

Clinically studied probiotics for dairy innovations: challenges, solutions and opportunities

Arja Laitila, Preben Jorgensen, Arthur Ouwehand & Johanna Maukonen

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Nutrition & Biosciences

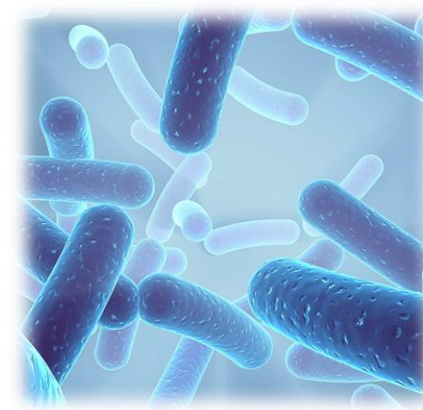
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Content

- Probiotics –setting the scene
- Importance of clinically studied probiotics
- Challenges related to probiotics in food and beverage production
- Probiotics in dairy applications and future outlook



Probiotics



- In 2001, FAO defined **probiotics** as *“live microorganisms, which when administered in adequate amounts confer a health benefit on the host”*. (1,2).
- Since then, additional guidelines for the use of the word probiotics have been published. (3, 4) – “which” replaced with **“that”**

Implications of the probiotic definition, set forth by Hill *et al* (3), Table adapted from Fenster *et al* 2019 (5)

1	are microbes	Although most commercial probiotics are lactobacilli and bifidobacteria, they can be other microbes and do not need to be bacteria.
2	need to be alive	When administered; while it may be desirable that they are alive in the gastrointestinal tract, it is not required.
3	need to be administered	This does not imply they must be eaten; other routes of administration are possible.
4	in sufficient amounts	At the end of shelf life, there are still at least as many viable microbes in the product as were used in a clinical study.
5	need to have a health benefit	This benefit should be shown in the target host population.

1. Food and Agricultural Organization of the United Nations and World Health Organization. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. (2001).

2. Food and Agricultural Organization of the United Nations and World Health Organization. [Joint FAO/WHO working group report on drafting guidelines for the evaluation of probiotics in food](#). (2002).

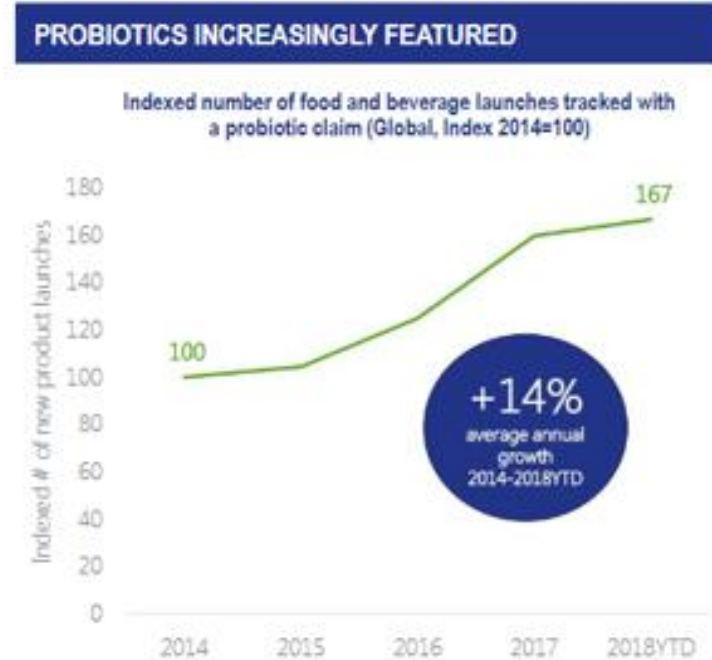
3. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint, HJ, Salminen S, Calder PC, Sanders ME. [The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic](#). Nature Rev Gastro Hepatol. advance online publication 10 June 2014; doi: 10.1038/nrgastro.2014.66

4. Reid, G., Gadir, A., Dhir, R. 2019. Probiotics: Reiterating what they are and what they are not. Frontiers in Microbiology, 10:424

5. Fenster et al. 2019. The production and delivery of probiotics: a review of practical approach. Microorganisms, 7, 83

Probiotics in the market

- Probiotics are positively perceived by consumers and associated with positive effects on general well-being, gut comfort, maintenance of immune protection and other health benefits
- Demonstration of scientific evidence of the benefits of probiotic strains has been and still is a growing area of clinical research
- **Dietary supplements:** beads, capsules, tablets, sachets
- **Food and beverages:** Yogurts and fermented milk products
 - Cheese, ice cream, chocolate, cereals, snacks, peanut butter, beverages etc.
 - Increasing trend with plant-based alternatives



Ref. Nutrition focus Probiotics. Innova Market Insights, Jan 2019.
2018 YTD = up to Dec 2018



Nutrition & Biosciences



Importance of clinical results and documented stories !

Clinical Trials - The Essence of DuPont Nutrition & Biosciences

- Any kind of research that involves human participants.
- Are intended to study the safety and efficacy of a product, or the characteristics of a population, eg. epidemiology.
- The first objective is to safeguard the integrity and safety of the participant.
- DuPont Clinical Trials must follow strict international, local and internal rules:
 - Declaration of Helsinki - WMA
 - Auditable Good Clinical Practice – ICH
 - Regional Guidelines for Health Claims
 - DuPont Human Studies Committee
 - DuPont Standard Operating Procedures

**Trial
duration:
2-5 years
(average)**



Things to consider when conducting probiotic clinical trials

The importance of trial setting

- Correct strain / one strain or several strains
- Dose
- Length of the intervention
- Age of the host (infant/child/adult/senior)
- Analysis methods
- Population differences, i.e. target population
- **Good Clinical Practise**



NIH Image Gallery on Flickr

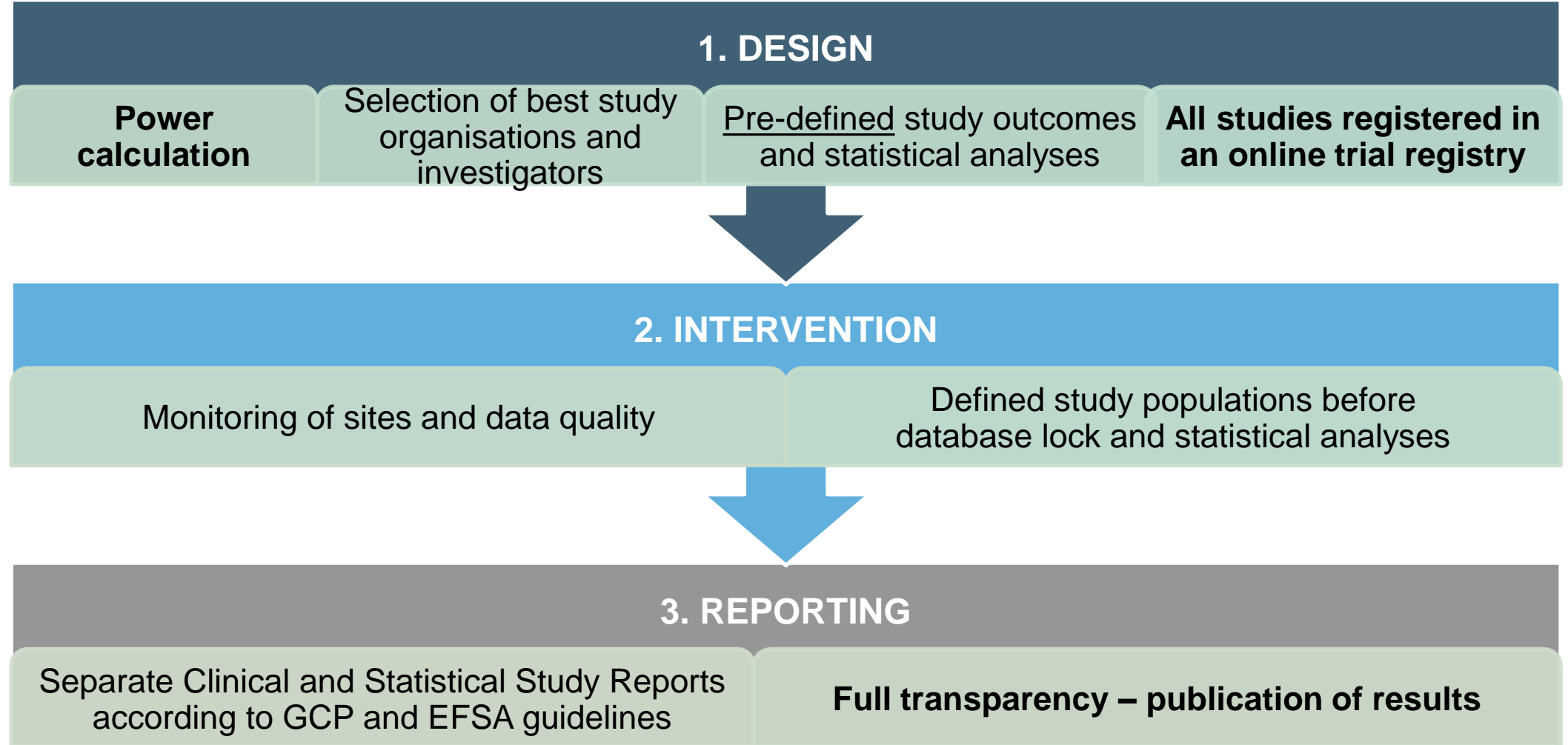
Difficult to compare results from trials with different settings as can be seen from older probiotic trials → inconsistent results → possible positive effect not detected reliably

Clinical substantiation

with highest quality standards since 2011



22 Clinical Standard Operating Procedures to date



Probiotic health effects: meta-analyses

Meta-analysis = statistical procedure for combining data from multiple sources

Digestive health

- Improved symptoms of Irritable bowel syndrome (IBS)
- Reduced risk of diarrhea
 - Antibiotic associated
 - *Clostridioides difficile* associated
 - Necrotizing enterocolitis
 - Infectious
- Benefits on Inflammatory Bowel Disease (IBD) are unclear



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Metabolic health

- Total and LDL cholesterol lowering
- Preventing obesity
- Preventing type II diabetes
- Improved hepatic encephalopathy
- Non-alcoholic fatty acid liver disease

Immune health

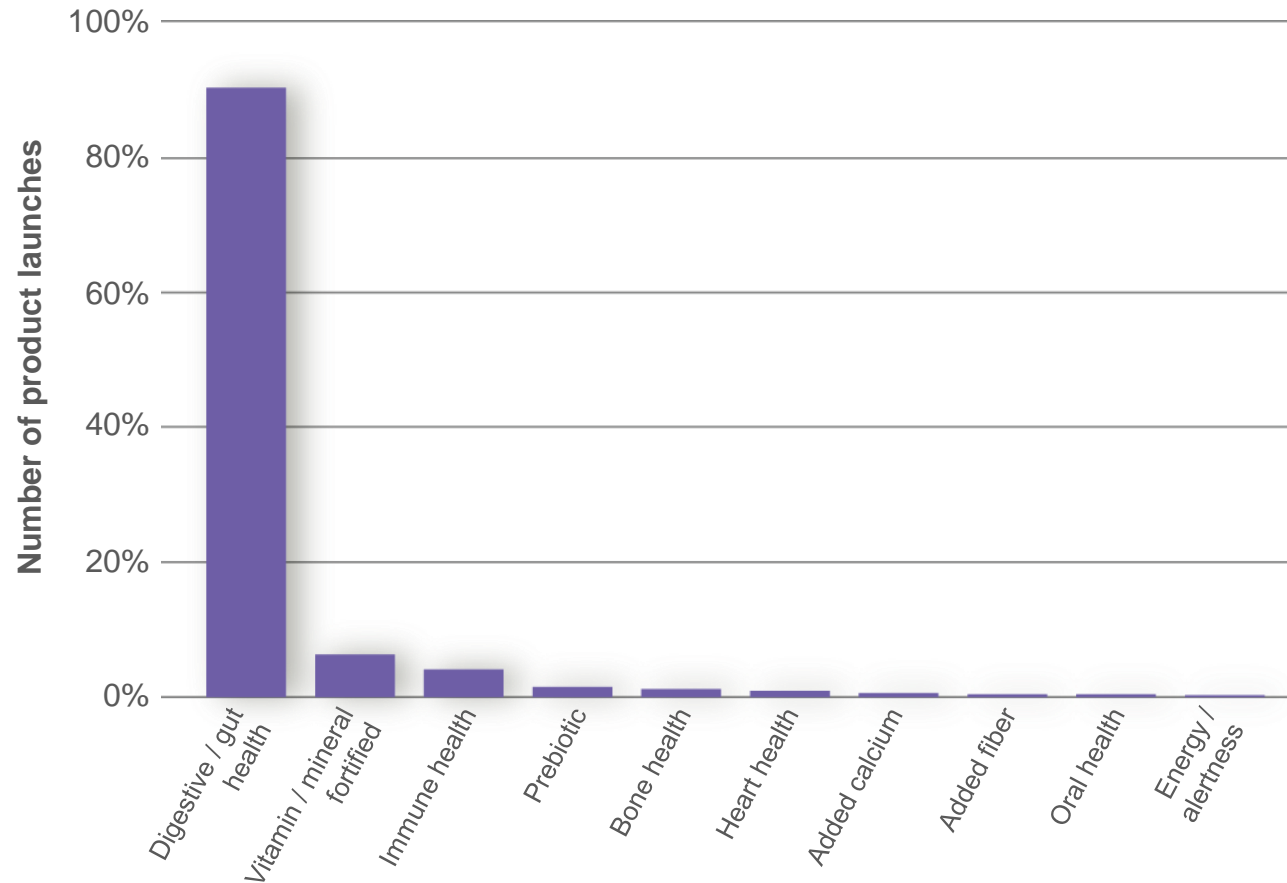
- Reduced risk of cold
- Potentially alleviation of allergic symptoms
- Reduced risk of eczema and atopic dermatitis especially if probiotics started during pregnancy

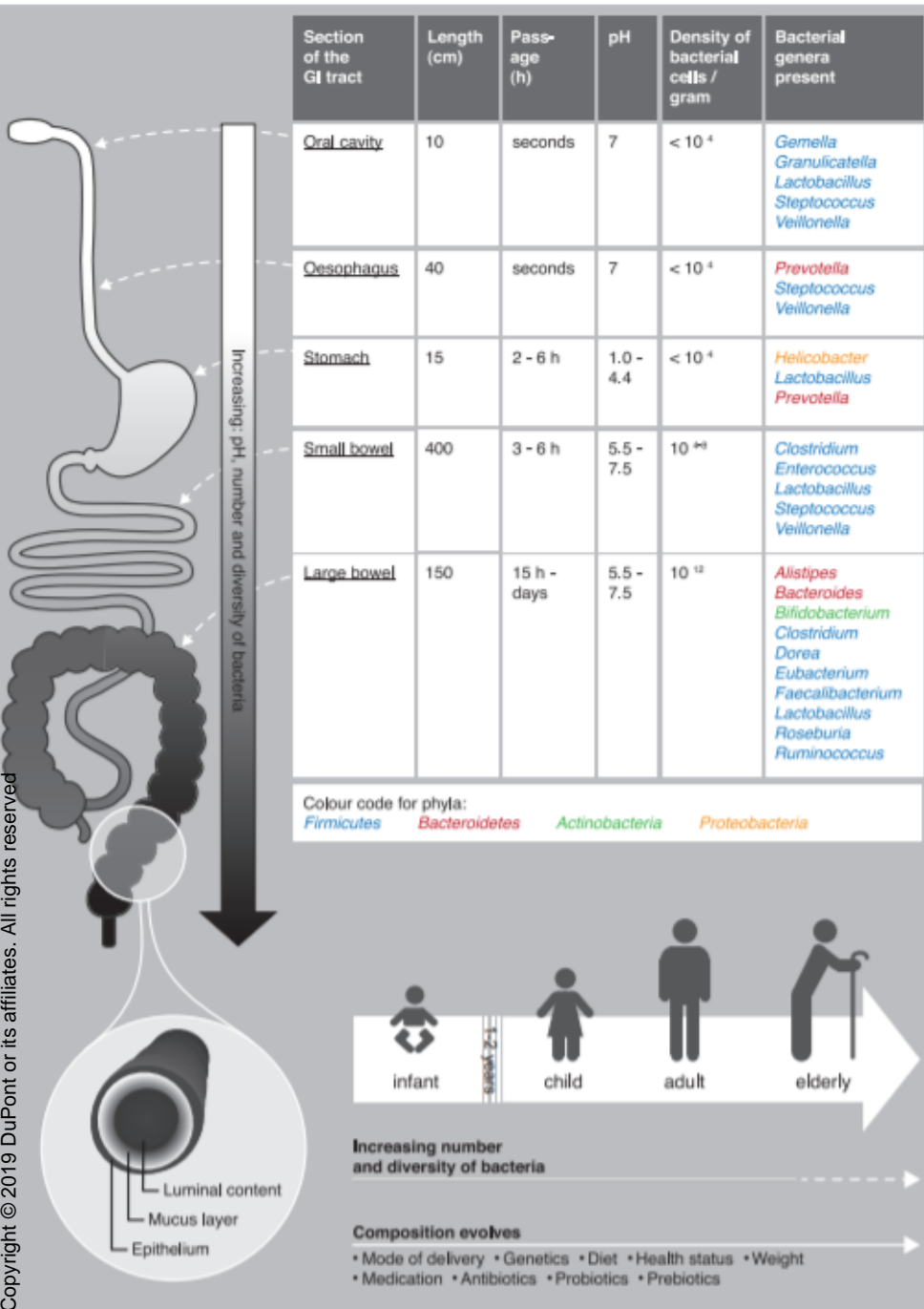


Digestive wellness is the top trend

■ When a health benefit is communicated in new product launches, gut health represents 90% of the messages to consumers ⁽¹⁾

Top 10 health benefits associated to probiotic yogurt launches





Characteristics of the human GI tract and Microbiota

- More than a 100 trillion microbes, more than 1000 different species
- More bacterial genes than human genes
- Our digestive system is more complex than it looks
- Microbial population change throughout the GI tract
- Unlike human genes, gut microbiota can be altered by drugs, diet, other.
- Opportunity to improve health with probiotics and prebiotics

→ **Future opportunities with next generation probiotics**

Sender et al 2019. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell*, 28: 337-340

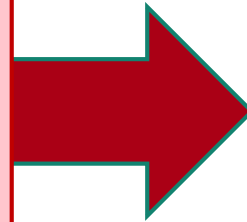


The intestinal microbiota → This delicate equilibrium is threatened by external factors

Gut-brain axis
When we mess microbes
– we change the behaviour of the host (Prof. Cryan 27.5.2019)

Our modern daily life is source of numerous external influences that can disrupt our intestinal microbiota:

- ✓ Antibiotic therapy
- ✓ Poor or irregular diet
- ✓ Food/water borne chemicals
- ✓ Alcohol consumption
- ✓ Ageing
- ✓ Stress
- ✓ Illness
- ✓ Travels...



That can lead to:

- ✓ **Digestive disturbances**
Constipation, diarrhea, abdominal pain
- ✓ **Low immune defenses**
More frequent cold & flu infections
- ✓ **Disease states that have been associated with altered GI microbiota**
Atopy & Asthma, Diabetes (type 1 & 2), Inflammatory Bowel Disease (IBD), Irritable Bowel Syndrome (IBS), GI infections, antibiotic associated diarrhea, etc.



Probiotics can help you to rebalance your microbiota and find back your equilibrium.

Our approach → individualised probiotics to meet consumer expectations



	Immune			Immune + Digestion	Digestion			Female Health		Weight	Oral Health
	Cold & Flu + URT	Performance	Optimal Start in Life	Combined Immune & Digestive health	Constipation	IBS	GI Restoration	Vaginal health	Immune (mother & baby) + Vaginal & Mood benefit (mother)	Reduce Waist Circumference	Gum Health
Pregnancy			HOWARU® Protect Early Life HN001						HOWARU® Protect Prenatal+ HN001 + La-14		
Infants			HOWARU® Protect Early Life HN001								
Kids	HOWARU® Protect Kids NCFM + BI-07			HOWARU® Balance NCFM+HN019							
Adult	HOWARU® Protect Adult BL-04			HOWARU® Balance NCFM+HN019	HOWARU® Transit HN019	HOWARU® Dophilus NCFM	HOWARU® Restore NCFM+BI-04+Bi-07+LPC-37			HOWARU® Shape B420 + Litesse	HOWARU® Smile HN019
Seniors	HOWARU® Protect Senior HN019			HOWARU® Balance NCFM+HN019		HOWARU® Dophilus NCFM					
Women								HOWARU® ProFem HN001+LA-14	HOWARU® Protect Prenatal+ HN001 + La-14		
Athletes		HOWARU® Protect Sport NCFM+BI-07									

OBS Indications mentioned above is not claim language but rather product positioning

Case: *Bifidobacterium lactis* HNO19

- Well-studied probiotic in infants, pregnant mothers, children, adults, seniors
- **Improves gastrointestinal health and well-being**
- Improved gastrointestinal transit and occasional constipation
 - An average **reduction** in colonic transit time ⁽¹⁾
 - 28 hours at 1.7×10^{10} dose ($p < 0.05$)
 - 18 hours at 1.8×10^9 dose ($p < 0.05$)
 - A significant improvement in digestive discomfort symptoms ⁽¹⁾
 - Constipation ($p < 0.01$)
 - Abdominal pain ($p < 0.01$)
 - Irregular bowel movement ($p < 0.05$)
 - A significant improvement in stool number for people with ≤ 3 stools/week (i.e. constipated) with doses of 10^9 and 10^{10} CFU per day ⁽²⁾
- **Beneficial modulation of immune functions**

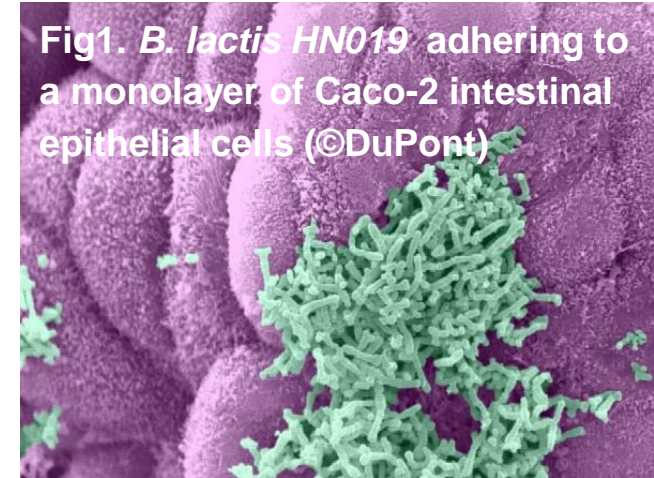


Fig1. *B. lactis* HNO19 adhering to a monolayer of Caco-2 intestinal epithelial cells (©DuPont)

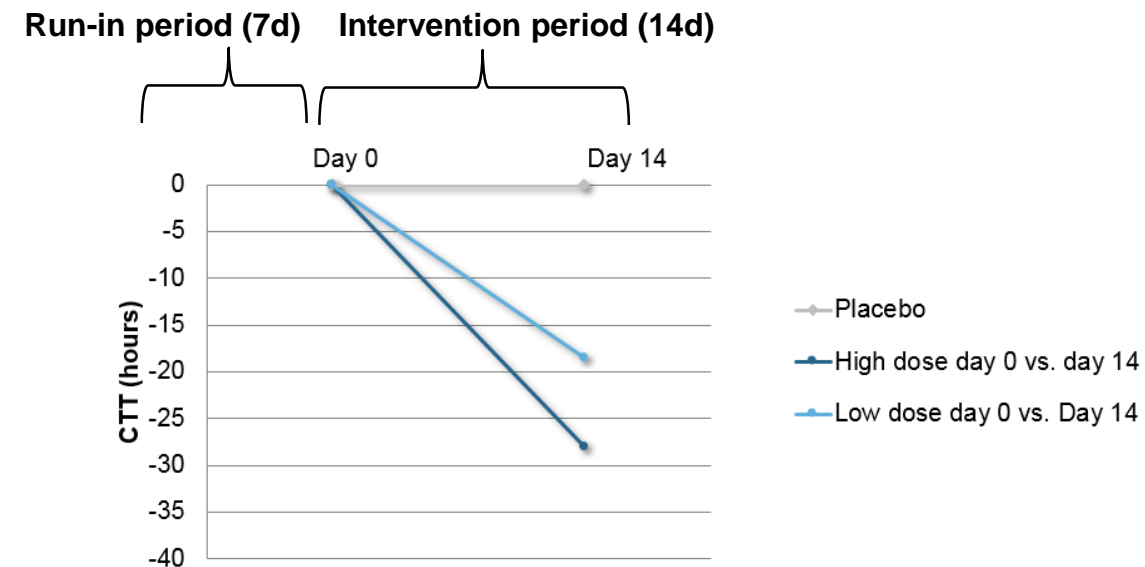


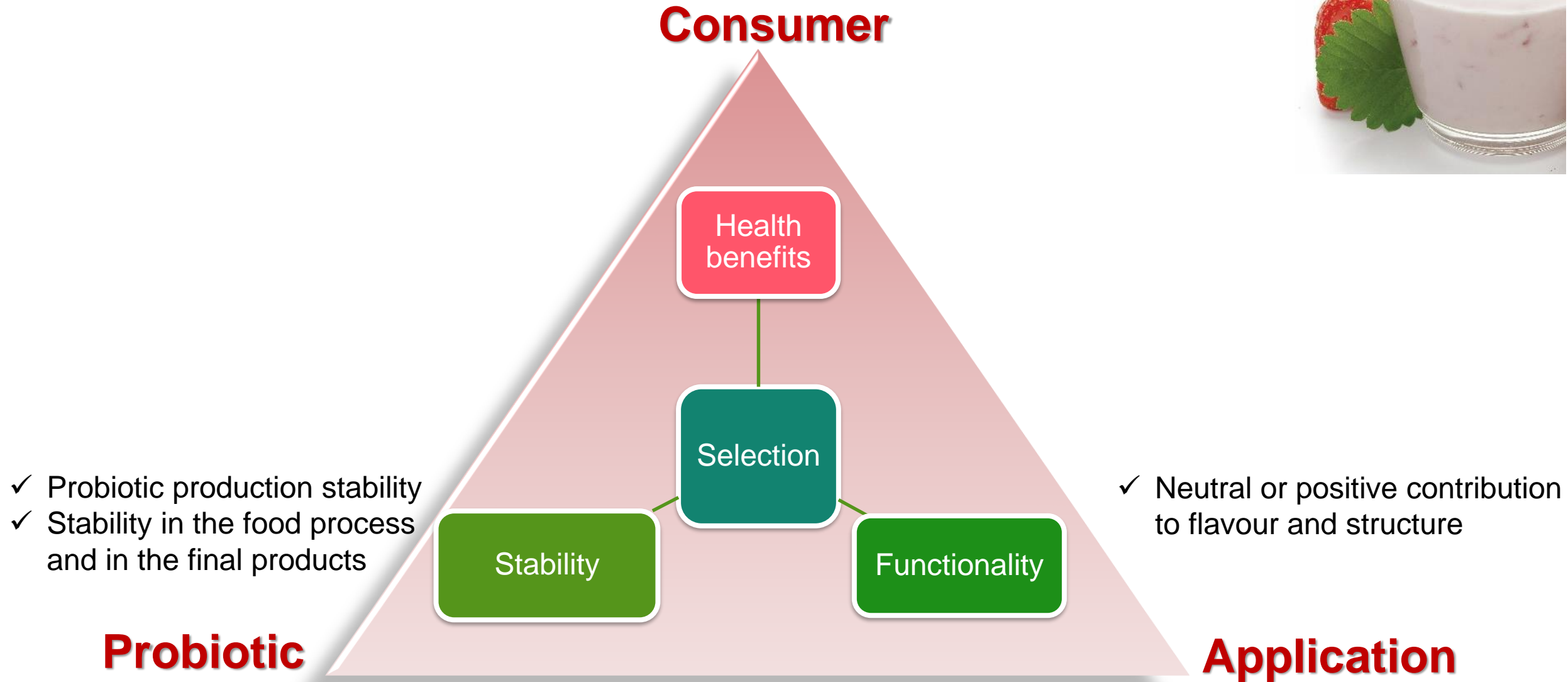
Fig 2. HNO19 significantly improved Colonic Transit Time (CTT) at both low and high dose

14 day, Triple blind, randomised, placebo controlled study with 100 healthy adults (Weller et al 2011)



Challenges of probiotics in food and beverage industry ?

Technological challenges related to selection of probiotics into food and beverage production

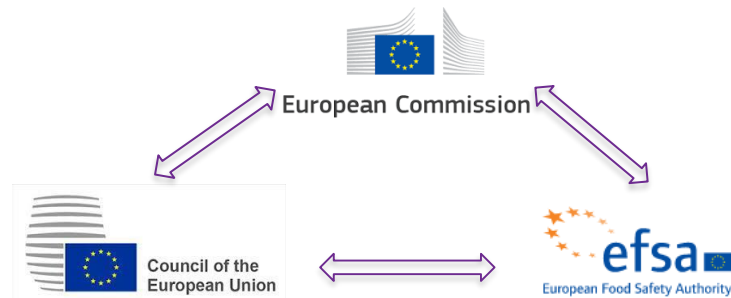


Country-specific regulatory for approved health claims

Nutrition & Health Claims Regulation (EC 1924/2006) regulates the claims-labelling of probiotics

This Regulation covers three aspects

- Nutrient profiles – *Not agreed yet*
- Nutrition claims – Authorization not needed. Listed in Annex of Regulation e.g. **high [name of vitamin/s] and/or [name of mineral/s]**
- Health Claims – Pre-market approval from Commission after assessment by EFSA



No Health Claim has been approved, despite >300 applications, and numerous positive clinical studies with probiotics (except a generic claim on live yoghurt.)

Live yoghurt cultures	Live cultures in yoghurt or fermented milk improve lactose digestion of the product in individuals who have difficulty digesting lactose	In order to bear the claim, yoghurt or fermented milk should contain at least 10 ⁸ Colony Forming Units live starter micro-organisms (<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> and <i>Streptococcus thermophilus</i>) per gram.		2010:8(10):1763	1143, 2976
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14 December 2007

GUIDANCE ON THE IMPLEMENTATION OF REGULATION N° 1924/2006 ON NUTRITION AND HEALTH CLAIMS MADE ON FOODS
CONCLUSIONS OF THE STANDING COMMITTEE ON THE FOOD CHAIN AND ANIMAL HEALTH

The term "contains probiotics" is considered a Health Claim, according to the Guidance of the EU Commission of 2007, as it "implies a health benefit".

Member state approaches


- Many (most) Member States implement the opinion 2007 Guidance.
- Some EU Member States have developed **their own guidances**, including Italy and Czech Republic. These guidances contradict the Commission Guidance of 2007.



Ministero della Salute

 **Italian** Guidance for Probiotics – provides guidelines on probiotics and prebiotics – last updated March 2018.



 **Czech Republic** National recommendations consider reference to containing a probiotic to be a nutrition claim fulfilling the conditions for use of the claim "contains (name of nutrient or other substance)"
(Guidance acknowledges that the approach to this labelling may vary in the other EU Member States)

The fresh dairy industry communicates on probiotics to consumers by using different options

- **Country-specific** regulatory-approved health claims
- Reference to documented benefits of the probiotic strains
- Communication relying on the name, the origin of the probiotic strain
- Communication based on biodiverse multiple probiotic strains or the high dose of probiotic strains (Obs. contains high dose of probiotics – is not permitted in the EU)

Growth opportunities in a fast growing market can be facilitated when approved by local regulatory authorities



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Industry has developed criteria to define "Probiotics" as an Industry Best Practice



Opportunity of country specific regulation to communicate on probiotic – Approved Health Claim in Switzerland

- Nutrition or health claims laid down in Labeling & Advertising regulation are generally harmonised with EU. Other claims can be authorised by FSVO (Federal Food Safety and Veterinary Office).
- Various product specific authorised claims related to probiotics including *Bifidobacterium lactis* HN019



<https://produits.migros.ch/marques/bifidus>

D: *Bifidobacterium lactis* HN019 leistet einen Beitrag zu einer normalen Verdauung, indem die Darmpassagezeit verkürzt wird
 F: *Bifidobacterium lactis* HN019 contribue à normaliser la digestion, en réduisant le temps de transit
 I: *Bifidobacterium lactis* HN019 contribuisce a normalizzare la digestione, riducendo il tempo di transito.

D: Mindestens 1 Mia. lebende Keime pro Tagesportion
 Im Rahmen einer ausgewogenen Ernährung und eines gesunden Lebensstils
 F: Au moins 1 milliard germes vivants par portion journalière dans le cadre d'une alimentation équilibrée et d'un mode de vie sain
 I: Almeno 1 mld di germi vivi per porzione quotidiana, nell'ambito di un'alimentazione bilanciata e uno stile di vita sano

DuPont Achieves Probiotic Health Claim in Switzerland

Email Print Share

3 June 2014 --- Nutrition and health expert [DuPont](#) has become the first ingredients company to receive a probiotic health claim in Europe. The company hopes that the approval, which was granted in Switzerland, will pave the way for other European nations to follow suit.



DuPont, which worked in close collaboration with a major Swiss Grocer, received approval from Switzerland's Federal Food Safety and Veterinary Office (FSVO) to market Danisco HOWARU *Bifidobacterium* HN019 probiotic as supporting digestion by reducing transit time.

"This is only the beginning," Global Public Affairs Leader, Cathy Andriadis told **FoodIngredientsFirst**. "DuPont now has a positive claim within Switzerland, and we hope to soon have a broader positive claim within the EU. It is now, more than ever, important to partner with companies that can achieve regulatory approvals in key markets like the EU. DuPont Nutrition & Health is a global leader in probiotics and we continue to provide our customers with the broadest portfolio of food ingredients in the industry. We have established that position through strong, credible science and dedicated technology. We are confident that our studies will strengthen the scientific evidence supporting our DuPont Danisco range."

Andriadis went on to explain that despite the regulatory challenges in Europe, "DuPont continues to deliver scientifically proven data on the efficacy of our probiotics". She also noted that, "this approval validates the strong evidence we have on the link between digestive health and probiotics – support for an important milestone that we hope prompts other countries to follow suit. Digestive comfort is an important benefit for consumers globally, and this approval creates new opportunities for food, beverage and dietary supplement marketers to promote this benefit to consumers."

DuPont has carefully studied EFSA studies and worked proactively with Swiss authorities, she said. The company's range of products includes Litesse and other benefits.

Approved Health Claim



Switzerland in 2014:
 "Bifidobacterium lactis HN019 contributes to a normal digestion by reducing the intestinal transit time"

Dose: 1,8x10e9/day



” The central message is that, despite numerous challenges that probiotic faced in the review period, they continue to have strong consumer appeal and are therefore worthy of industry investment. ”

Ewa Hudson

*Head of Health & Wellness research at Euromonitor International
Conference at PROBIOTA, Amsterdam, February 2016*



Probiotics in dairy applications and future outlook

What Inoculation? Application Optimization

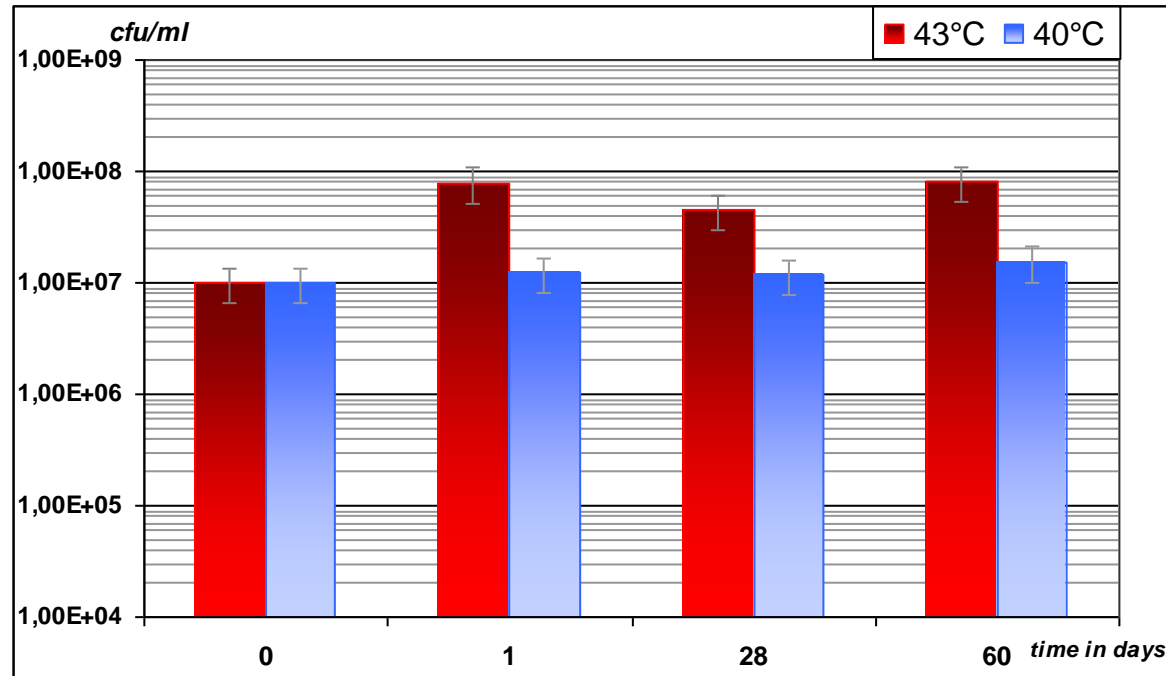
Probiotic Calculator	
<i>Red is input data</i>	
Probiotic culture	
type	x
Units / DCU in pouch	500
Cell count per unit/DCU	1,00E+11
Cell count in total pouch	5,00E+13
Product	
Product volume in ml (for daily dosage)	125
Daily Dose in Billion CFU	5
No of doses per Unit/DCU	20
No of doses per pouch	10.000
Needed cell count per ml (end of shelflife)	4,00E+07
Growth factor during fermentation	1
Decrease factor during shelflife	1
Net growth factor	1
Dosage	
DCU per 100 litre	40,0
Actual inoculation cell count/ml	4,00E+07

Critical parameters:

- Formulation, fruit type
- Water activity (powdered)
- Temperature
- Storage time
- Oxygen content
- pH
- Osmotic pressure
- Mechanical stress



Bifidobacterium lactis HN019 in application 1: Stability in model fermented milk and impact of the incubation temperature



- *B. lactis* HN019 shows a perfect stability during 60 days of storage at 6°C/43°F.
- Better results at 43°C (8x) vs. 40°C (1x) (no impact of inoculation rate)
- Up to 2x decrease of cell count during storage time (regrowth 30→60 days not further validated)
- Warning : *Process conditions, recipe, storage temperature... could impact differently the growth and survival of B. lactis* HN019.

Description of the pilot-scale conditions

Inoculation rate :

100 DCU of Howaru Bifido for 1000 liters / 264 gallons of milk

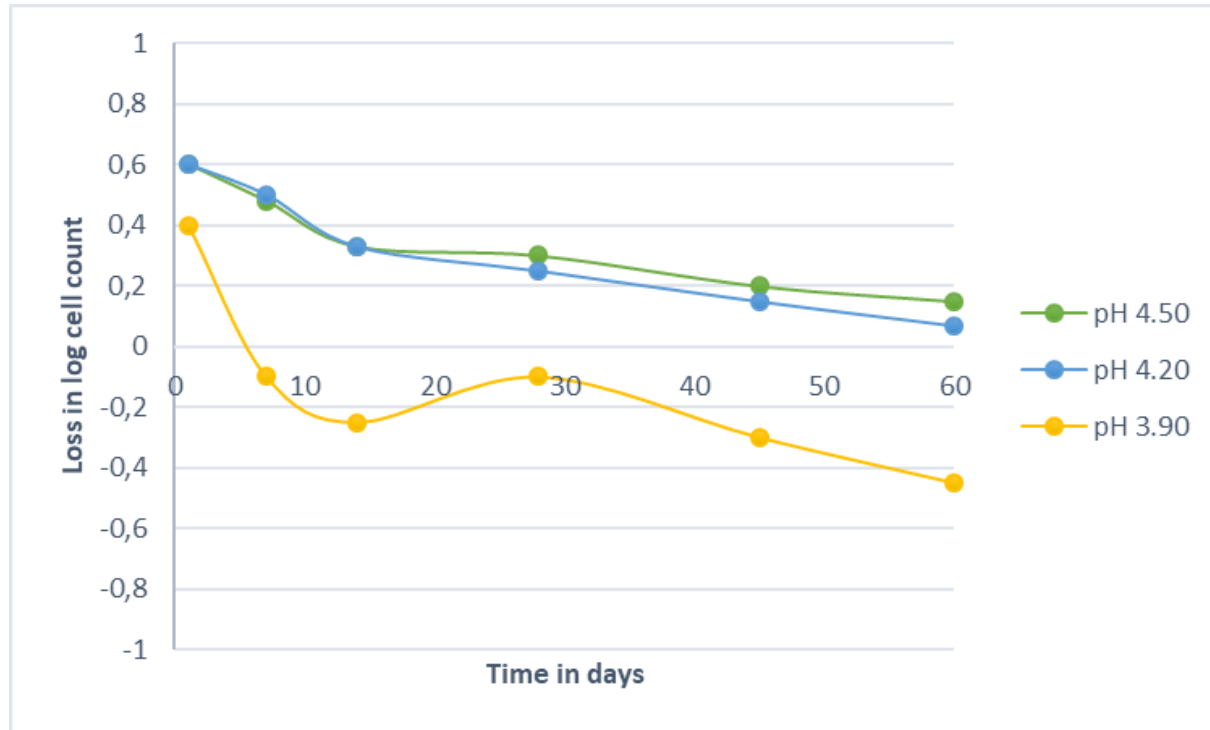
200 DCU of Yo-Mix Real Mild cultures for 1000 l / 264 gallons of milk

Fermentation temperature : 43°C/109°F or 40°C/104°F / Mix: UHT semi skimmed milk + 3% SMP – 10 min at 90°C/194°F.

The fermentation is stopped at 4,60 and then the products are cooled and stored at 6°C/43°F.

At the end of the shelf life the pH is around 4,20.

***Bifidobacterium lactis* HNO19 in application 2: Impact of pH on the stability in GDL acidified milk (HN019 added after acidification)**

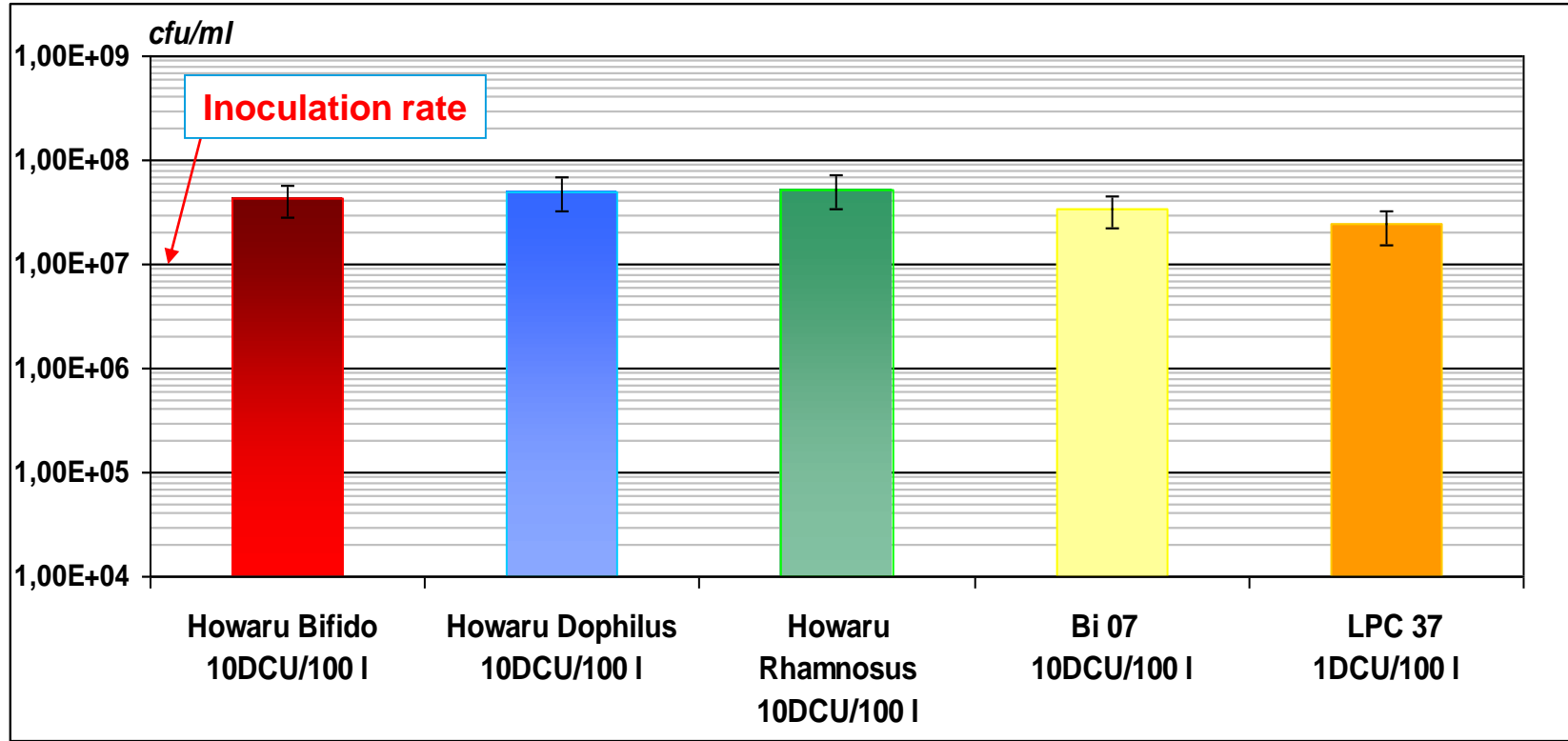


- *B. lactis* HNO19 show an good stability during 60 days of shelf life, especially in the pH range 4.2-4.5.
- A slight decrease is observed after 28 days at pH 3.9.

Description of the pilot-scale conditions

UHT milk is acidified with GDL (D-gluconic acid lactone) at different pH: 4.5 ; 4.2 ; 3.9
The Howaru Bifido is inoculated after the acidification at the same standard level for each pH.
Then the inoculated milk is stored 60 days at 6°C/43°F.
The numerations are conducted at D1, D7, D14, D28, D45 and D60.

Stability of different probiotic strains in yogurt, 28 days shelf life (Application 3)



Howaru Bifido = *B.lactis* HN019

Howaru Dophilus = *L. acidophilus* NCFM

Howaru rhamnosus = *L. rhamnosus* HN001

Bi-07 = *B. lactis* Bi-07

Lpc-37 = *L. paracasei* Lpc-37

Description of the pilot-scale conditions

Inoculation rate : 100 DCU of Howaru Bifido, Howaru Dophilus, Howaru Rhamnosus, Bi07 for 1000 litres / 264 gallons of milk

or 10 DCU of LPC37 for 1000 litres / 264 gallons of milk

200 DCU of Yo-Mix Real Mild cultures for 1000 l / 264 gallons of milk

Fermentation temperature : 43°C/109°F or 40°C/104°F (for LPC37) / Mix: UHT semi skimmed milk + 3% SMP – 10min 90°C/194°F.

The fermentation is stopped at 4,60 then the products are cooled and stored at 6°C/43°F. After 28 days, the pH is > 4,20.

Vegan Fermented Products – Now also with Probiotics

Danisco® VEGE Culture	Nut matrix (coconut, almond, cashew, ...)	Cereal matrix (oat, rice,....)	Fruit & vegetable matrix (apple, carrot, pumpkin,...)	Bean matrix (Pea, Chickpea, Lentils, ...)	Soy matrix	Other fermented Foods matrix
Danisco® VEGE ST LYO series	●●●	●●		●	●●●	●
Danisco® VEGE 030 LYO series	●●●	●●●		●●●	●●●	●
Danisco® VEGE 050 LYO series	●●●	●●●	●	●●	●●●	●
Danisco® VEGE 040 LYO series	●●●	●●●			●●●	
Danisco® VEGE 020 LYO series Danisco® VEGE 060 LYO series	●●●	●●			●●●	
Danisco® VEGE 010 LYO series Danisco® VEGE 090 LYO series			●●●			●●●
Danisco® VEGE 080 LYO series		●●●				●●●
Danisco® VEGE C-100 LYO series	Flavor adjunct	●●●	Flavor adjunct	●●●	Flavor adjunct	Flavor adjunct
HOWARU® VEGE BIFIDO LYO HOWARU® VEGE DOPHILUS LYO	●●●	●●●	●●●	●●●	●●●	●●●
HOLDBAC® VEGE YM FRO HOLDBAC® LC LYO	●●●	●●●	●●●	●●●	●●●	●●●
Recommendation of use : ● suitable ●● more suitable ●●● most suitable						

✓ Many people are moving towards plant-based alternatives for various reasons including health issues, vegan diet, to reduce their impact on planet

Vegan cultures:

- no allergens in final culture
- Adhere to all required certification for vegan products (produced in vegan approved raw materials A-Z)



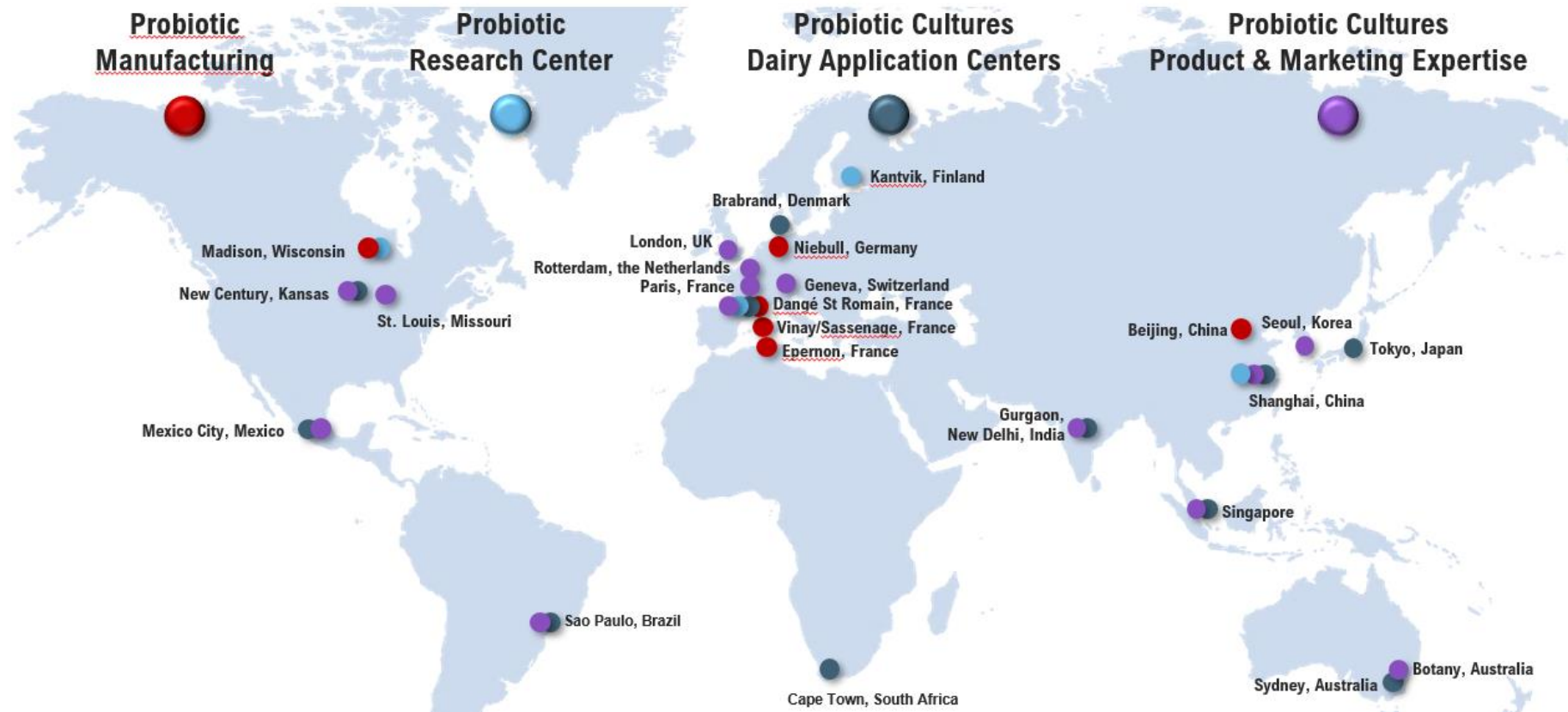
Take home messages

- Increasing consumer awareness on microbiomes and probiotics
- Importance of probiotics with science behind benefits
- Gut health is top of mind for many consumers
- Regulatory issues vary country to country
- It is possible to apply probiotics to a wide range of products
- Nutrition, health, wellness getting personalised → Targeted probiotics for targeted products



Acknowledgements

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- Karen Vokes (Regulatory Affair Manager)
- DuPont™ Danisco® Probiotic Cultures:



Thank you



“

We supply every
3rd probiotic product
sold globally ”



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