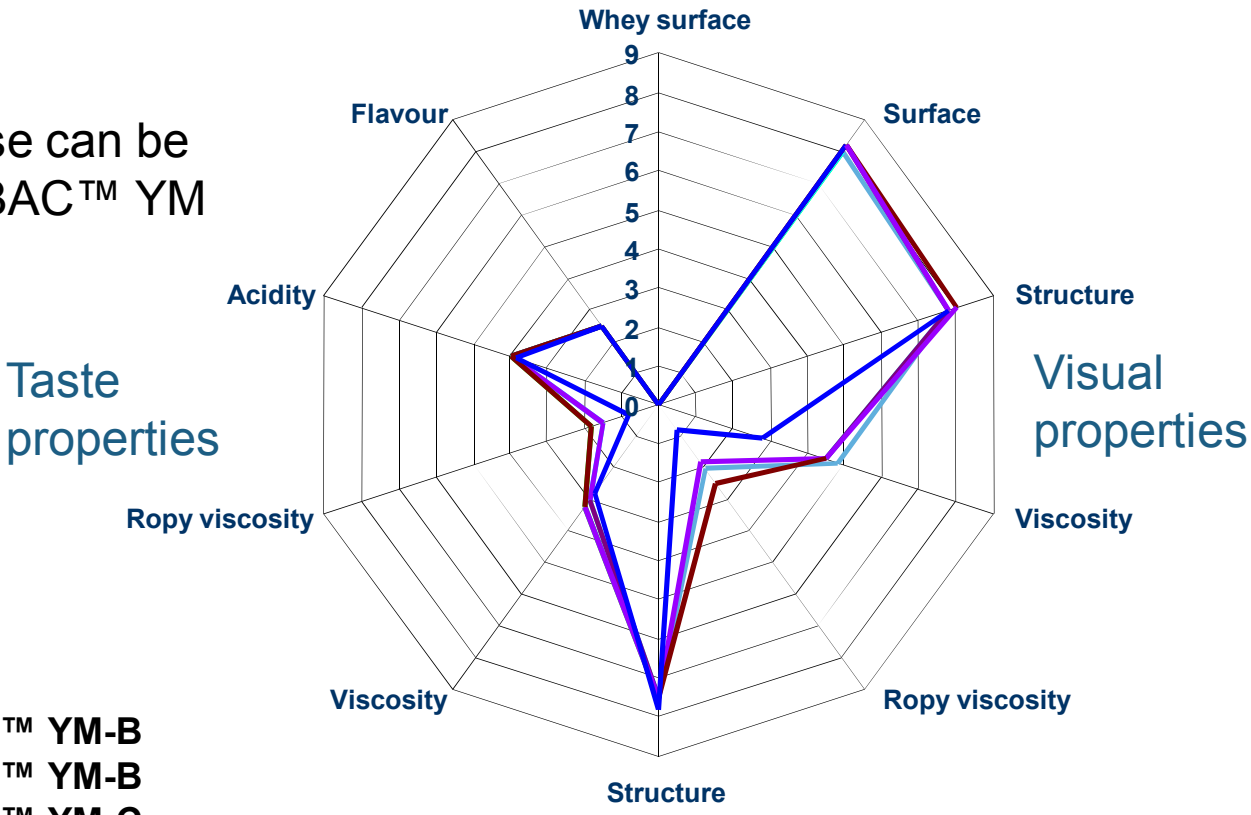
Reducing waste and returns
- extend shelf-life/minimize fluctuations



Dosage	HOLDBAC™ YM-C added at 10 DCU / 100 l milk
Mould challenge	Pool of four mould strains at 100 spores / ml
Test conditions	Stored at 10°C for 65 days

Maintain taste freshness
- yogurt

Some viscosity increase can be obtained using HOLDBAC™ YM

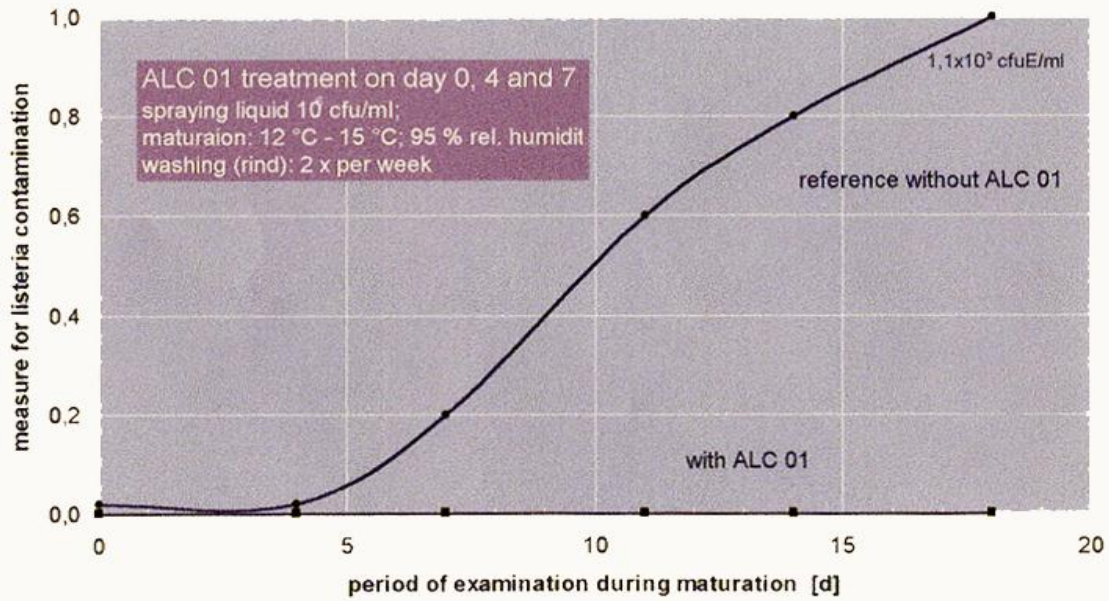


- Reference
- 10 DCU/100 l HOLDBAC™ YM-B
- 50 DCU/100 l HOLDBAC™ YM-B
- 10 DCU/100 l HOLDBAC™ YM-C

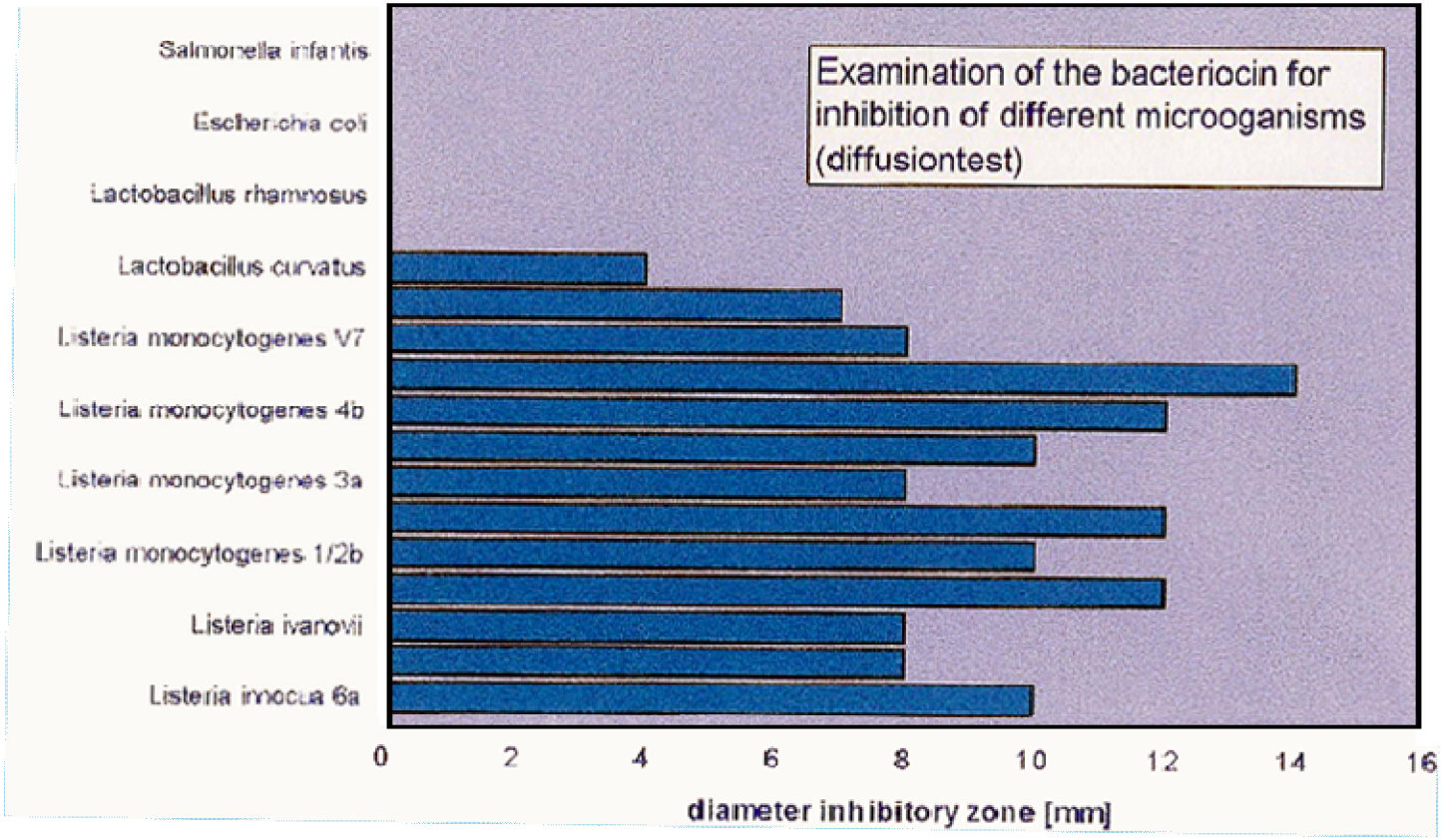
Dosage	HOLDBAC™ YM added at 10 and 50 DCU/100 l milk
Starter used	YO-MIX™ 400 series
Test conditions	Evaluated by a trained panel after 45 d at 6°C

Effect of Holdbac Listeria

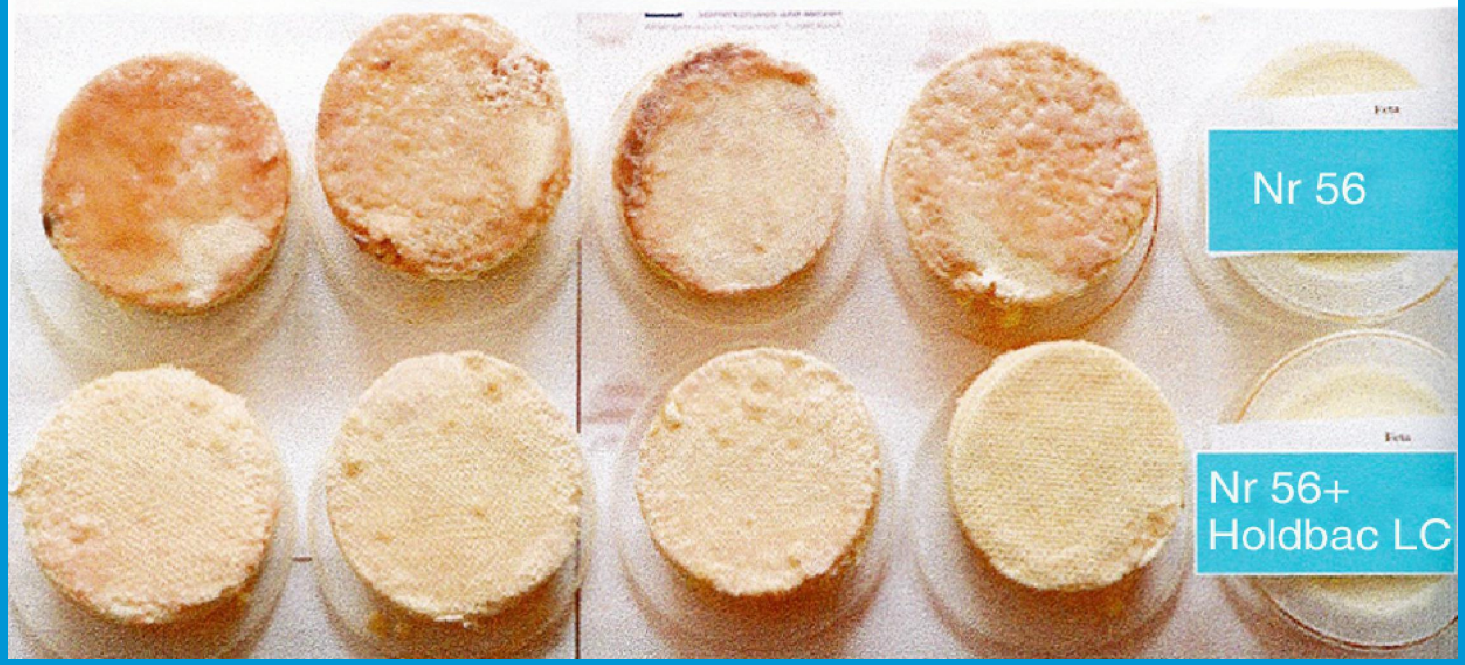
on Munster naturally contaminated with listeria



Efficiency of the bacteriocin formed by Holdbac Listeria



Effect of Holdbac LC
on the yeast counts in Feta



Effect of Holdbac LC
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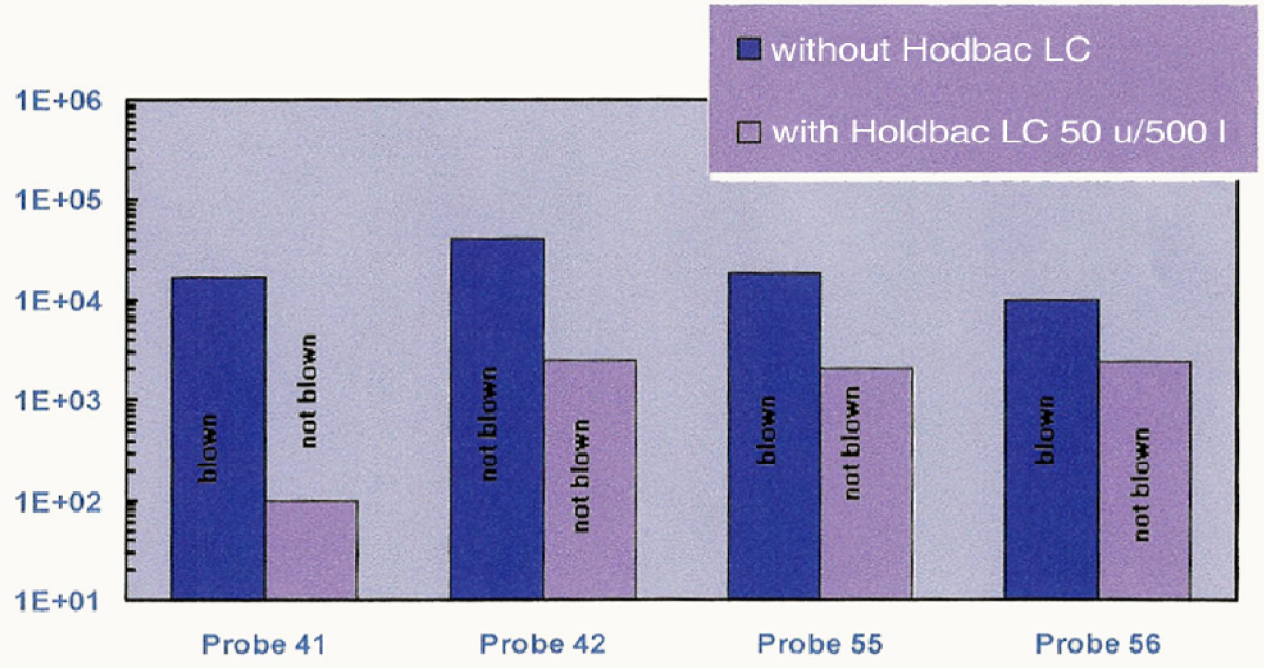


Molds

Penicillium roqueforti

Penicillium candidum (album)

Geotrichum candidum

Trichothercium domesticum (cylindrocardon sp) Mycodore

Yeast

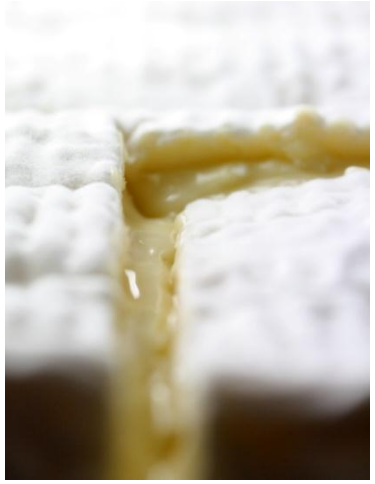
Sacchromyces

debaryomyces

kluyvermyces (telomorph of candida)

candida

Mould ripening : a step wise technology



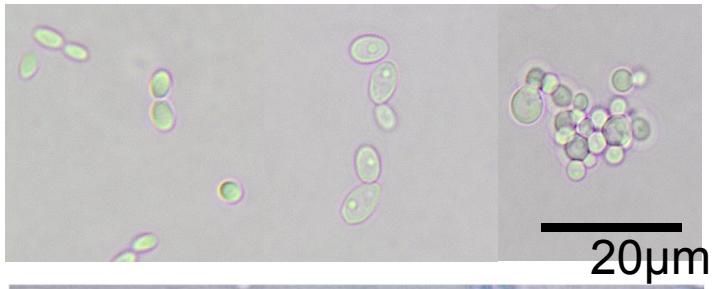
2 main steps

- pH increase up to 5.8 (D1 to 5)
  - driven by lactic acid consumption (e.g by yeast)
  - slower inside
  - Can be influenced by dry salt (*Geotrichum*)
  - From this step, surface flora is critical for final product quality

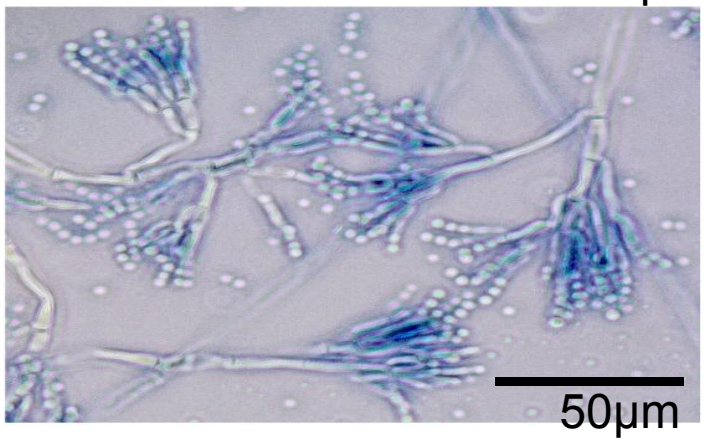
- Then ripening (from day 5)
  - Change of internal pH
  - Visible growth of surface flora, lactose and lactate consumption,
  - Lipolysis and fatty acids degradation– Protéolysis and amino acid degradation

CHOOZIT™ range for white moulded cheese

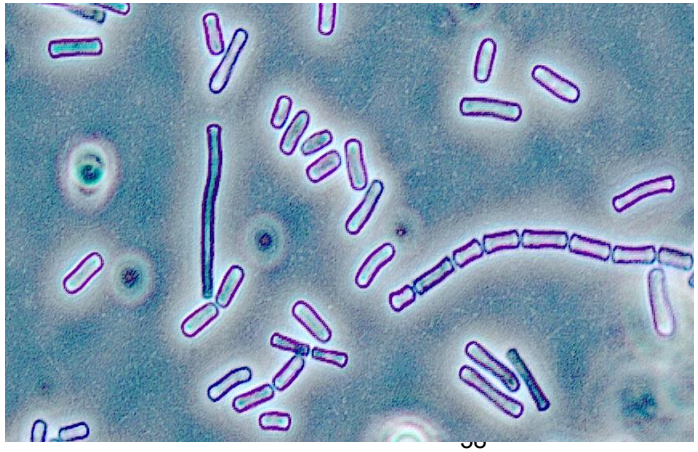
Yeast



Penicillium camemberti



Geotrichum candidum



Yeast functionalities

Consume residual sugars (lactose, galactose)

Consume lactate, thereby neutralising cheese surface which stimulate mould growth

Proteolysis: wide range of proteolytic actovities (caseinolytic, aminopeptidasic, carboxypeptidasic…)

Contribute to lipolysis (limited)

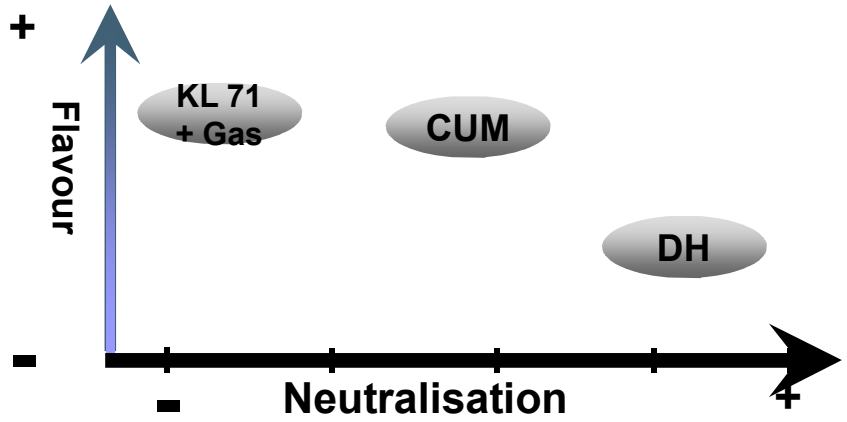
Produce gas (potentially)

Produce flavour compounds

Interact with other microorganisms: stimulate *G. candidum growth*

CHOOZIT™ Yeasts

CHOOZIT™ product	Compo.	Pop. per dose	Assimilation			Fermentation /CO_2 production on			Salt tolerance	Neutralising power	Proteolysis potential	Flavouring potential	Comment
			Glucose	Gal /Lactose	DL Lactate	Glucose	Gal /Lactose	Lactate					
DH	*Debaryomyces hansenii*	*1D= 1.10^{10}*	*yes*	*yes*	*yes*	*no*	*no*	*no*	++	+++	+	+/-	
CUM	*Candida utilis*	*1D=5.10^{9}*	*yes*	*no*	*yes*	*yes*	*no*	*no*	+	++	+/-	+	
KL71	*Kluyveromyces lactis*	*1D= 2.10^{9}*	*yes*	*yes*	*yes*	*yes*	*yes*	*yes*	+	+	+/-	+	*Typically used for microper sillage in soft cheese*



Penicillium candidum : functionalities

Form the typical white mould cover on Brie or Camembert type cheese

Neutralisation : consume lactic acid >> pH increase

Proteolysis: complex and very active system

- Aspartyl protease ou protease acide : pH optimum 5.00 on casein
- Metalloproteases: pH optimum ph 6.00 (degradation of αS1 casein)
- Carboxypeptidases: pH optimum ph de 6.00 à 6.50
- Aminopeptidases: pH optimum entre 6.00 and 8.00 >> release free AA antribute to reduce bitterness

Lipolysis : contribute to typical white mould cheese flavour.

Flavour production : methyl ketones, secondary alcohols, esters, aldehydes, amines and ammonia

Interactions with other micro-organismes: very fast growth, competing with the growth of indesirable moulds.

Penicillium candidum : growth parameter

Temperature

- Optimum between 20 et 25° C,

Used in between 8 et 14° C

pH

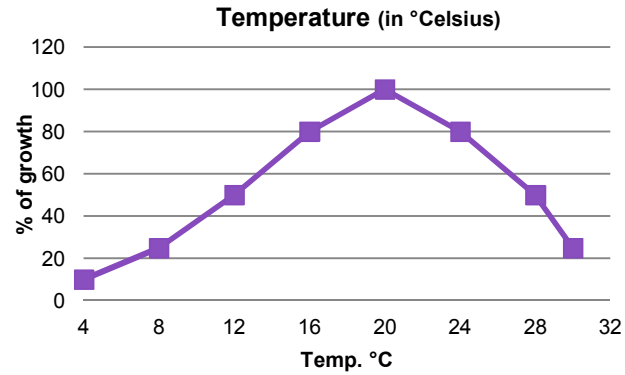
- Not very sensitive in the range pH 4.5 à 5.2

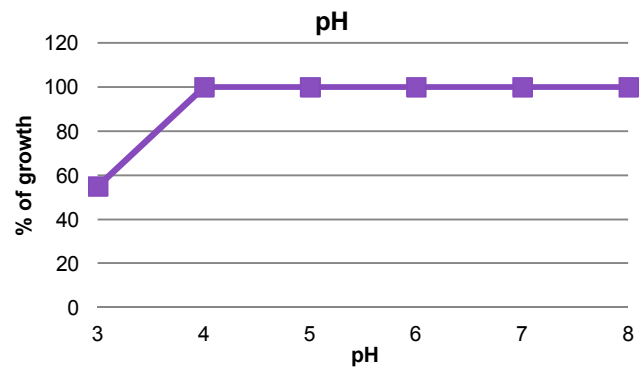
Halotolerance

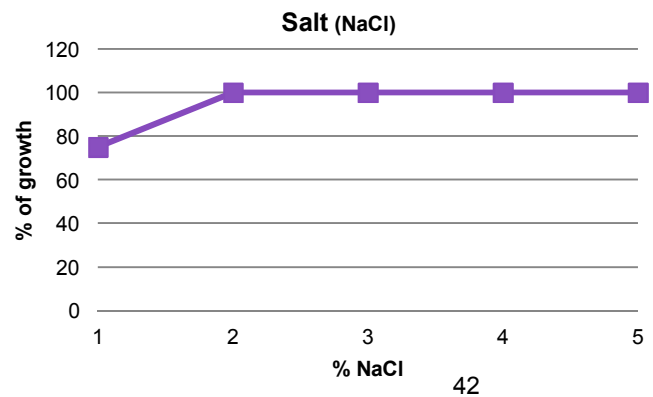
- High salt resistance (dry salting or brine salting

Nutritional need

- Sugar, lactates…







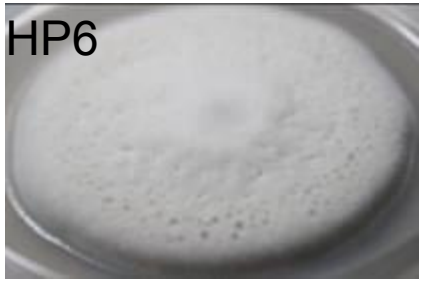
CHOOZIT™ PC range : aspect

CHOOZIT™ PC02

CHOOZIT™ PC12

CHOOZIT™ PC22

CHOOZIT™ PC HP6



CHOOZIT™ PC Neige

CHOOZIT™ PC SAM3

CHOOZIT™ PC VB

CHOOZIT™ PC VS

Pictures have been taken after inoculation (2µl of a solution containing 1.E+06 spores/ml) and incubation on Potatoe Dextrose Agar (PDA) for 5 days at 25° C

CHOOZIT™ PC: selection guide

CHOOZIT™	Whiteness	Growth speed	Thick-ness	Proteolysis	Lipolysis	Other benefits	Target techno.
PC 02	+	++	+	+	+		Solubilized techno. High HFD
PC 12	+	++	++	++	+		
PC 22	+++	++	++	++	++		
PC VS	+++	+++	+	+++	++		Traditional lactic curd
PC HP6	+++	+++	++	+++	++		
PC NEIGE	++	+	++++	+++	+++		
PC VB	++	+	++++	++++	+++		
PC SAM 3	++	+	+++	++	++	Anti-mucor	

1 dose de PC = 2.10^9 spores

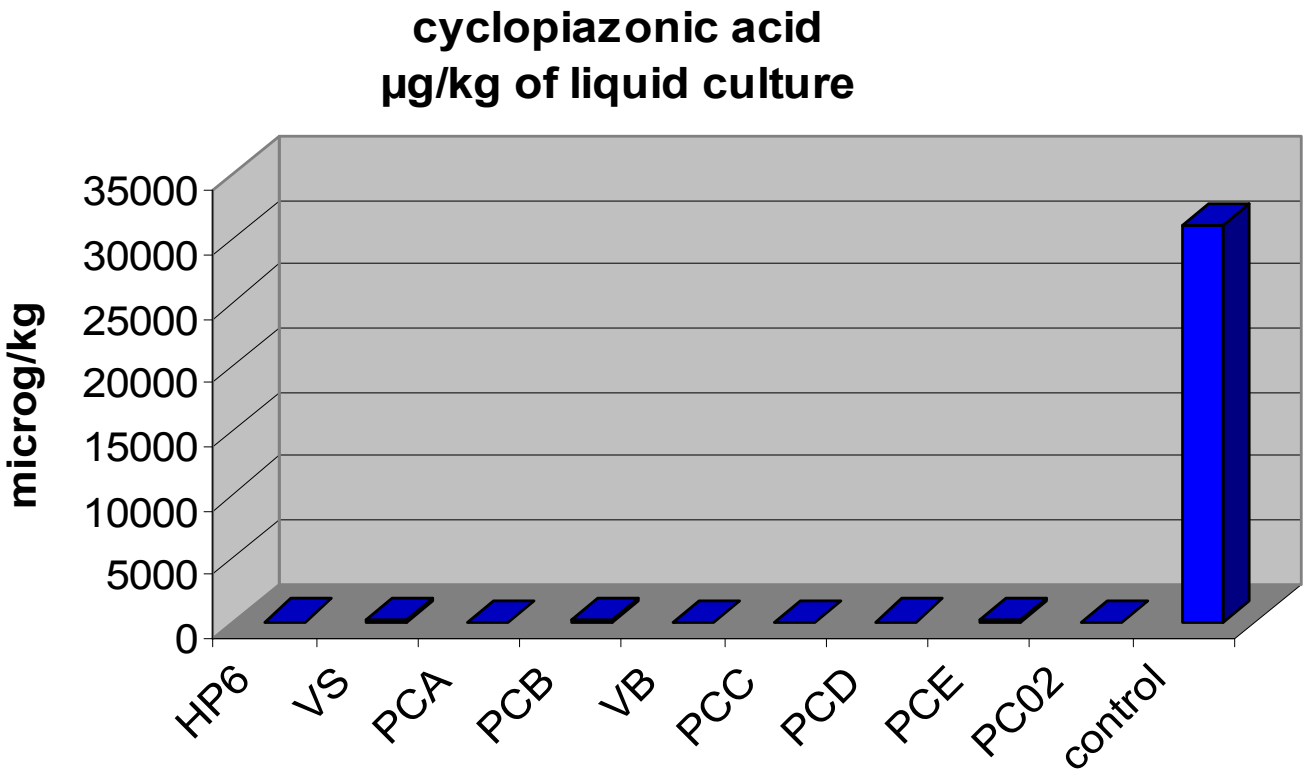
CHOOZIT™ PC range: controlling mycotoxin risk

Penicillium camemberti species can produce only one mycotoxine: cyclopiazonic acid

Strain dependent feature

All our industrial strains of P. *camemberti* were tested

Not detectable or very low for all (far below toxicity level) in toxinogenic conditions



Geotrichum candidum: functionalities

Thin surface fine, white to cream colour

Influence rind look, texture and flavour

Neutralise the surface by consuming lactate, contributing to texture improvement and to stimulate acido-sensitive flora

Contribute to stabilise the rind

Contribute to protéolysis (much less than PC)

Contribute to release amino-acids and to reduce bitterness (less than PC)

Contibute to lipolysis (strain dependant feature)

Produce flavour compounds (methyl ketones, sulfury compounds…)

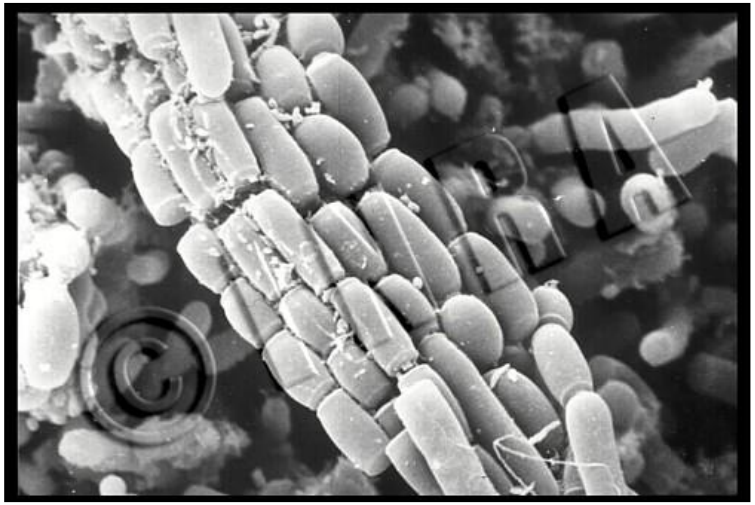
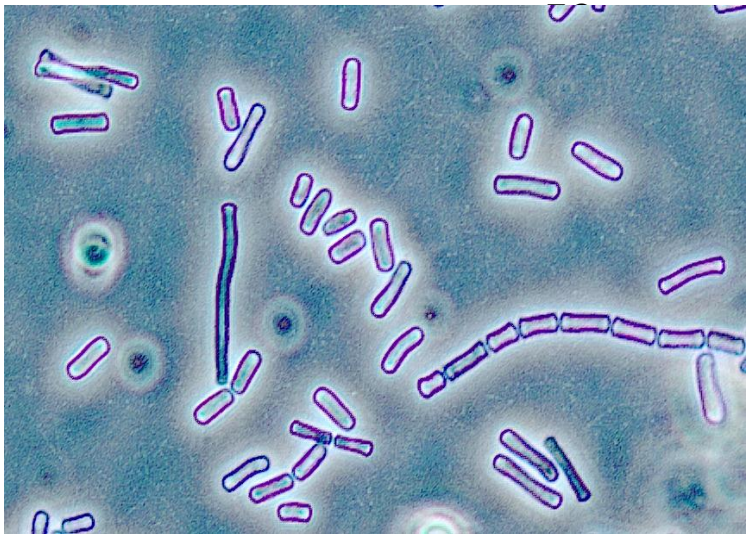


Photo INRA



Geotrichum candidum – 3 morphology [Gueguen, 1984]

- Type 1 : yeast like
  - Cream colour T° C optimum: 22 to 25° C, used from 10 to 15° C
  - Little mycelium, many arthrospores low proteolytic activity
- Type 2 : Intermediate
- Type 3 : Mould like
  - White T° C optimum: 25 to 30° C, used from 10 to 15° C
  - Proteolytic

1. Yeast like

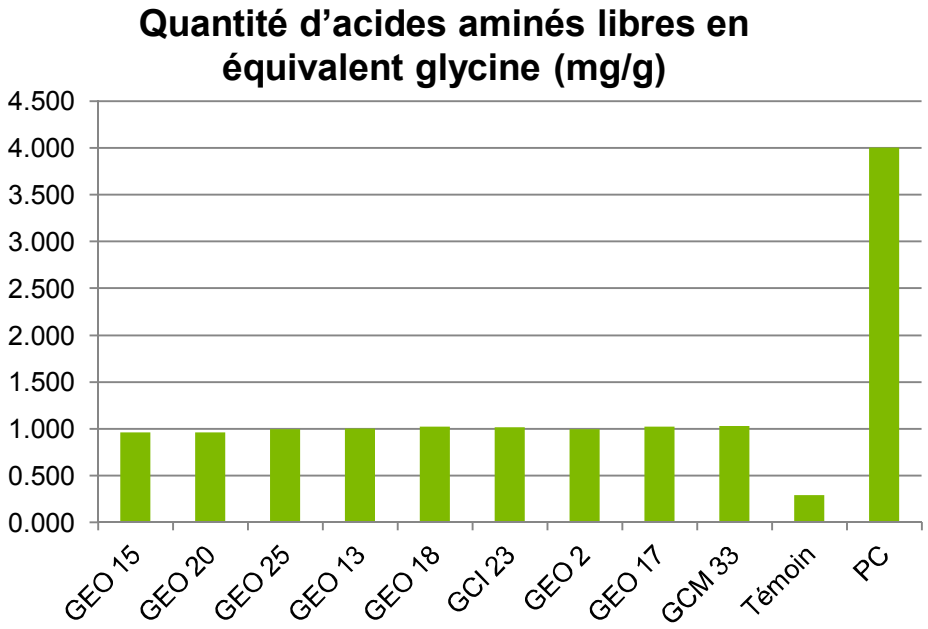
2. Intermediate

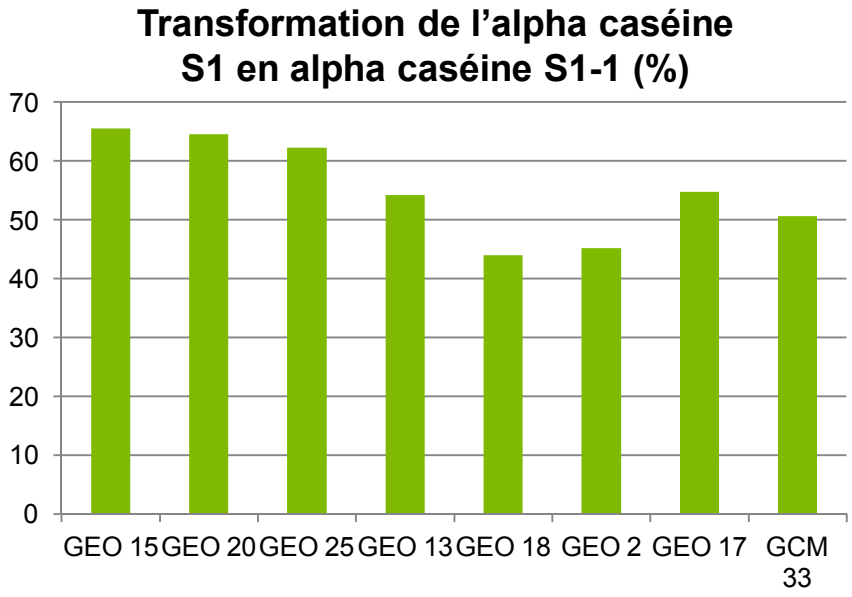
3. Moulds like

CHOOZIT™ Geo : Proteolysis

Light difference in between strains (slightly stronger for mould-like ones)

But in any case much lower than for PCs!





CHOOZIT™ Geo : Selection guide

Genre: *Geotrichum* Species: *G. candidum*

CHOOZIT™	Format	Morphology	Growth speed	Flavour
GEO 13 LYO	Lyophilised	Intermediate	+	+
GEO 15 LYO	Lyophilised	Yeast like	++	+
GEO 17 LYO	Lyophilised	Mould like	++	+

* Freeze-dried formats are strongly advised for shipment outside Western Europe

PC/GEO combination

Benefits to cheese quality:

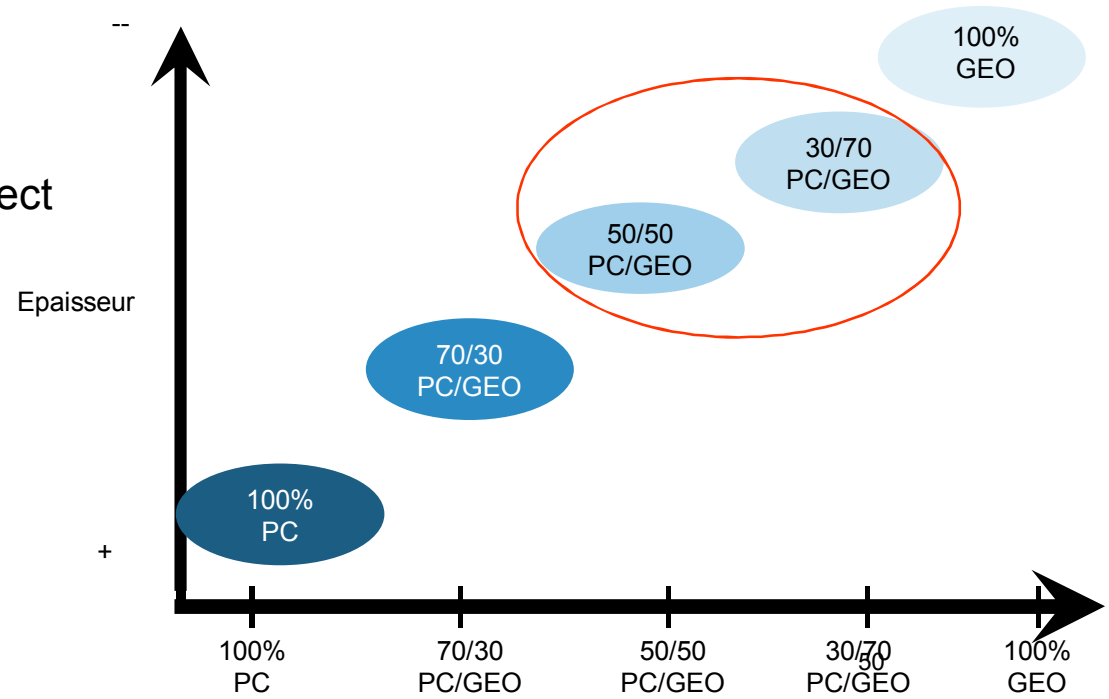
Faster surface flora development,

Better protection against contaminants,

More regular & thinner rind,

Better stability from the rind during shelf-life,

Reduced bitterness and flavour defect (mushroom, ammonia...)



CHOOZIT for blue cheese – Selection guide

	Culture	PACKAGING SIZES	Phage alternative	ACIDIFICATION SPEED	FLAVOUR	GAS FORMATION	APPLICATIONS		
							STILTON	LACTIC BLUE type) CHEESE (Roquefort	SOFT BLUE CHEESE	GORGONZOLA TYPE
DEFINED HETEROFERMENTATIVE MESO				***Leuconostoc***					
Freeze dried									
	CHOOZIT LM 57	20-50DCU	CHOOZIT LM79 - LD106	Medium	++	++++	+	+	+
	CHOOZIT LM 79	50DCU	CHOOZIT LM57 - LD106	Medium	++	++++	+	+	+
	CHOOZIT LD106	50DCU	CHOOZIT LM79 - LM57	Medium	++	++++	+	+	+
			Proteolysis	Lypolysis	Color				
BLUE MOULDS				***Penicillium roqueforti***					
Freeze dried									
	P. roqueforti CB2 LYO	5D	++	++		Blue			
	P. roqueforti PA LYO	10D	+	+		Blue grey			
	P. roqueforti PJ LYO	10D	+	+		Light green			
	P. roqueforti PV LYO	10D	+++	+++		Dark blue-green			

CHOOZIT™ range for smear cheese ripening

Yeast :

- Consume residual sugars,
- Neutralise (bring pH up), enabling the growth of acid sensitive flora
- Contribute to proteolysis, lipolysis and to flavour development (depending on species)
- Contribute to smear look

Brevibacterium :

- Mesophilic, optimal temperature: 25° C à 30° C – minimal temp.: 10-12° C
- Acid-sensitive: minimum pH = 6.00 or 5.80 for CHOOZIT™ latest generation
- Salt -tolerant : till 12% NaCl for some strains
- Strong production of sulfury flavour compounds (feet, garlic, egg smell)

Other bacteria and blends : positive interaction with Brevibacterium

Potential use in combination with Geo or PC

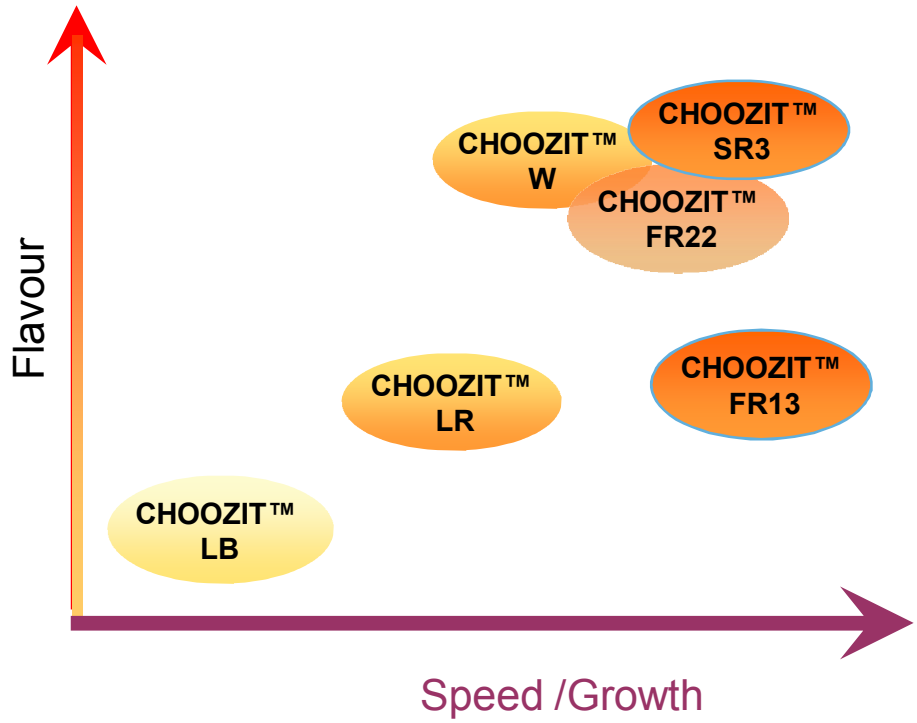
CHOOZIT™ Brevibacterium

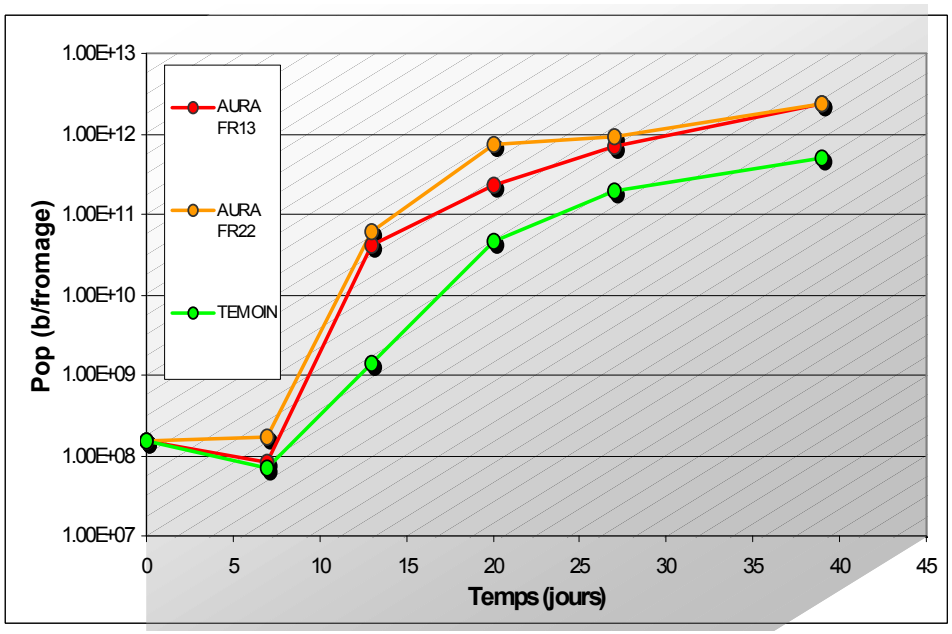
Culture	Composition	Formats	
CHOOZIT™ FR13	*B. linens*	Lyo 10-50-100D	1 Dose = 5.10^{10}cfu
CHOOZIT™ FR22	*B. linens*	Lyo 2-10-50D	
CHOOZIT™ SR3	*B. linens*	Lyo 2-10-100D	
CHOOZIT™ LR	*B. linens*	Lyo 10-100D	
CHOOZIT™ LB	*B. linens*	Lyo 10-100-300D	
CHOOZIT™ Linens W	*B. linens*	Fro 500g	

CHOOZIT™ Other bacteria or blends

Culture	Composition	Format	
CHOOZIT™ MVA	*Staphylococcus xylosus*	Lyo 2-10-100D	1 Dose = 1.10^{11} cfu
CHOOZIT™ MVS	*Staphylococcus carnosus*	Lyo 10D	
CHOOZIT™ MGE	*Arthrobacter nicotiniae*	Lyo 10-100D	1 Dose = 5.10^{10} cfu
CHOOZIT™ PLA	*A. nicotaniae, B. linens, D. hansenii, G. candidum*	Lyo 2-10D	
CHOOZIT™ OFR9	*B. linens, D. hansenii, G. candidum, C. utilis, B. casei*	Liquide	
CHOOZIT™ ARO 21 – HA	*P. membranaefaciens, K.lactis, L. paracasei, S. carnosus, B. linens*	Lyo 10D	

CHOOZIT™ Brevibacterium – Growth & colour features

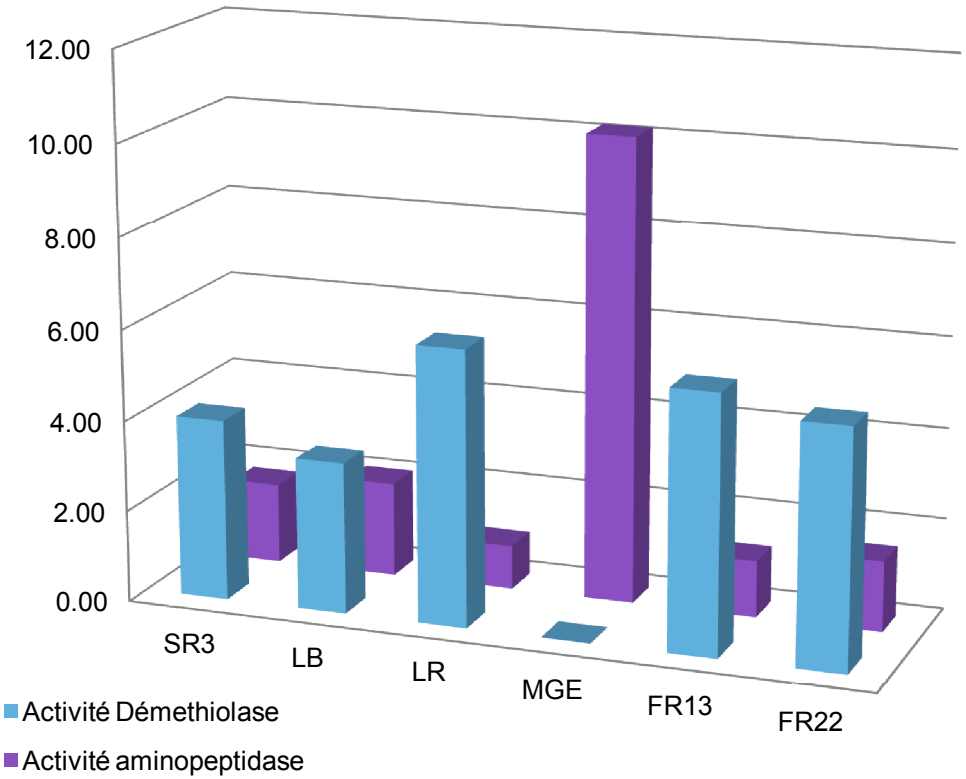




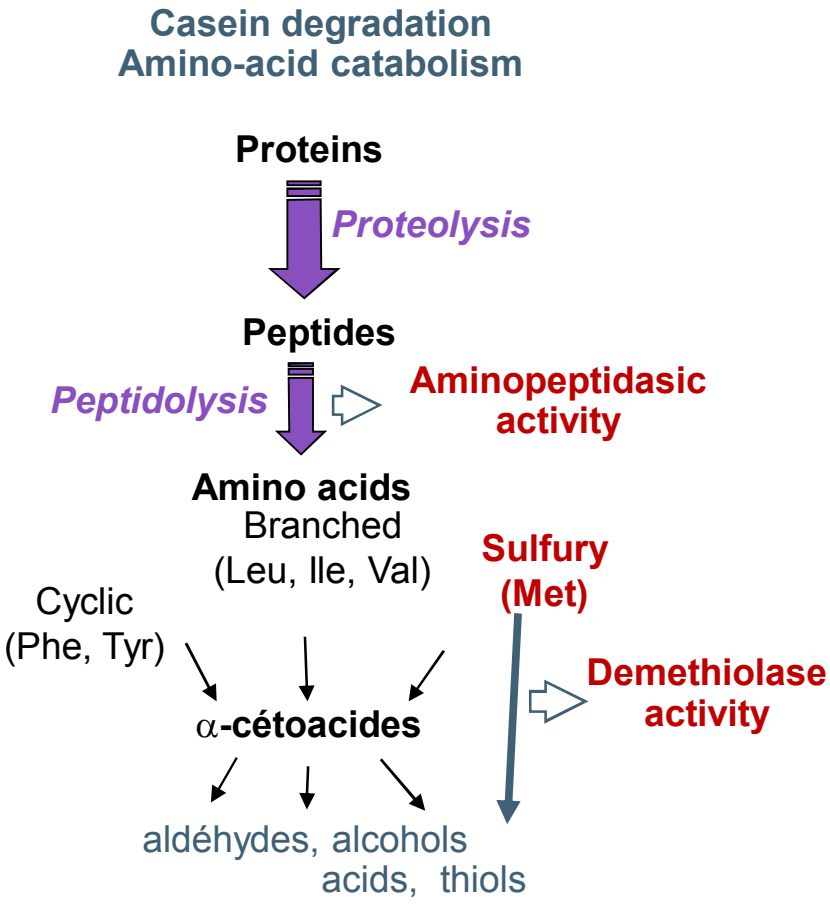
Growth of CHOOZIT™ FR13 et FR22 (latest generation) on thermophilic matrix (buffered, low pH), with same amount of yeast

CHOOZIT™ Brevibacterium – Enzymatic features

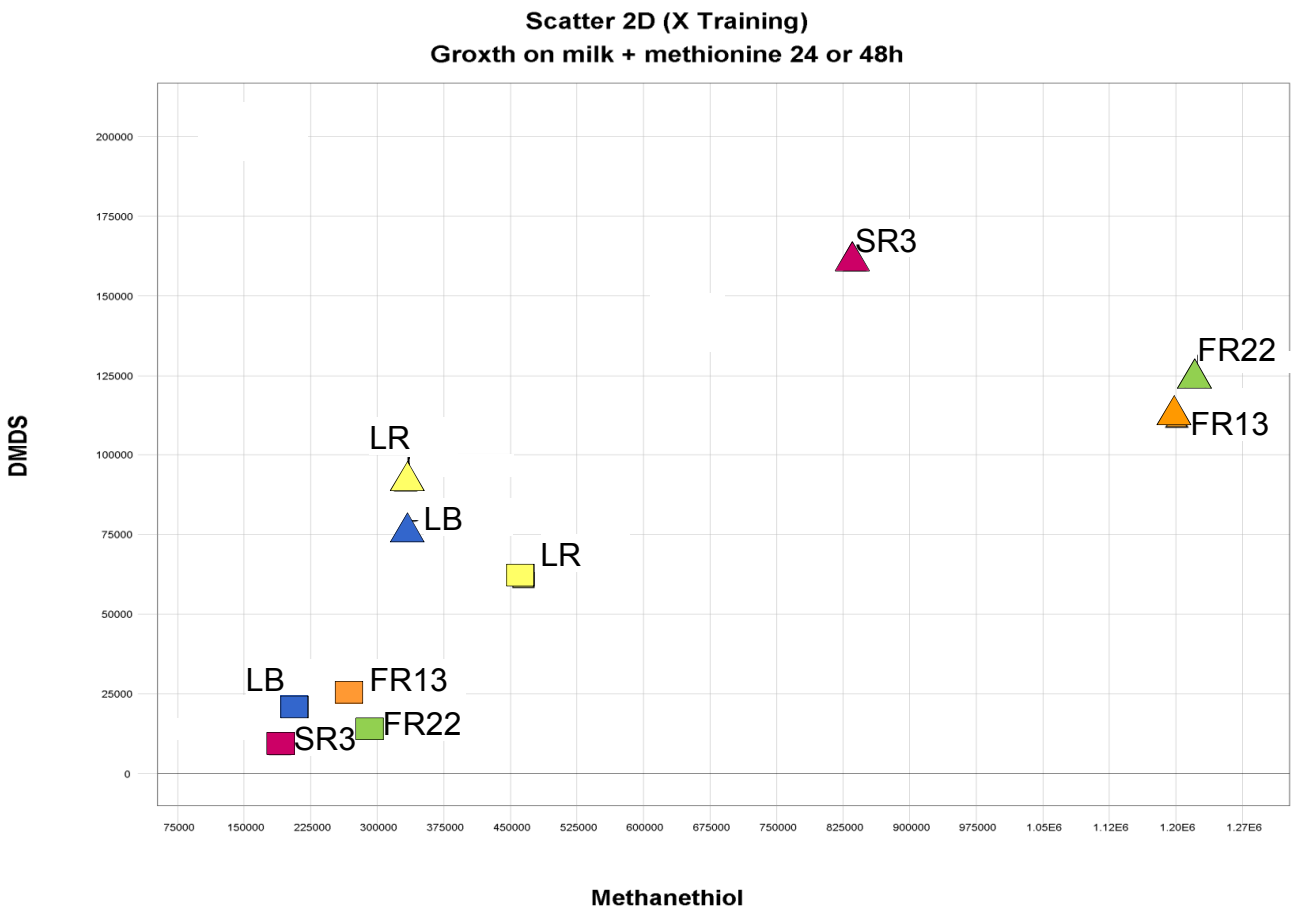
Enzymatic activities



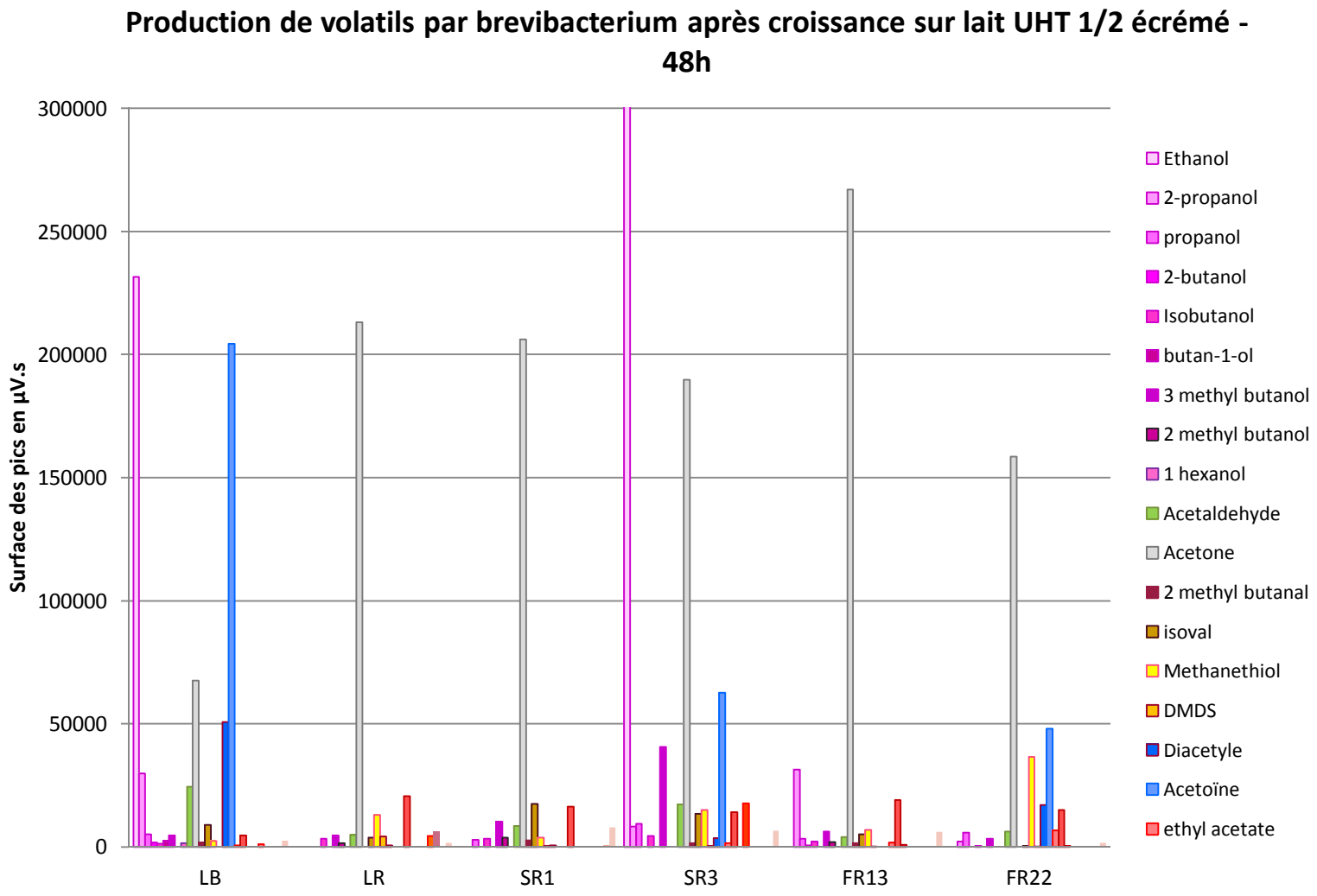
Mesure of demethiolase and aminopeptidase activities : activities are expressed as total activity per biomass used in the assay (measured DO 410 nm / DO650 nm of culture at 1/10th dilution)



CHOOZIT™ Brevibacterium – Flavour features: production of sulfury compounds (MM & DMDS) on milk+methionine



CHOOZIT™ Brevibacterium – Flavour features : non sulfury

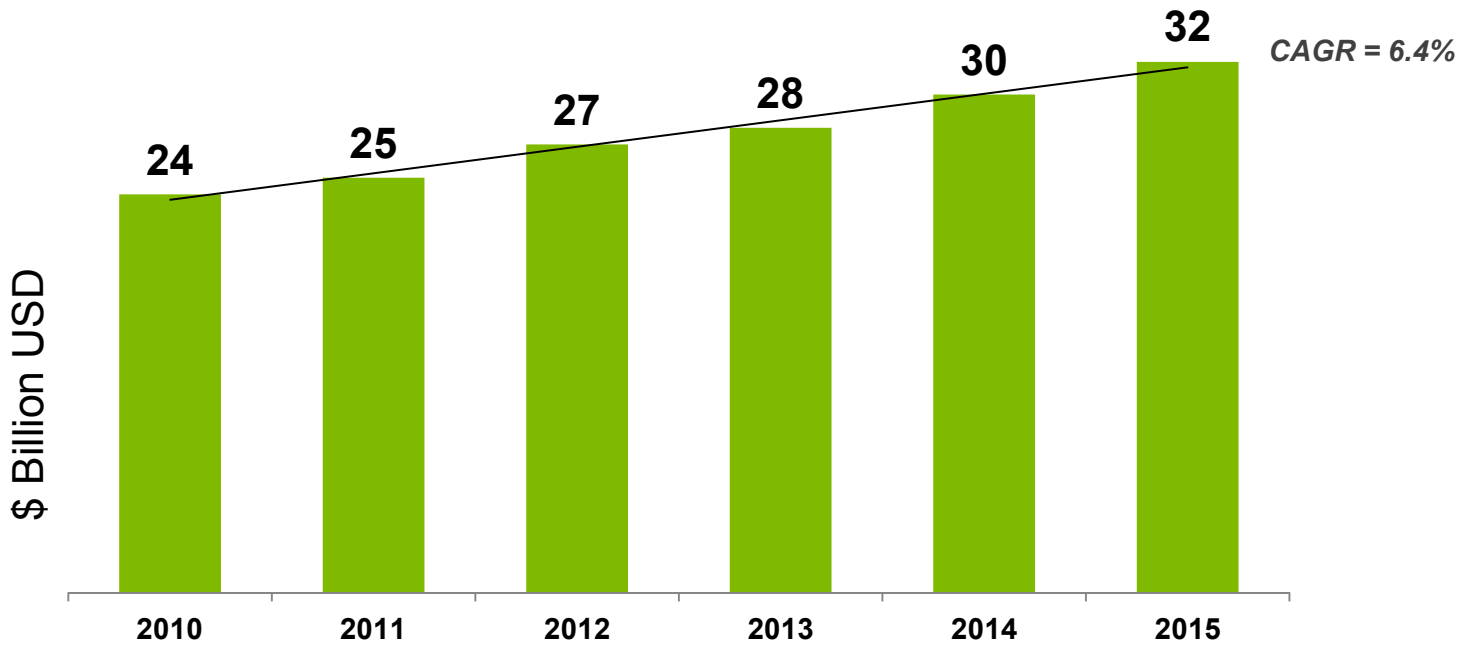


CHOOZIT™ Selection guide – Smear ripened cheese

Ferment CHOOZIT™	Colour	Sulfury flavour	Growth speed	Acid-tolerance	Other benefits	Target techno.
CHOOZIT™ FR13		++	++++	5.8	No colour déviation during ripening	All technologies, esp. solubilized
CHOOZIT™ FR22		+++	+++	5.8		
CHOOZIT™ SR3		+++	++++	6		Traditional smear ripened lactic curd
CHOOZIT™ LR		++	++	6		
CHOOZIT™ LB		++	+	6		
CHOOZIT™ MVA		-	++++		Competitive exclusion – Improve surface appearance	
CHOOZIT™ MVS		-	++++			
CHOOZIT™ MGE					Boost brevi – act. Aminopep.	
CHOOZIT™ OFR9				6	Blend	
CHOOZIT™ PLA				6	Blend	

Probiotics represent one of the fastest growing sectors within the global functional food market

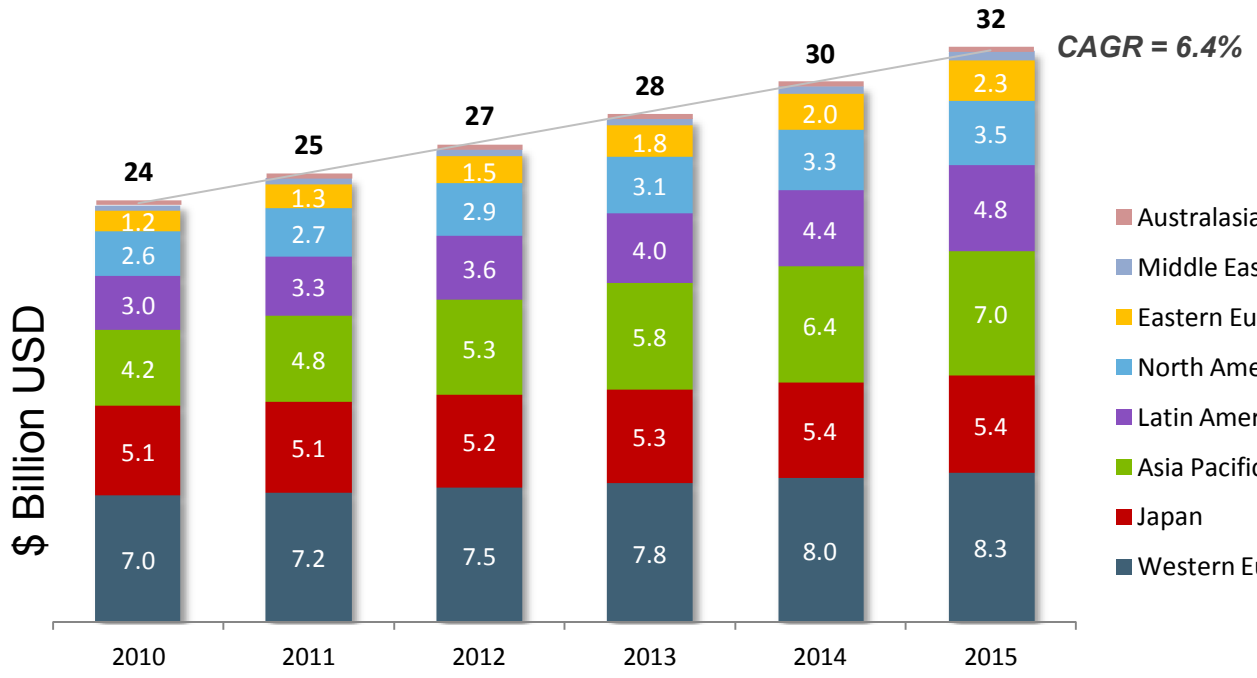
- In 2011, estimated total finished product sales was $25 billion USD.
- A strong growth rate is expected to raise this size to $32 billion by 2015.



Source: Euromonitor 2011

North America, Latin American, and Asia Pacific have strong growth forecasted

- These regions' market sizes will significantly increase in the coming years.
- Western Europe & Japan are the most developed markets for probiotics, and growth is slower than the average



	2010 Sales in Billion USD	CAGR 2010-2015
Australasia	0.3	10.4%
Middle East & Africa	0.3	2.5%
Eastern Europe	1.2	13.9%
North America	2.6	6.6%
Latin America	3.0	10.0%
Asia Pacific	4.2	10.8%
Japan	5.1	1.4%
Western Europe	7.0	3.4%

Source: Euromonitor 2011

Cheese examples

Said to inhibit harmfuld bacteria as well as promote improved	Cheese with Probiotics	Contains lactobacilli that help regulate the digestive system	Contains probiotics	Contains probiotics that contribute to a healthy digestive system	Contains more than 1 billion probiotics per serving to contribute to healthy digestion
Dean Foods	Colun	Paranhos	Interdeli	Agropur	Altiments Ultima

Source: Mintel GNPD 2011

Cheese : a potential "functional food" ?

Research findings	Comments
Production of Crescenza Cheese by Incorporation of Bifidobacteria **M. Gobbetti 1, A. Corsetti 1, E. Smacchi 1, A. Zocchetti 1, and M. De Angelis 1** 1 Institute of Dairy Microbiology, Agriculture Faculty of Perugia, University of Perugia, Italy	When added individually, the cell counts of *B. bifidum, B. longum*, and *B. infantis* were $\log^{10}$ 8.05, 7.12, and 5.23 cfu/g, respectively at the end of the ripening (14days).
Viability of Probiotic Argentinian Fresco Cheese (Vinderola et al.2000,Universidad Nacional del Litoral, Santa Fe, Argentina)	Different combinations *of B.bifidum B.longum*, *L.acidophilus*, and *L. casei* were evaluated as probiotic adjuncts. Counts decreased <1 log order for bifidobacteria but no decrease was detected for L. casei. B. bifidum was the most resistant organism.
Probiotic white cheese with Lactobacillus acidophilus KasImoglu, A; Goencueoglu, M; Akguen, S International Dairy Journal [Int. Dairy J.]. Vol. 14, no. 12, pp. 1067-1073. Dec 2004.	Survival of L. acidophilus during ripening of the cheese stored in vacuum or in brine was studied. On ripening in vacuum pack, L. acidophilus survived to numbers $>10^7$ cfu/ g
Incorporation and survival of Bifidobacterium sp. strain Bo and Lactobacillus acidophilus strain Ki in a cheese product (Gomes et al. Univ. católica Portuguesa, escola superior biotecnologia, 4200 Porto, PORTUGAL)	During the whole storage period studied, the average numbers of L. acidophilus strain Ki decreased by two log cycles to 0.2 x 10[7] cfu/g, whereas those of Bifidobacterium sp. strain Bo decreased by less than one log cycle to 6-18 x 10[8] cfu/g.Incorporation of Bifidobacterium spp. and L. acidophilus as starters in a Gouda-type cheese is feasible and can offer an alternative and interesting route of administering them to human beings.

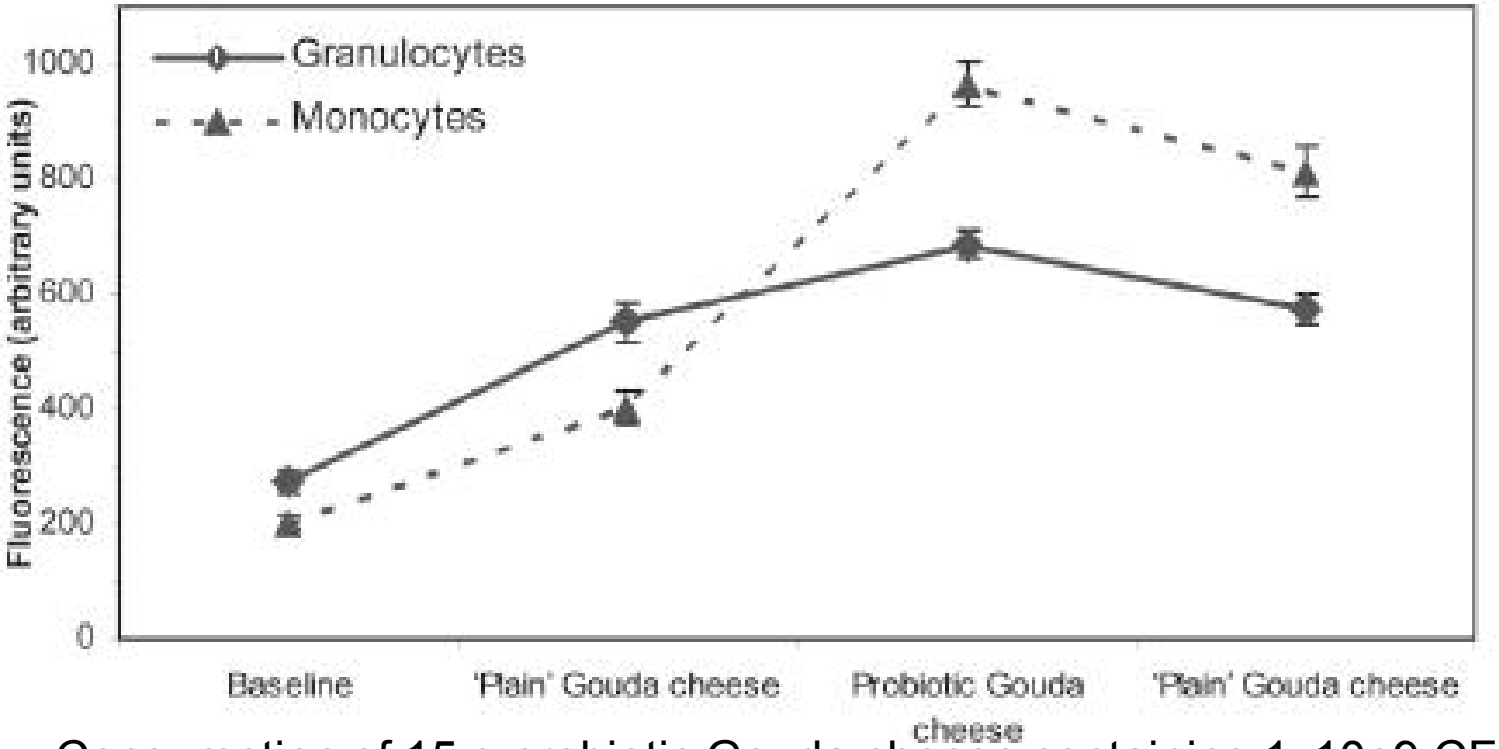
18 Publications available showing addition of probiotic to cheese

Cheese type	Probiotic bacteria[1]	Viability log10 (CFU/g)	Effects	Ref.
Brine-ripened cheese	*L. acidophilus*	≥8.5 after 90 days	Maintained viability, *L. acidophilus* can be used to give good flavour, texture, and a high level of proteolysis.	21
Cheddar	*Bifidobacterium* and *Lactobacillus*	7.5 after 24 weeks	Significant effects depending on the type of probiotic used, ripening time, ripening temperatures and their interactions on the concentration of lactic and acetic acids in the cheeses	22
Cheddar	*L. acidophilus, L. casei, L. paracasei* and *Bifidobacterium spp.*	>7.5 after 24 weeks	All probiotic adjuncts survived the manufacturing process and maintained their viability	23
Cheddar	*B. bifidum*	>7.1 after 24 weeks	No adverse effect on flavour, texture or appearance	24
Cheddar	*B. animalis* Bb-12	≥ 8 after 6 months	Partial negative effect, moisture level = 40 percent, which is slightly above the legal limit permitted for Cheddar cheese	25
Cheddar	*B. longum* BB536	5 after 6 months	No adverse effect on the cheese composition	25
Cheddar	*E. faecium* PR88	8.6 after 15 months	Cheddar cheese compares very favourable with fresh yogurt as a delivery system for viable probiotic microorganisms to the gastrointestinal tract	12
Cheddar	*L. acidophilus, Bifidobacterium* sp., *L. casei, L. paracasei* and *L. rhamnosus*	≥7.6 after 32 weeks for all except *L. acidophilus* (3.7)	Cheddar cheese is a good vehicle for a variety of commercial probiotics but survival of *L. acidophilus* strains will need to be improved	5
Cheddar-like	*B. infantis*	6.4 after 84 days	Excellent viability, metabolically active	26
Cottage	*B. infantis*	>0.5 after 22 days	Loss of viability, metabolically active	27
Crescenza	*B. bifidum, B.longum* and *B. infantis*	8.05, 7.12 and 5.23 after 15 days	Lower viability after 14 days	28
Fresco	*Bifidobacterium, L. acidophilus* and *L. casei*	<1 log order in 60 days	Cheese found to be an adequate carrier of probiotic bacteria	9
Minas	*L. acidophilus*LAC4	≥ 6.23 after 28 days	*L. acidophilus* remained viable during the shelf life of the products and no negative affect was detected	29
Gouda	*B. lactis* Bo & *Lb. acidophilus* Ki	6.5 after 70 days	Effect on cheese flavour after 9 weeks ripening	30
Gouda	*L. acidophilus, L. rhamnosus*	8.5 after *in vitro* simulation	Probiotics maintained their viability both in cheese and during passage through the upper gastrointestinal tract	10
Gouda	*L. acidophilus, L. rhamnosus*	>4 after 4-week intervention	Probiotics survived the gastrointestinal transit, and enhanced immune responses was detected	14
Semi-hard	*L. acidophilus*and *L. paracasei*	>7.78 after 60 days	Increase in the production of short peptides and free amino acids, and modification of peptide profiles	31
Semi-soft	*L. fermentum*	7.7 after 54 days	Probiotic strain survived the technological processing of cheese, retaining moderate antimicrobial and high antioxidative activity throughout ripening and storage	32

[1]L, *Lactobacillus*; B, *Bifidobacterium* and E=*Enterococcus*

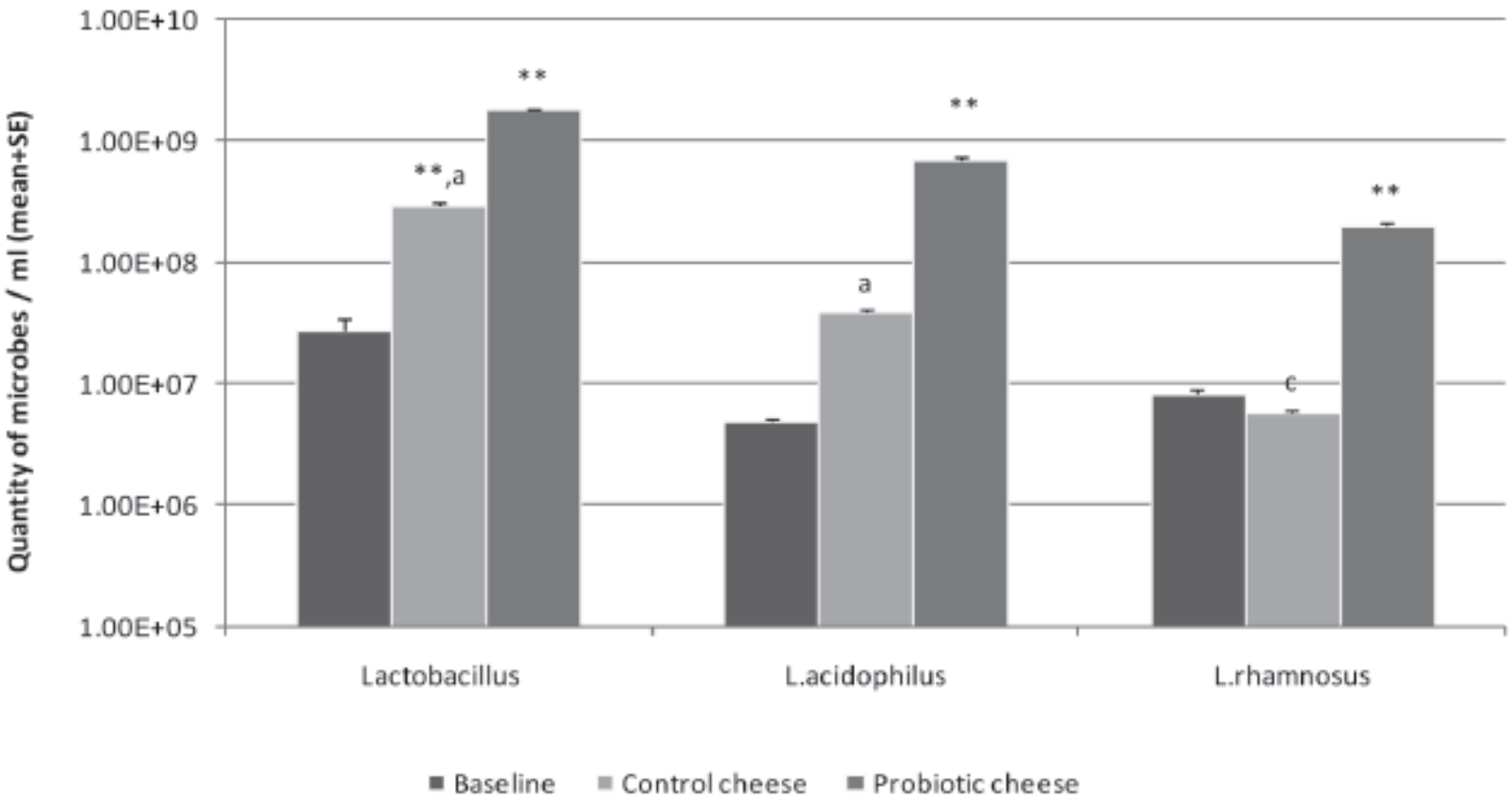
Table 1. Examples of viability of probiotics in different types of cheeses (modified after 7).

Consumption of Probiotic Gouda cheese significantly enhanced immune health markers in elderly



Consumption of 15 g probiotic Gouda cheese containing 1x10e9 CFU *L. rhamnosus HN001 and 1x10e7 CFU L. acidophilus NCFM in an elderly population (mean age 85 years).*

Probiotic Cheese enhance GI microflora vs baseline and control cheese



Measured quantities of microbes in the simulator vessel fluids (modified after 10).Values are sums of microbes indicated in the 4 vessels of the simulator combined (mean copy number of the target gene ± SE).
**Significant difference from baseline $p < 0.01$

Probiotics in cheese GOUDA

Objectives:

- Evaluation of probiotic strains' survival in gouda technology

Inoculation level used in the preliminary work

- Acidification blend:
- CHOOZIT™ 712: 1 DCU/100l
- Probiotic strains:

5.10^6CFU/ml of milk

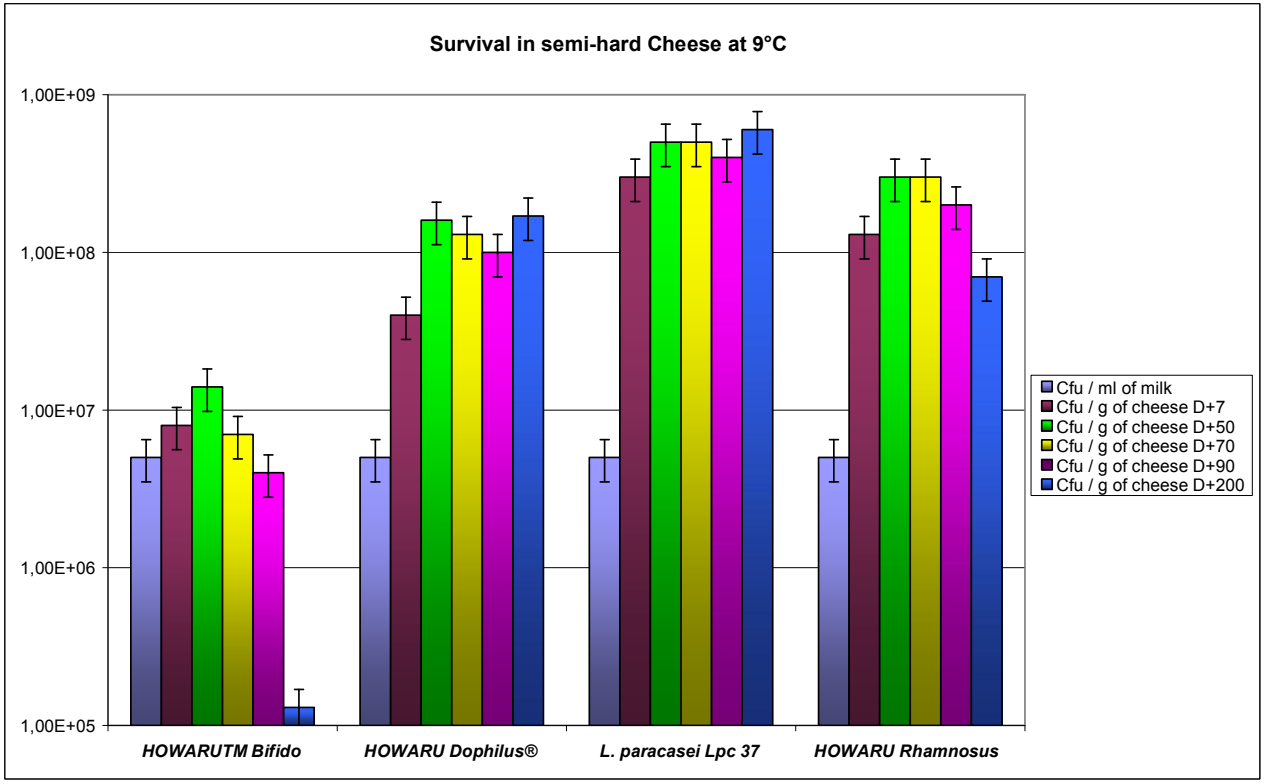
5 DCU / 100 litres

GOUDA cheese physico chemical comparisons

	Vat 1	Vat 2	Vat 3	Vat 4
Blend	*HOWARU™ Bifido LYO*	*HOWARU™ dophilus LYO*	*L. paracasei Lpc 37 LYO*	*HOWARU™ Rhamnosus FRO*
pH before Lactose removal	6,55	6,55	6,55	6.55
pH moulding	6,47	6,48	6,48	6,49
Time to reach pH 5,50	4h30	4h30	4h30	4h30
Dry mater	54 %	54,5 %	53,7 %	53,1 %
Fat	24,6 %	25 %	25 %	24 %
Fat on dry mater	45,6 %	45,8 %	46,5 %	45,2 %
pH	5,47	5,48	5,41	5,41
NaCl	1,74 g/100g	1,75 g/100g	1,80 g/100g	2,17 g/100g
Ca/ESD	3.08 %	3.01 %	3.07 %	3.16 %
Lactose	< 0,2g/100g	< 0,2g/100g	< 0,2g/100g	< 0,2g/100g
Galactose	< 0,2g/100g	< 0,2g/100g	< 0,2g/100g	< 0,2g/100g

- The cheeses have very closed composition, and we don't observe a modification of the acidification kinetic during the process.

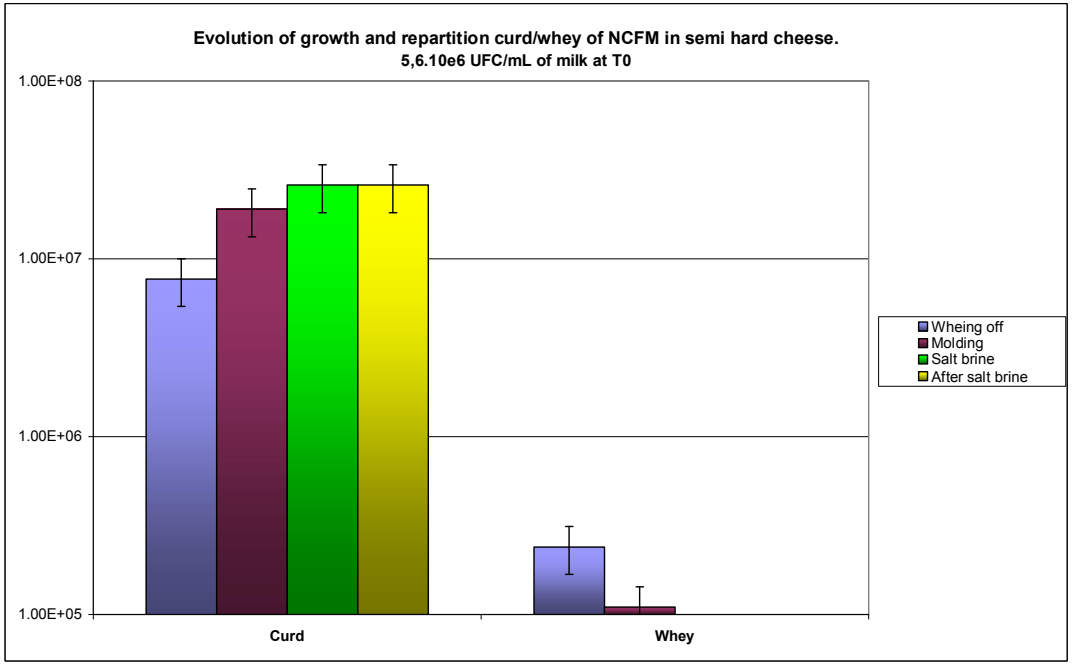
Gouda example – Danisco internal study
Inoculation level : 5 x10^6/ ml of milk



- Cheeses made on microfiltered milk powder in controlled bacteriological conditions
- High reproducibility
- Very good survival until 6 months

	Vat 1	Vat 2	Vat 3	Vat 4
DANISCO probiotic strain	*HOWARU™ Bifido*	*HOWARU Dophilus®*	*L. paracasei Lpc 37*	*HOWARU Rhamnosus*
Cfu / ml of milk	5,00E+06	5,00E+06	5,00E+06	5,00E+06
Cfu / g of cheese **D+7**	8,00E+06	4,00E+07	3,00E+08	1,30E+08
Cfu / g of cheese **D+50**	1,40E+07	1,60E+08	5,00E+08	3,00E+08
Cfu / g of cheese **D+70**	7,00E+06	1,30E+08	5,00E+08	3,00E+08
Cfu / g of cheese **D+90**	4,00E+06	1,00E+08	4,00E+08	2,00E+08
Cfu / g of cheese **D+200**	1,30E+05	1,70E+08	6,00E+08	7,00E+07

What do we lose in the whey? HOWARU™ Dophilus



	Population per gram or ml	
	Curd	Whey
Wheying off	7.7 10^6	2.4 10^5
Molding	1.9 10^7	1.1 10^5
Salt brine	2.6 10^7	/
After salt brine	2.6 10^7	/

- The quasi total L.acidophilus stay in the curd during the gouda technology
- Laloy and Vuillemard et al. showed that the fat content of cheese milk directly influenced the number of starter cells retained in the curd (Int.Dairy Journal 6 (1996) 729-740.)

Probiotics in cheese CHEDDAR

Objectives:

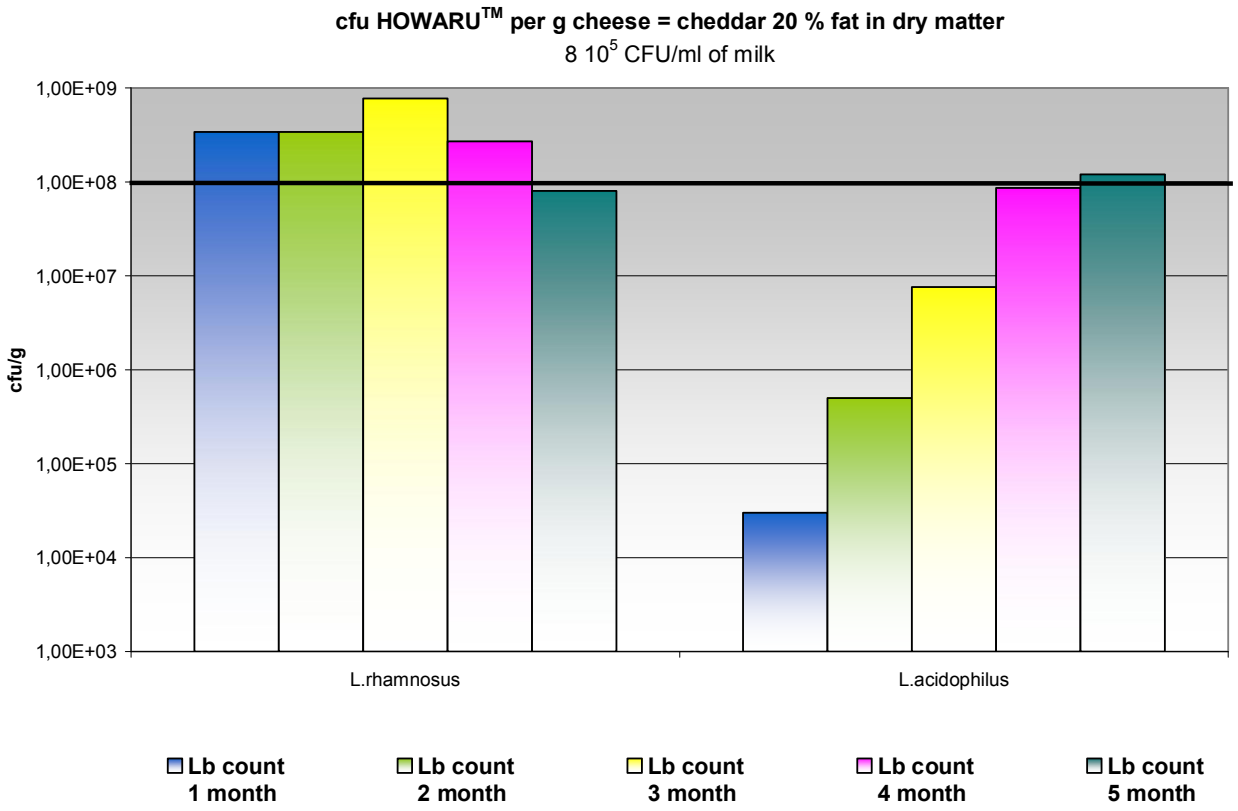
- Evaluation of probiotic strains' survival in cheddar technology

Inoculation level used in the preliminary work

- Acidification blend:
- CHOOZIT™ RA021: 5 DCU/100l
- Probiotic strains:

1 DCU/100 litres of milk

Cheddar example – A commercial Example



- Semi industrial cheddar cheese trials
- Inoculation rate : 8.10^5 CFU/ ml of milk
- High reproducibility (3 vats done per trial)
- Very good survival until 5 months

Culture used	Lb count* 1 month	Lb count 2 month	Lb count 3 month	Lb count 4 month	Lb count 5 month
L.rhamnosus	3,40E+08	3,40E+08	7,70E+08	2,70E+08	8,00E+07
L.acidophilus	3,00E+04	5,00E+05	7,60E+06	8,60E+07	1,20E+08

** CFU/ gram of cheese*

Probiotics in cheese

Objectives:

- Evaluation of probiotic strains' survival in caciotta technology

Inoculation level used in the preliminary work

- Acidification blend:
- CHOOZIT™ TA061: 5 DCU/100l
- Probiotic strains:

5.10^6CFU/ml of milk

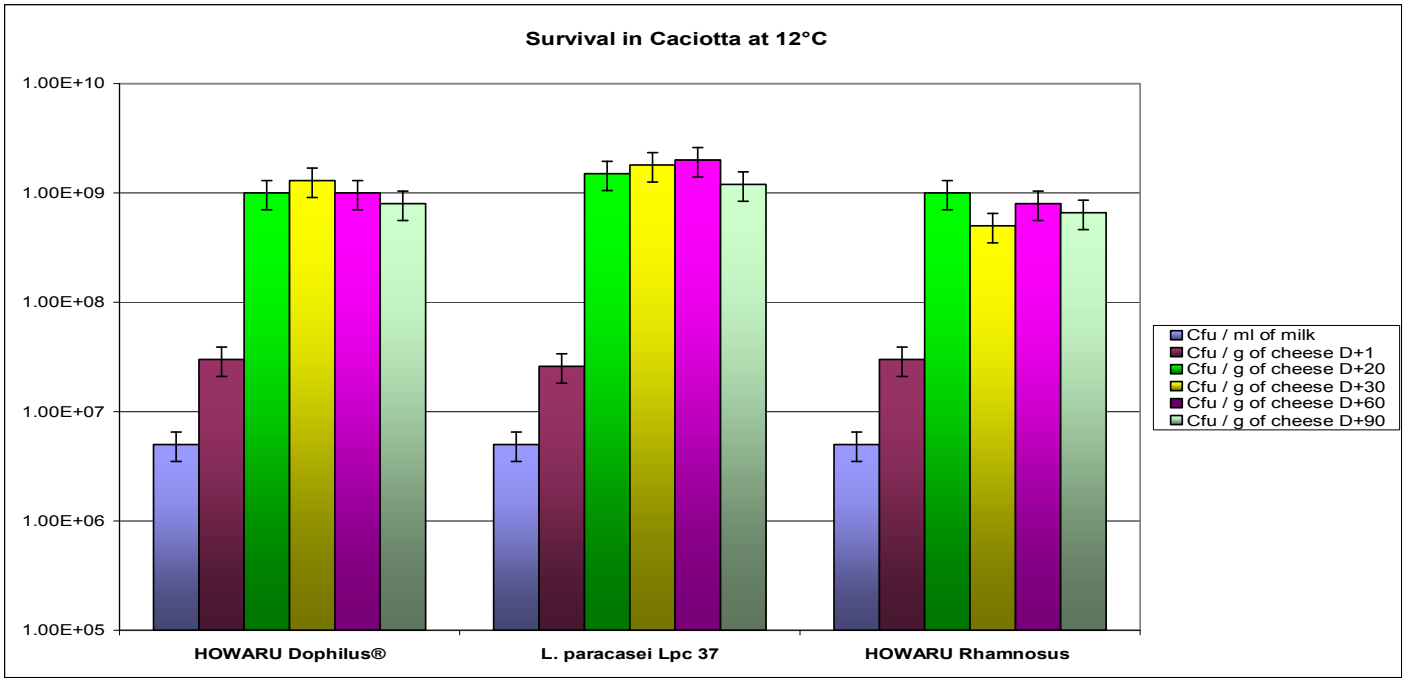
Cheese physico chemical comparisons

Cheeses results :

Blend	Commercial control CHEESE	HOWARU™ Dophilus LYO	L. paracasei Lpc 37 LYO	HOWARU™ Rhamnosus FRO
pH moulding		6.40	6.40	6.40
Time to reach pH 5,10		4h40	4h40	4h40
Dry mater	60.7%	58.8 %	58.2 %	57.2 %
Fat	31.4%	31.9 %	31.4 %	30.4 %
Fat on dry mater	51.7%	54.3 %	53.9 %	53.1 %
pH	5.33	5.33	5.30	5.30
NaCl	1.40g/100g	1.06 g/100g	0.85 g/100g	1.08 g/100g
Ca	862mg/100g	837mg/100g	807mg/100g	838mg/100g

- Analysis of the cheese models have been done at 15 days
- The composition of our cheese model is close to the industrial one
- Good reproducibility between the lab vats
- Salt is slightly lower compared to the control

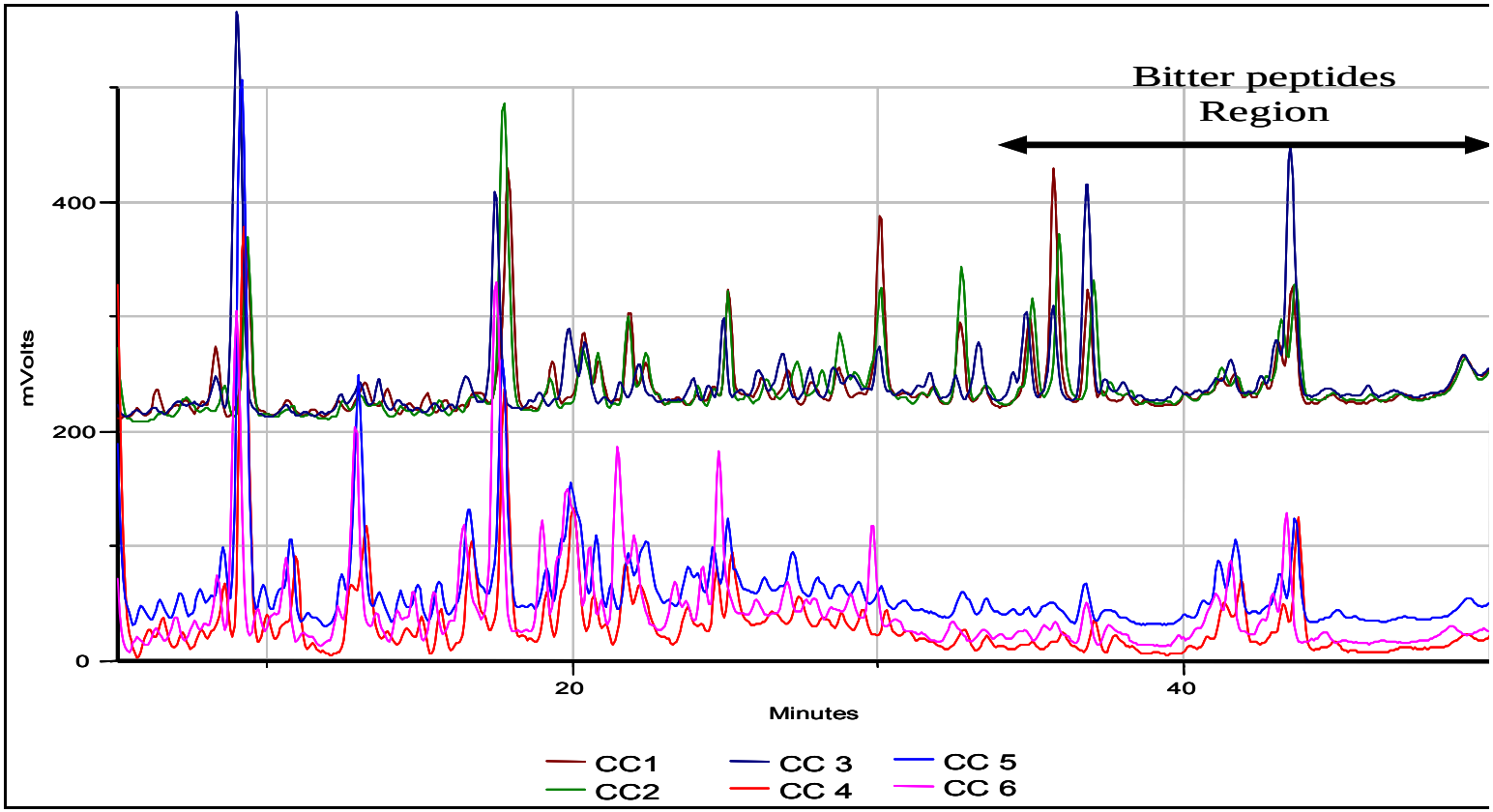
Survival in cheese after 90 days



Blend	HOWARU™ Dophilus LYO	L. paracasei Lpc 37 LYO	HOWARU™ Rhamnosus FRO
Cfu / ml of milk	5.10^{6}	5.10^{6}	5.10^{6}
Cfu / g of cheese **D+1**	3.10^{7}	$2,6.10^{7}$	3.10^{7}
Cfu / g of cheese **D+20**	1.10^{9}	$1,5.10^{9}$	1.10^{9}
Cfu / g of cheese **D+30**	$1,3.10^{9}$	$1,8.10^{9}$	5.10^{8}
Cfu / g of cheese **D+60**	1.10^{9}	2.10^{9}	8.10^{8}
Cfu / g of cheese **D+90**	8.10^{8}	$1,2.10^{9}$	$6,6.10^{8}$

How does it influence the peptides profiles ?

Peptides Profiles:



Commercials samples have more hydrophobic peptides and less hydrophilic peptides. They are probably more bitter than probiotics trials which have a lot of hydrophilic peptides coming from an advanced proteolysis.

Conclusions

Very good survival of the probiotic strains all the cheese exaples during ripening. Using selected Danisco HOWARU™ probiotics together with cheese application expertise allows you to get 10^8 to 10^9 live probiotics per gram after 5/6 months shelf-life at 12° C, therefore a minimum of 10^9 live probiotics per serving of 10 g of cheese.The three lactobacillus grow to 1.10^8 to 10^9 cfu/g and stay at this level until 6 months in the gouda technology.

Cheddar and gouda are excellent carrier of the Danisco health promoting bacteria

Caciotta is a good vehicle for those probiotics strains thanks to the favourable composition and the low constraints

The taste profile was not affected but more investigations need to be done in order to control the exact flavour however we have observed protection against bitterness.

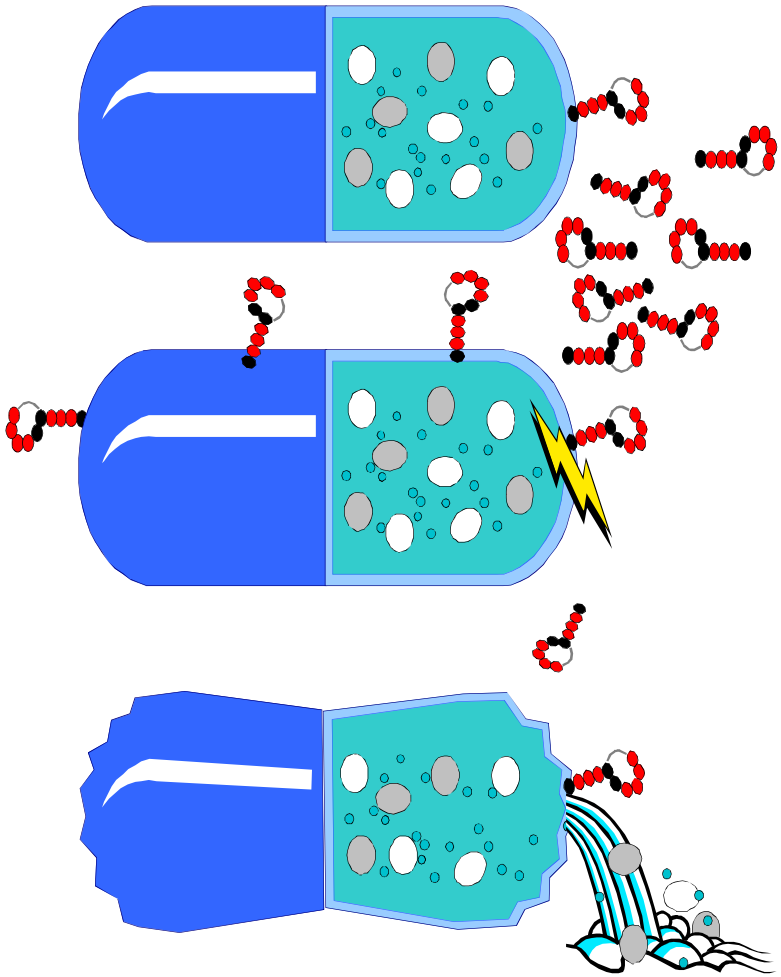
CFU: Colony forming unit

CONCLUSION / How much to consume ?

Using selected DuPont HOWARU™ probiotics together with cheese application expertise allows you to get 10^8 to 10^9 live probiotics per gram after 5/6 months shelf-life at 12° C, therefore a minimum of 10^9 live probiotics per serving of 10 g of cheese.

CFU: Colony forming unit

Mode of Action: Peptide (Bacteriocins)



Cell Wall Binding

↓

Cellular Membrane Destabilization

↓

Molecular Leakage

↙ ↘

Cellular Lysis = BACTERICIDAL FUNCTIONALITY

No Growth = BACTERIOSTATIC FUNCTIONALITY

Mesophilic Cultures (52-104 ^{o}F)

Lactococcus lactis ssp. Cremoris (Streptococcus)

Lactococcus lactis ssp. Lactis

Thermophilic Cultures (86-122° F)

Streptococcus thermophilus

Nisin

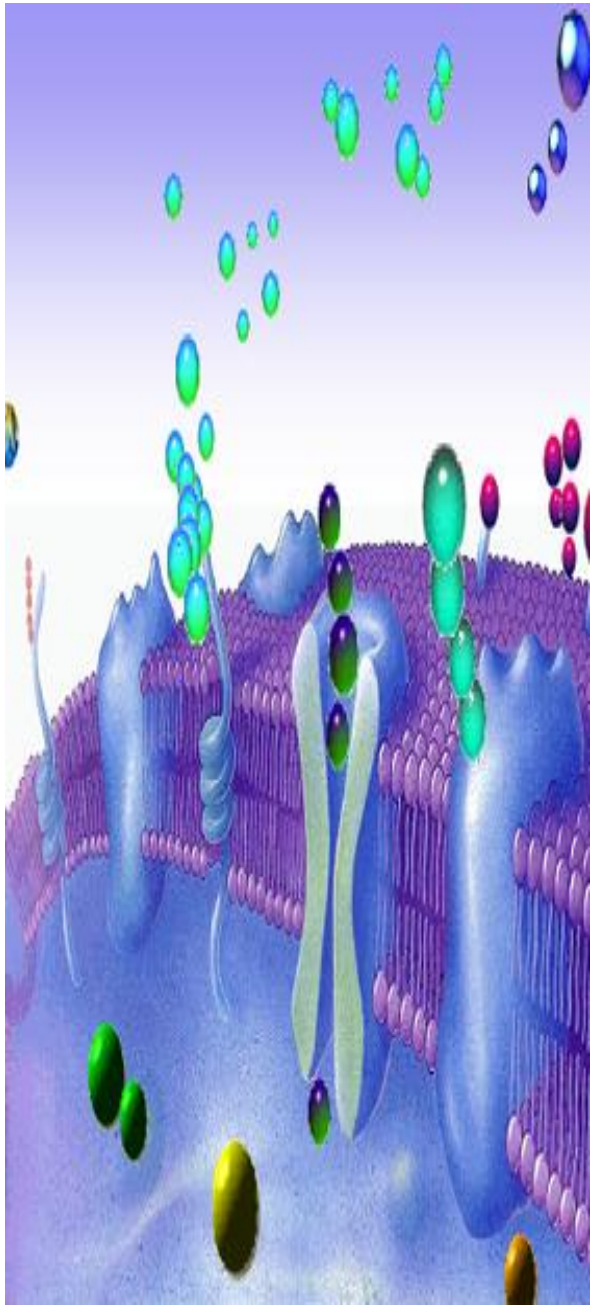
1953: First commercial nisin preparation

1969: joint FAO/WHO expert committee recognised nisin

as a safe and legal food additive

Authorised as a food additive in > 50 countries world-wide.
GRAS status

- Producer strain regarded as safe (food-grade)
- Non toxic
- No cross-resistance related to therapeutic antibiotics
- Degraded during digestion
- Heat stable at low pH



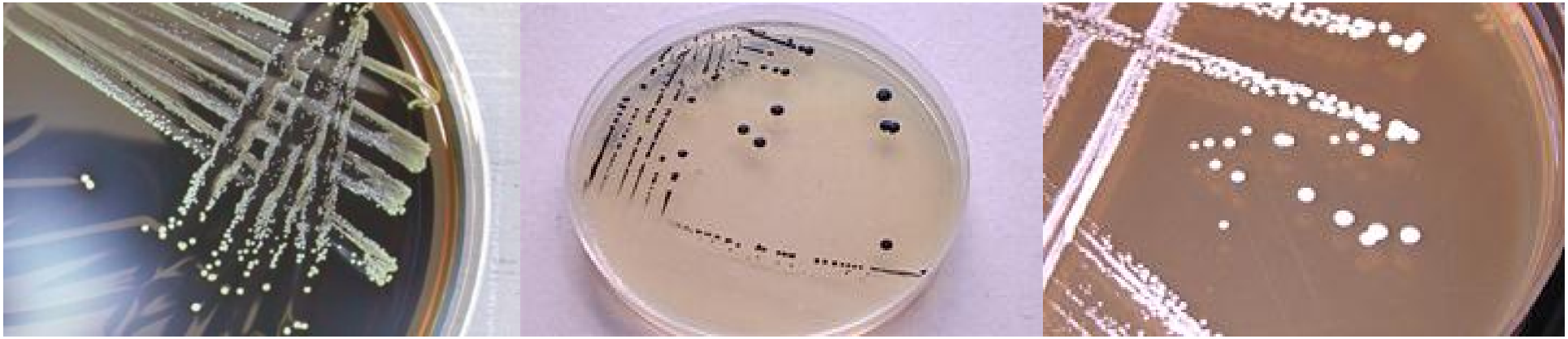
Nisin: Mechanism of Activity

Vegetative cells

- Adsorbs to cytoplasmic membrane, forms transient pores
- Low molecular weight compounds leak from cell causing loss of energy
- pH gradient across the membrane dissipated
- Collapse of proton motive force

Heat-resistant endospores

- Spores affected after germination, preventing outgrowth
- Does not normally kill bacterial spores



Antimicrobial – Nisaplin

Product	Comp.	Key Properties	Applications (US)	Levels
Nisaplin®	nisin preparation	Against Gram + bacteria	Pasteurized Cheese Spreads	10,000 ppm
			Liquid Egg Products	600 ppm
			Sauces (+/- meat or poultry)	600 ppm
			Non-standard salad dressings, dips, spreads	600 ppm
			Chilled Soups	400 ppm
			Chilled Soups with Meat	400 ppm
			RTE Processed Meats	400 ppm
			Natural, Imitation, Cream Cheese	350 ppm
			RTE Vegetarian Sides	300 ppm
			Select “Fruit” Juices	50 ppm

Canada - Not Allowed

Mexico – Limited (Ripened and processed cheeses)

Natamycin

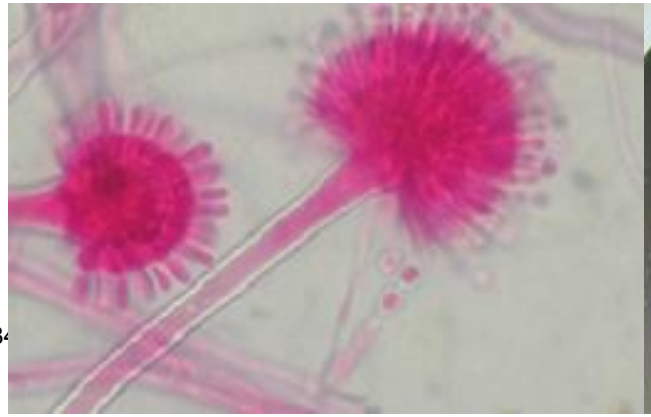
Natural antifungal

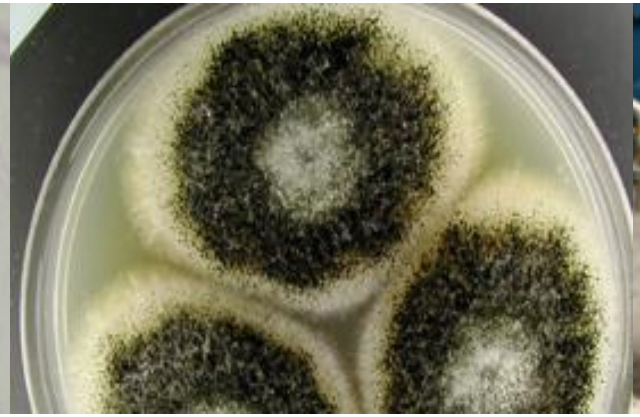
Produced by ***Streptomyces natalensis*** (soil bacteria).

Used commercially as a food preservative since 1970s, eliminating or reducing yeast and mold and preventing mycotoxin formation.

Very powerful; no development of resistant strains.

DuPont's brand: Natamax®





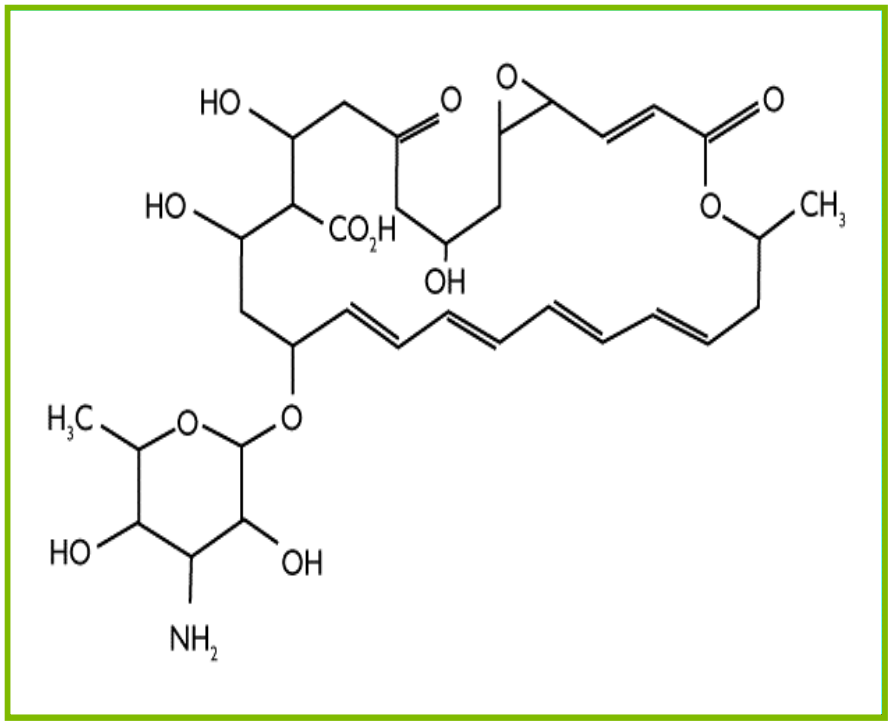
Structure of Natamycin

Empirical formula $C_{33}H_{47}NO_{13}$.
Molecular weight: 665.7
Amphoteric. Isoelectric point 6.5.

Neutral aqueous suspensions relatively heat stable (50 - 100°C).

Stability affected by exposure to UV light, extreme pH values, peroxides, oxidants, chlorine and heavy metals.

Low water solubility:
approx. 30 - 50 ppm natamycin.



Heat and pH Stability of Natamycin

Dry powder: Very stable - over 1 year at 20 ^{o}C

Heat stability of aqueous suspensions

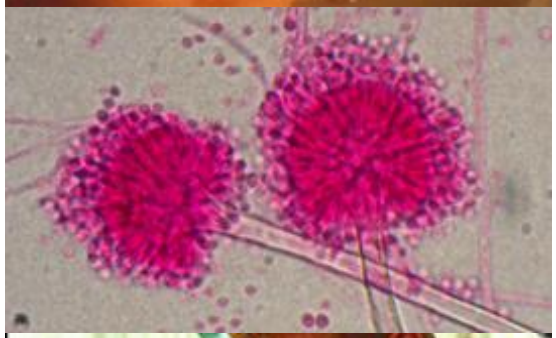
- Ambient temperature - stable
- Optimum heat stability at pH 5 - 9
- Heat-cool cycle (72 and 10 ^{o}C) gave no loss in activity

  over 10 cycles, even in brine (20% NaCl)

pH stability

- Optimum range: pH 5 -7
- Active over range pH 3 - pH 9,

  but stability may be compromised at low pH



Natamycin: Dairy Applications

Typically applied to cheese surfaces. Effective levels 7-10 ppm (Max. 20 ppm) or 2-4µg/cm^2. Applied as aqueous suspension spray or dip.

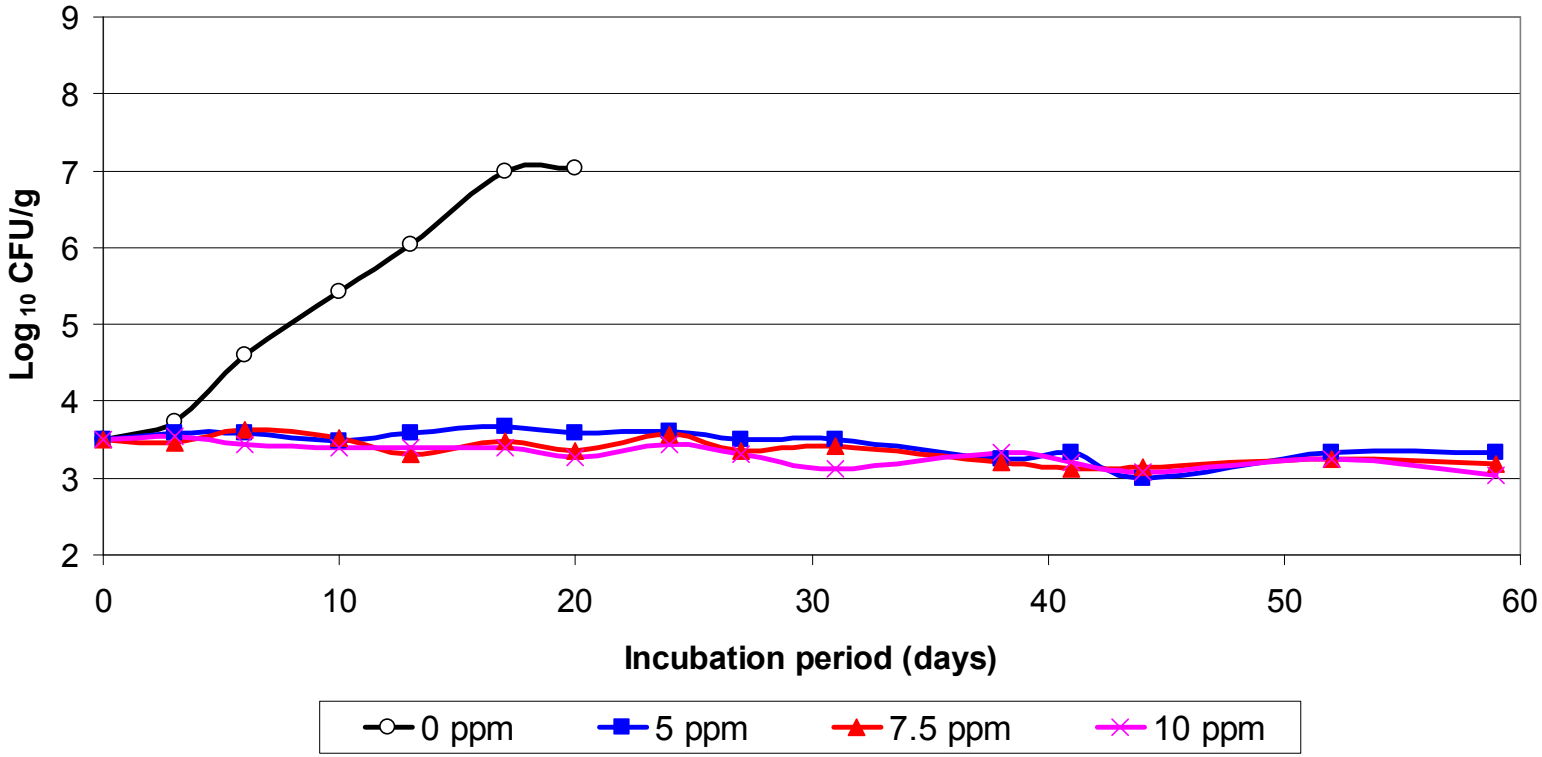
Effective antimycotic in fresh cultured dairy (yogurt, sour cream, cottage cheese, cream cheese @ 5-7ppm). Applied directly to formulation

Natamycin Versus Sorbate

Natamycin	Sorbate
Natural	Chemical
Fungicidal	Fungistatic
No effect on bacteria	Bactericidal
No migration into food	Penetrates into food
No flavor	Bitter flavor
Effective at 1-20 ppm	Effective at 1000 – 2000 ppm
Effective at pH 3 - 9	Effective only at acidic pH

Natamycin Against Yeast in Yogurt

Natamax™ achieves total inhibition of yeast (*S. cerevisiae)* growth at 8° C



Natamycin Inclusion in Yogurt Formulation

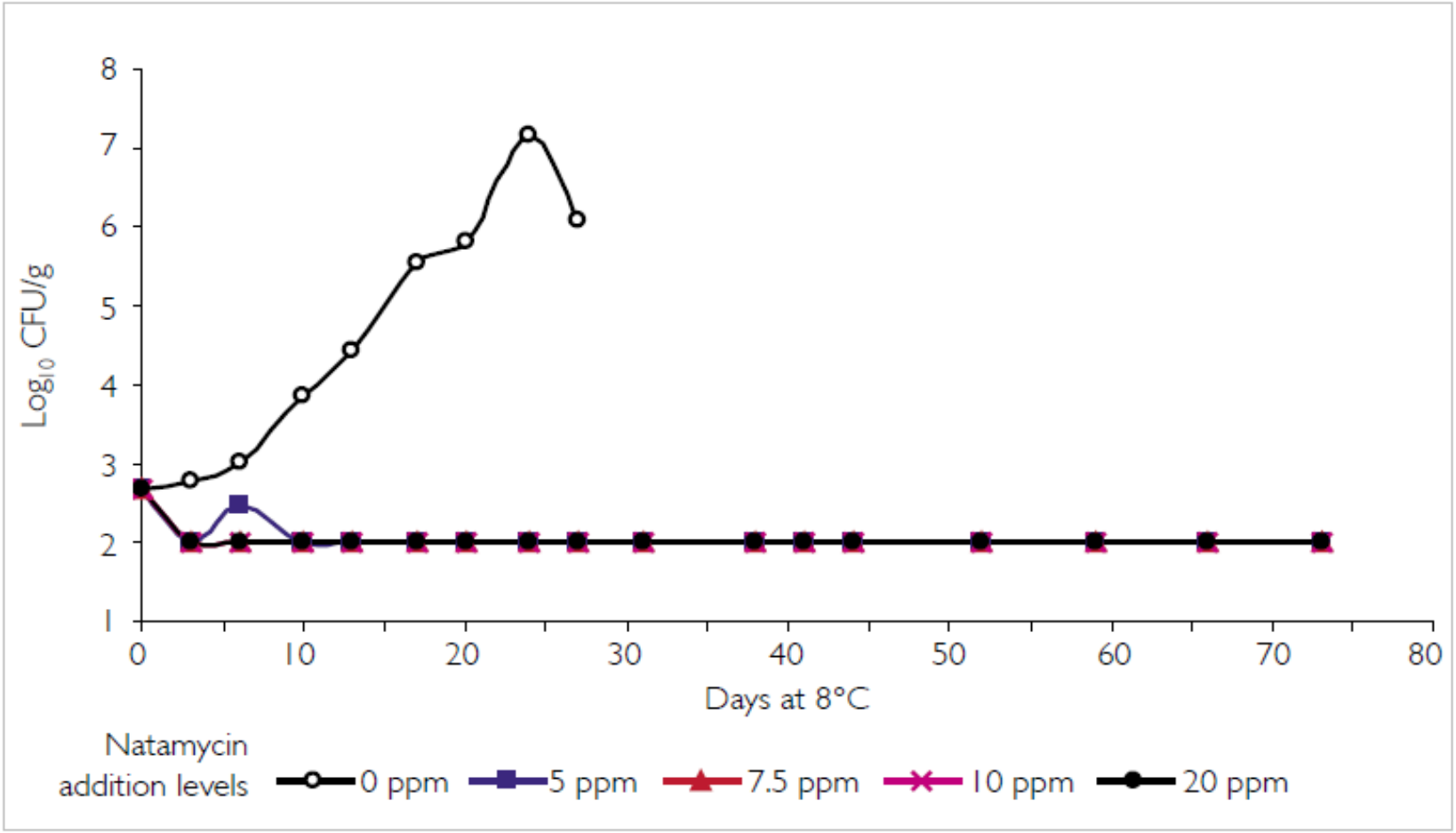


Figure 3. The effect of natamycin on the growth of *Rhodotolura mucilaginosa* CBS8161 in live yogurt at 8°C. Minimum detection 1.0×10^2 CFU/g.

The miracles of science

In May 2011, Danisco was acquired by DuPont

DANISCO®

Adjunct Cultures

Lactobacillus plantarum

Lactobacillus casei

Lactobacillus rhamnosus

Lactobacillus para-casei

Brevibacterium linens (corynebacteria)

Micrococci

Ripening what does it means? for cheese …

- Ripening general definition:

Giving an appearance, a texture and a flavour at an acid curd

- Two key technological steps
  - Curd formation: Acidification
  - Ripening: pH neutralisation
    Enzymatic actions
    Flavour surface and aroma formation

- *The physico-chemical curd consistency is essential for the ripening control*
- *Ripening don't allow to correct a technological or an acidifying problem*

Ripening what does it means? for cultures …

- A wide diversity of micro-organisms can play a role :

Lactic acid bacteria, Propionibacteria

Yeast

Moulds (white and blue moulds)

Brevibacteria and other red smear bacteria

- A wide range of functionalities

  Proteolysis, Peptidolysis, Lypolysis

  Catabolism of sugars, amino acids and fatty acids into flavour

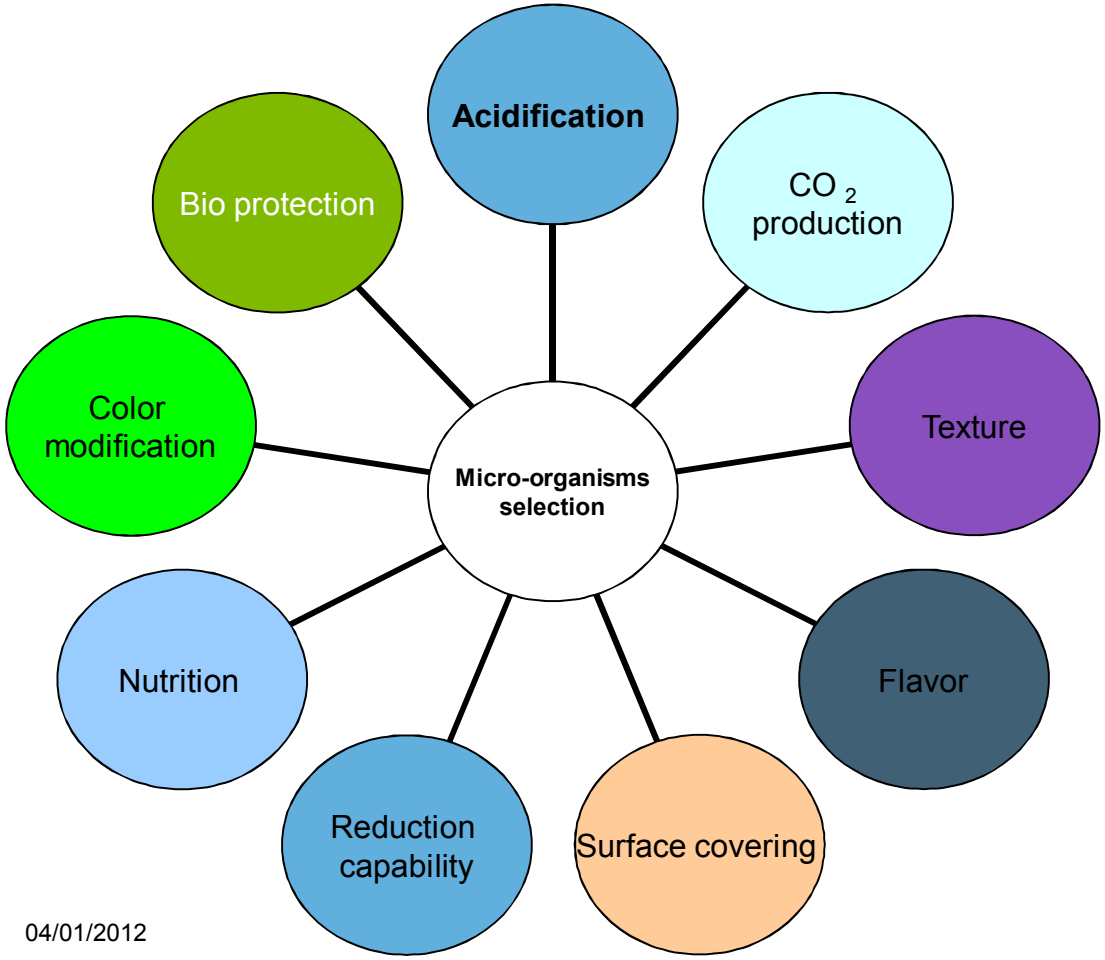
  Gas production

  Surface covering and colouring

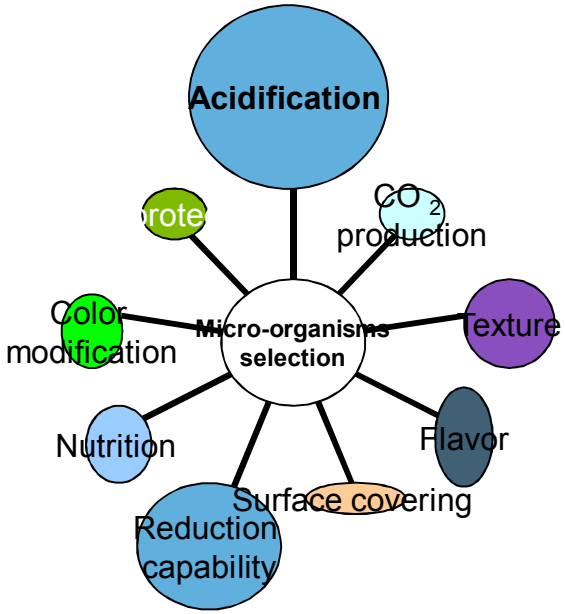
- Micro-organism can act by growing mainly in the cheese curd or mainly on the surface

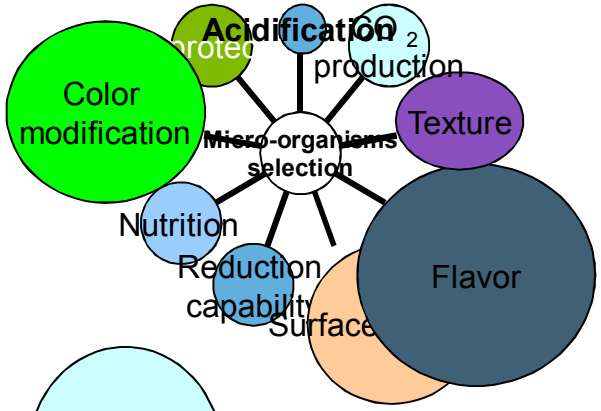
Ripening: a balancing act

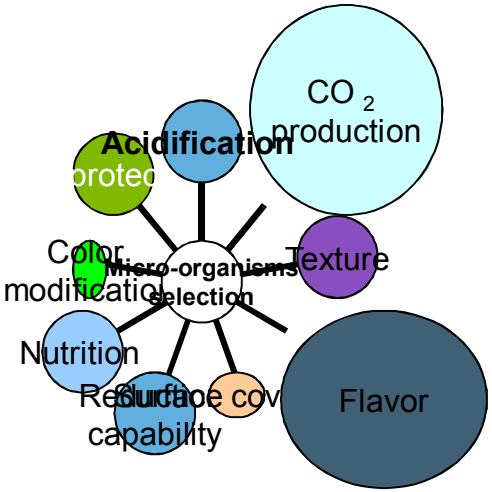
Cultures provide a wide range of functionalities
No micro-organism, no starter cultures has all functionalities



Ripening: a balancing act
Combination offer a wider functionality range





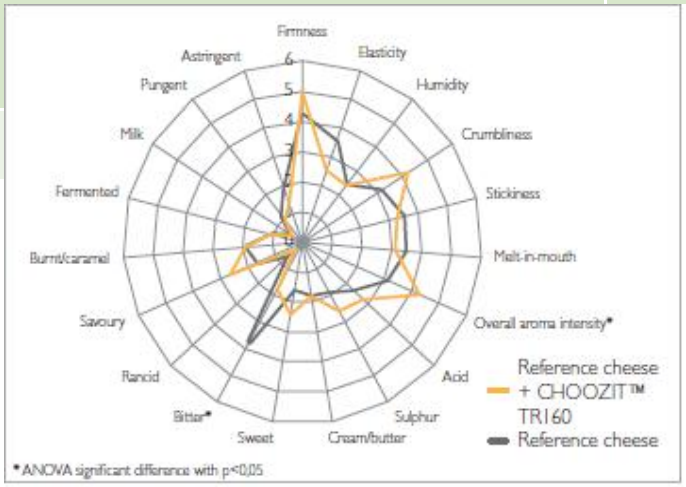


CHOOZIT™ Adjunct Cultures for hard and semi-hard cheese

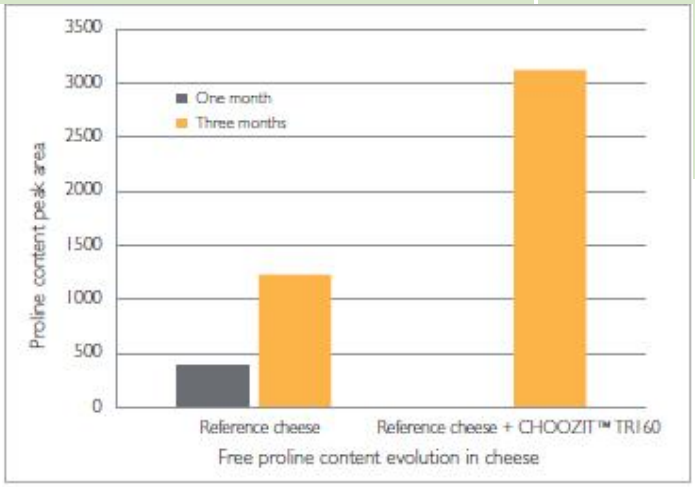
- Lactic acid bacteria with a strong enzymatic and flavour forming potential:
  - *Lactobacillus helveticus*
  - *Lactobacillus delbrueckii lactis*
  - *Lactobacillus casei*
  - *Lactobacillus paracasei*
  - *Lactobacillus rhamnosus*

- Gas forming cultures:
  - *Leuconostoc*
  - *Propionibacterium*

CHOOZIT™ TR160 : the sweetest choice

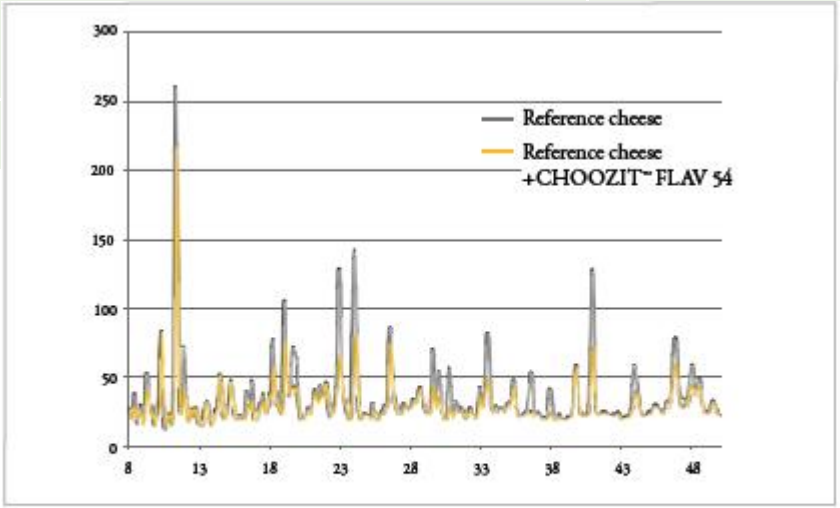


Sensory profile of 9 months old Cheddar cheese added with CHOOZIT™ TR160, as evaluated by a trained panel.

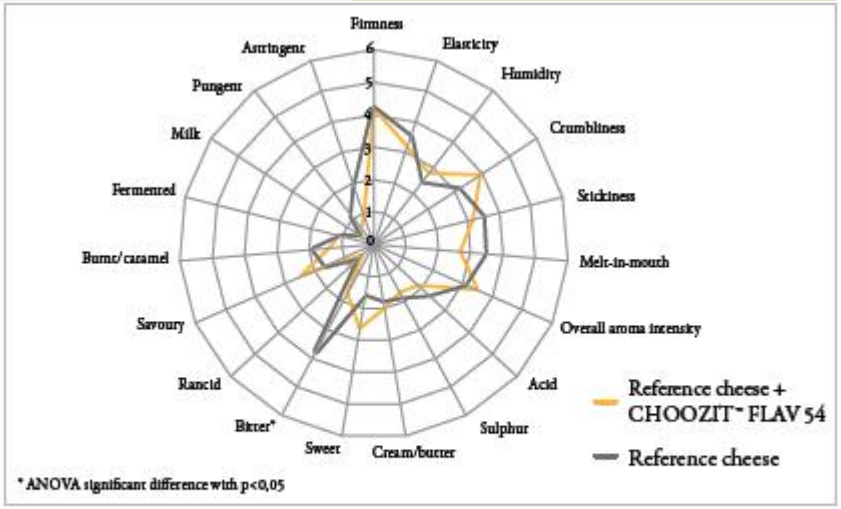


Free proline levels in cheese at 3 months

CHOOZIT™ Flav 54: Lift up your cheese flavour

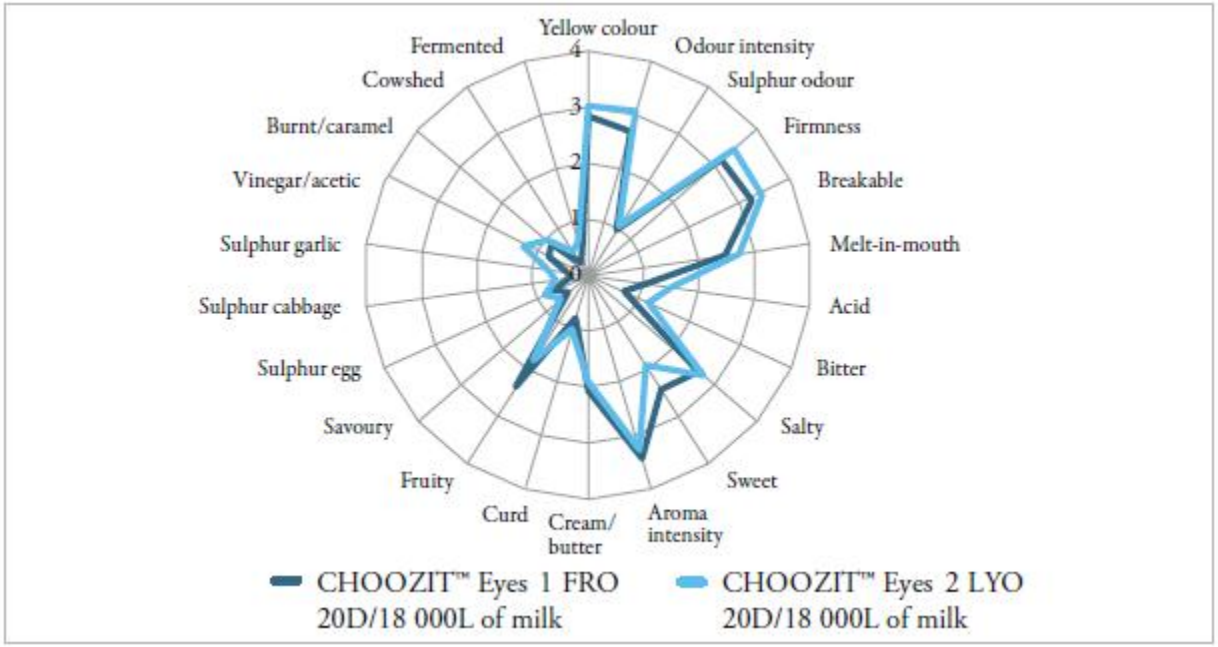


Comparative chromatogram of large and potentially bitter peptides extracted from Cheddar cheese at 8 weeks of ripening clearly showing reduced peptide levels in the cheese with CHOOZIT™ FLAV 54 added.



Sensory profile of 9 months old Cheddar cheese added with CHOOZIT™ FLAV 54, as evaluated by a trained panel.

CHOOZIT™ Eyes
Fast and robust culture for great tasting cheese



Sensory profiles of semi-hard cheese (ripened 7 days at 12-14° C +11 days at 21° C) produced with CHOOZIT™ Eyes 1 FRO and CHOOZIT™ Eyes 2 LYO, as evaluated by a trained panel

Back up : overview adjunct

Product	Culture	Principle Flavour Notes
CHOOZIT TR160	*Lactobacillus helveticus*	Sweetest
CHOOZIT FLAV 43	*Pediococcus acidilactici*	**Creamy, sweet**
CHOOZIT FLAV 54	*Lactobacillus helveticus*	**Savoury, sweet, de-bittering**
CHOOZIT LH100	*Lactobacillus helveticus*	**Rounded savoury**
CHOOZIT LH01	*Lactobacillus helveticus*	**Sweet savoury**
CHOOZIT LH11	*Lactobacillus helveticus*	**Strong savoury**
CHOOZIT LF302	*Lactobacillus casei*	**Meaty savoury**
CHOOZIT LF304	*Lactobacillus helveticus & casei mix*	**Sharp savoury**
CHOOZIT helv.A	*Lactobacillus helveticus*	**Sweet savoury**
CHOOZIT 220 to 240	Undefined Lactococcus	**Slow maturing traditional savoury**
PROBAT 222	Undefined mesophile	**Nutty, open texture for use in Red Leicester**