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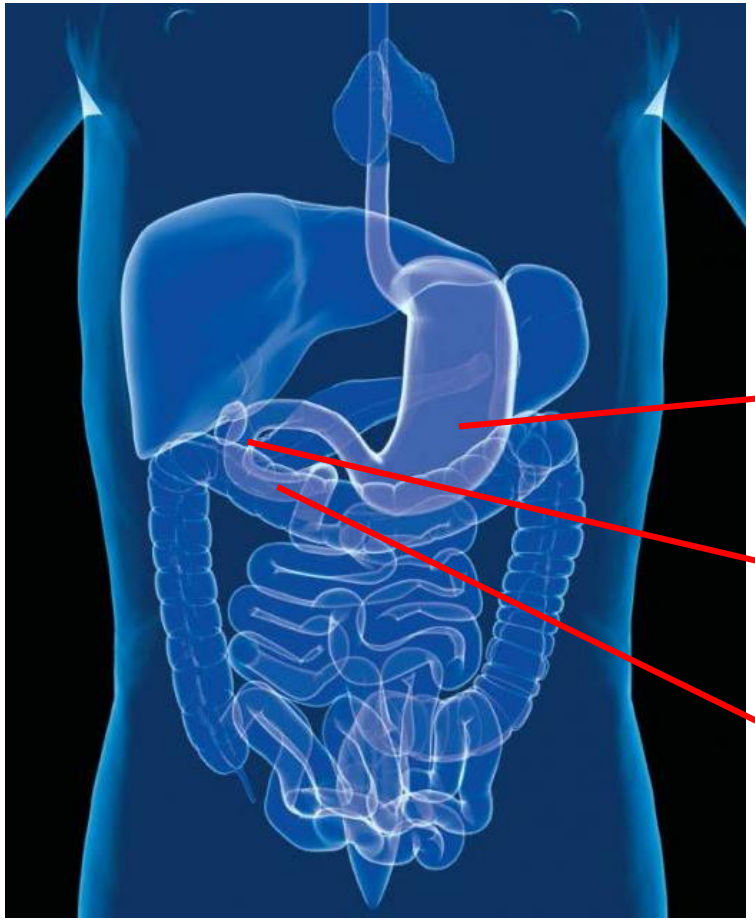
Mortality in the gastro-duodenal transit



The ingested microorganisms undergo inevitably a variable mortality when in contact with human biological fluids (gastric juice, bile acid and pancreatic juice)

Stomach: gastric juice (pH extremely low), pepsin (protein hydrolysis)

Duodenum: bile (emulsion and detergent action of the fats)

Duodenum: pancreatic juice (protides, glucies and lipids hydrolysis)

Mortality in the gastro-duodenal transit

Along the years, many research activities have studied the mortality dynamics of probiotic microrganisms in contact with these biological fluids

Mortality in contact with simulated gastric juice

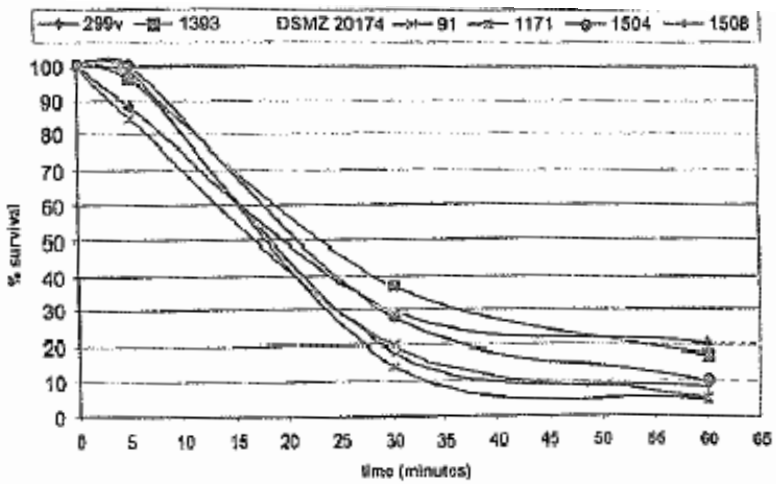


Fig. 2. Gastrointestinal resistance of various *L. plantarum* strains to simulated human gastric juice.

Mortality in contact with human gastric juice

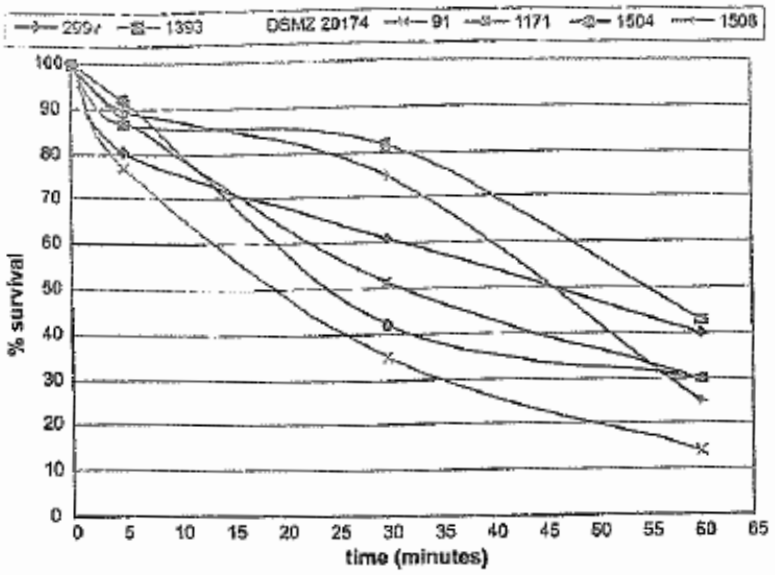
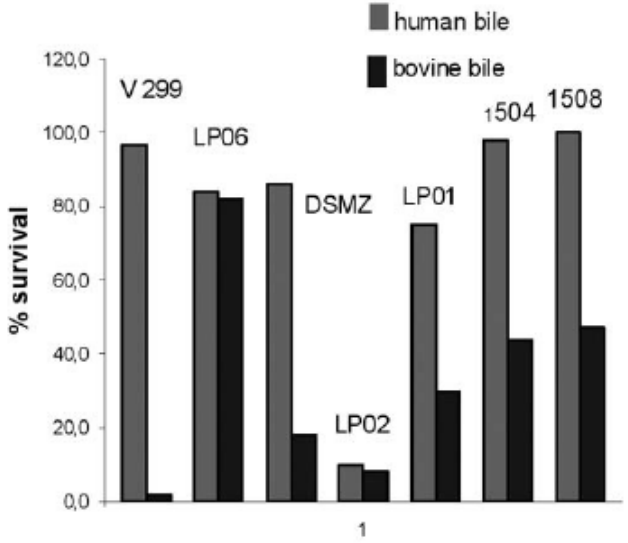


Fig. 3. Gastrointestinal resistance of various *L. plantarum* strains to human gastric juice.

After 60min, the survival rate is at most 20% of the microorganisms in contact with simulated gastric juice and from 15% to 45% when in contact with human gastric juice!

Mortality in the gastro-duodenal transit

Mortality in contact with bovine and human bile (60 min contact)



Conclusions: Most of the tested strains of probiotics were rapidly destroyed when exposed to bovine bile salts, while a significant viability was found after incubation with human bile. For this reason, it is possible that probiotical strains are not further studied, produced and marketed for their high in vitro sensitivity to bovine bile. To avoid this incongruity, we propose the use of human bile for in vitro testing of probiotics.

Survival of 42-83% in contact with human bile
and 5-48% in contact with bovine bile

Mortality in the gastro-duodenal transit

Mortality in contact with simulated and human pancreatic juice

TABLE 2. Effect of Simulated and Human Pancreatic Juice on the Viability of Selected *Lactobacillus* and *Bifidobacterium spp*

		Contact Time-related Mortality (%)					
		5 min		30 min		60 min	
Probiotic Strain	**n**	**Artificial**	**Human**	**Artificial**	**Human**	**Artificial**	**Human**
Lactobacillus acidophilus C.I.	1	12.9	8.6	28.4	22.4	39.9	33.8
Lactobacillus johnsonii C.I.	2	13.6	13.3	25.5	23.8	53.4	52.1
Lactobacillus rhamnosus C.I.	3	7.4	7.5	16.08	13.9	21.6	19.6
Lactobacillus acidophilus Probial LA02	4	9.9	8.2	25.4	20	35.5	34.2
Lactobacillus fermentum Probial LF06	5	11.1	10.3	23	21.4	32.07	29.7
Lactobacillus paracasei Probial LPC00	6	7.2	3.9	17.4	16.9	24.1	22.8
Lactobacillus plantarum Probial LP01	7	12.9	8.6	20.4	21.1	28	25.9
Lactobacillus rhamnosus Probial LR04	8	5.4	5.02	22.9	18.4	25.1	19.9
Bifidobacterium animalis subsp. lactis C.I.	9	8.9	12.5	27.9	22.7	48.7	51
Bifidobacterium breve Probial BR03	10	13.7	12	45.3	40.9	53.4	54.6
Bifidobacterium longum Probial BL03	11	6.5	8	38.3	34.6	42.1	42.9
Bifidobacterium adolescentis Probial BA02	12	2.9	4.2	21.8	17.3	26.2	29.1

After 60min, survival of 21-53% in contact with simulated pancreatic juice and 15-55% in contact with human pancreatic juice

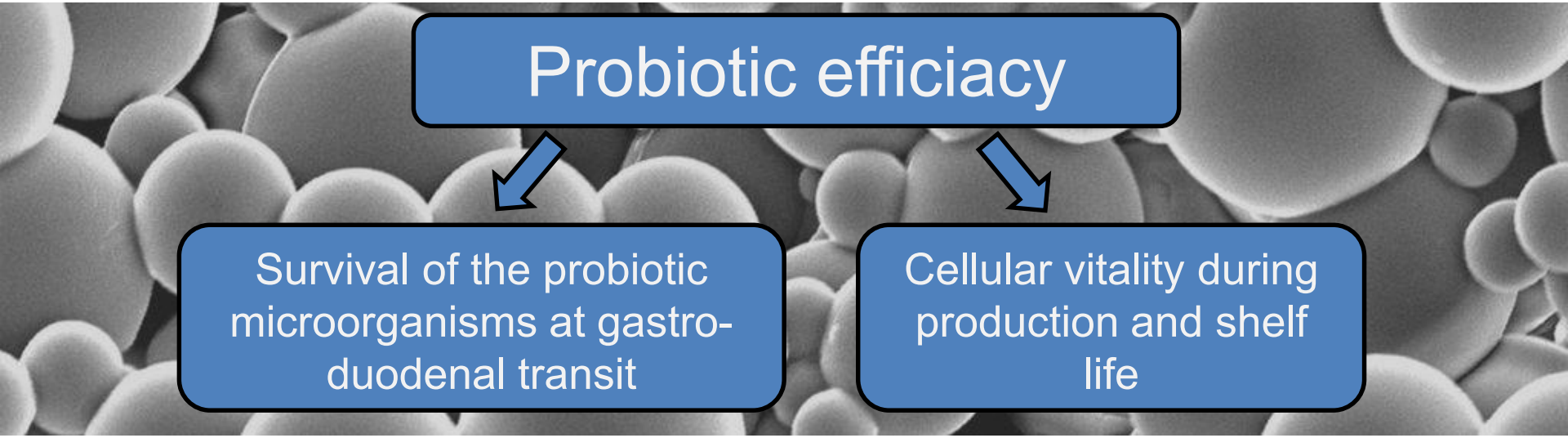
Mortality in the gastro-duodenal transit

General concept: the gastroduodenal transit implies an **unavoidable mortality** for the strains and depends on numerous factors including time of transit, physiological status, and mostly the strain's nature (**variability of resistance on a strain to strain basis**).

N.B. The analyses mentioned refer to the three distinct classes of biological juices (gastric juice, bile and pancreatic juice), **obviously the microorganisms come into contact with all three during transit and a higher mortablity is therefore expectable.**

How can we standardize these behaviours ?
Can we insure a better protection during the gastroduodenal transit ?

Advanced technology : microencapsulation



Microencapsulation: coating technology that covers every single cell with a fine protective lipidic layer.

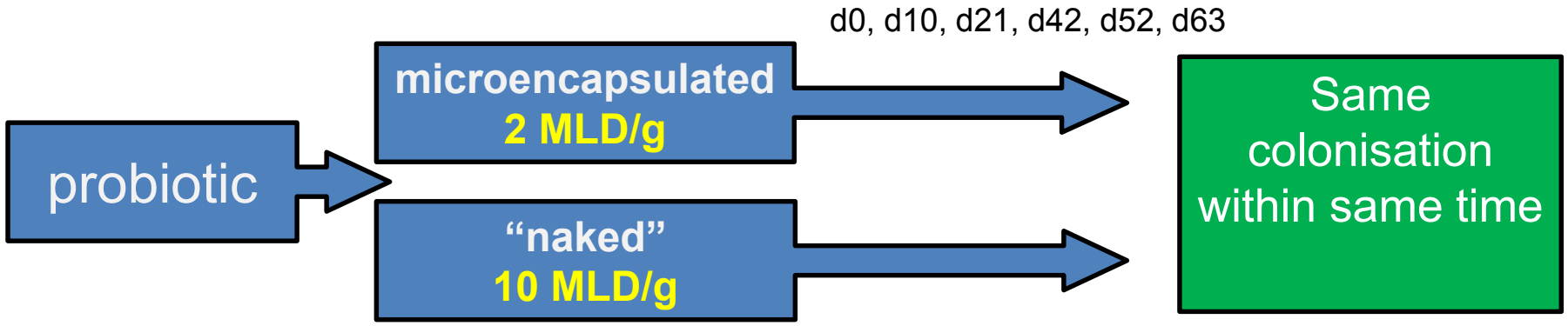
Microencapsulation increases the probiotic microorganisms' resistance during the gastro-duodenal transit, insuring its survival and biological activity.
In parallel, our microencapsulation protects the cells from degradation phenomena due to external factors such as humidity, acidity, osmotic pressure, oxygen and light.

Microencapsulation for an **improved** efficacy

1. Del Piano M., et al. "*Evaluation of the intestinal colonization by microencapsulated probiotic bacteria in comparison with the same uncoated strains." J Clin Gastroenterol. 2010 Sep;44 Suppl 1:S42-6.*

2. Del Piano M., et al. *"Is microencapsulation the future of probiotic preparations? The increased efficacy of gastro-protected probiotics." Gut Microbes. 2011 Mar-Apr;2(2):120-3.*

3. Del Piano M., et al., *"Comparison of the Kinetics of Intestinal Colonization by associating 5 Probiotic Bacteria Assumed either in a microencapsulated or in a traditional uncoated form." J Clin Gastroenterol 2012; 46:S85-S92*



The microencapsulated strains manages to colonize the gut within the same time at a dosage of one fifth of the same NON microencapsulated strain

Microencapsulation for an **improved** efficacy

Del Piano M., et al. *"Is microencapsulation the future of probiotic preparations? The increased efficacy of gastro-protected probiotics." Gut Microbes. 2011 Mar-Apr;2(2):120-3.*

NAKED 5 MLD/dose strain

MICROENCAPSULATED 1 MLD/dose strain

Table 1. Quantification of fecal Lactobacilli and Bifidobacteria (m ± SEM, $\log_{10}$ CFU/gram) before and after the two treatment periods, including the washout phase

Time		Group A		Group B		p¶ (A vs. B)
		log CFU/g	p§	log CFU/g	p§	
d_0						
Lactobacilli	LP01	5.53 ± 0.23	*	5.47 ± 0.20	*	0.85
Bifidobacteria	BR03	7.94 ± 0.23	*	8.25 ± 0.19	*	0.29
d_{10}						
Lactobacilli		6.89 ± 0.12	<0.0001	6.87 ± 0.19	<0.0001	0.92
Bifidobacteria		9.26 ± 0.13	0.0001	9.21 ± 0.17	0.0008	0.83
d_{21}						
Lactobacilli		7.32 ± 0.13	<0.0001	7.10 ± 0.14	<0.0001	0.26
Bifidobacteria		9.47 ± 0.10	<0.0001	9.43 ± 0.12	<0.0001	0.81
d_{42}						
Lactobacilli		5.61 ± 0.23	*	5.75 ± 0.21	*	0.53
Bifidobacteria		8.05 ± 0.23	*	8.44 ± 0.17	*	0.34
d_{52}						
Lactobacilli		7.13 ± 0.14	<0.0001	6.96 ± 0.15	<0.0001	0.41
Bifidobacteria		9.38 ± 0.09	0.0001	9.19 ± 0.16	0.003	0.30
d_{63}						
Lactobacilli		7.41 ± 0.13	<0.0001	7.20 ± 0.13	<0.0001	0.27
Bifidobacteria		9.63 ± 0.08	<0.0001	9.47 ± 0.08	<0.0001	0.18

CFU indicates colony-forming units. *Comparison reference time (d_0 for the first treatment period and d_{42} for the second one). §Comparison between time zero (d_0), or d_{42}, and the following analysis within each group. ¶Comparison between the two groups at d_0 and following analysis.

No statistically significant difference between group A and group B, meaning that colonisation dynamics are comparable

p <0.05 are considered significant

Microencapsulation for an **improved** efficacy

3. Del Piano M., et al., *"Comparison of the Kinetics of Intestinal Colonization by associating 5 Probiotic Bacteria Assumed either in a microencapsulated or in a traditional uncoated form." J Clin Gastroenterol 2012; 46:S85-S92*

TABLE 2. Quantification of Fecal Total *Lactobacilli*, Heterofermentative *Lactobacilli*, and Total *Bifidobacteria* (Mean ± SEM, log10 cfu/g) Before and After the 2 Treatment Periods, Including the Wash-Out Phase: Comparison Between the 2 Groups at d_0 and Following Analysis

	log cfu/g		
Time	Group A	Group B	*P* (A vs. B)*
d_0			
Total *Lactobacilli*	6.80 ± 0.17	6.78 ± 0.17	0.9063
Heterofermentative *Lactobacilli*	6.73 ± 0.22	6.61 ± 0.21	0.6589
Total *Bifidobacteria*	8.85 ± 0.20	8.94 ± 0.16	0.5158
d_{10}			
Total *Lactobacilli*	7.57 ± 0.19	7.62 ± 0.17	0.8121
Heterofermentative *Lactobacilli*	7.39 ± 0.19	7.53 ± 0.19	0.4535
Total *Bifidobacteria*	9.62 ± 0.14	9.61 ± 0.16	0.9394
d_{21}			
Total *Lactobacilli*	7.85 ± 0.12	7.82 ± 0.14	0.8502
Heterofermentative *Lactobacilli*	7.70 ± 0.12	7.71 ± 0.15	0.9383
Total *Bifidobacteria*	9.69 ± 0.12	9.81 ± 0.11	0.2673
d_{42}			
Total *Lactobacilli*	6.75 ± 0.17	6.98 ± 0.16	0.2133
Heterofermentative *Lactobacilli*	6.70 ± 0.22	6.89 ± 0.21	0.3195
Total *Bifidobacteria*	8.89 ± 0.14	9.02 ± 0.14	0.3004
d_{52}			
Total *Lactobacilli*	7.62 ± 0.20	7.67 ± 0.21	0.7843
Heterofermentative *Lactobacilli*	7.38 ± 0.19	7.51 ± 0.22	0.4906
Total *Bifidobacteria*	9.70 ± 0.14	9.63 ± 0.15	0.6589
d_{63}			
Total *Lactobacilli*	7.99 ± 0.13	7.91 ± 0.17	0.6617
Heterofermentative *Lactobacilli*	7.83 ± 0.13	7.78 ± 0.19	0.8045
Total *Bifidobacteria*	9.79 ± 0.11	9.85 ± 0.10	0.5607

*Comparison between the 2 groups at d_0 and following analysis.
cfu indicates colony forming units.

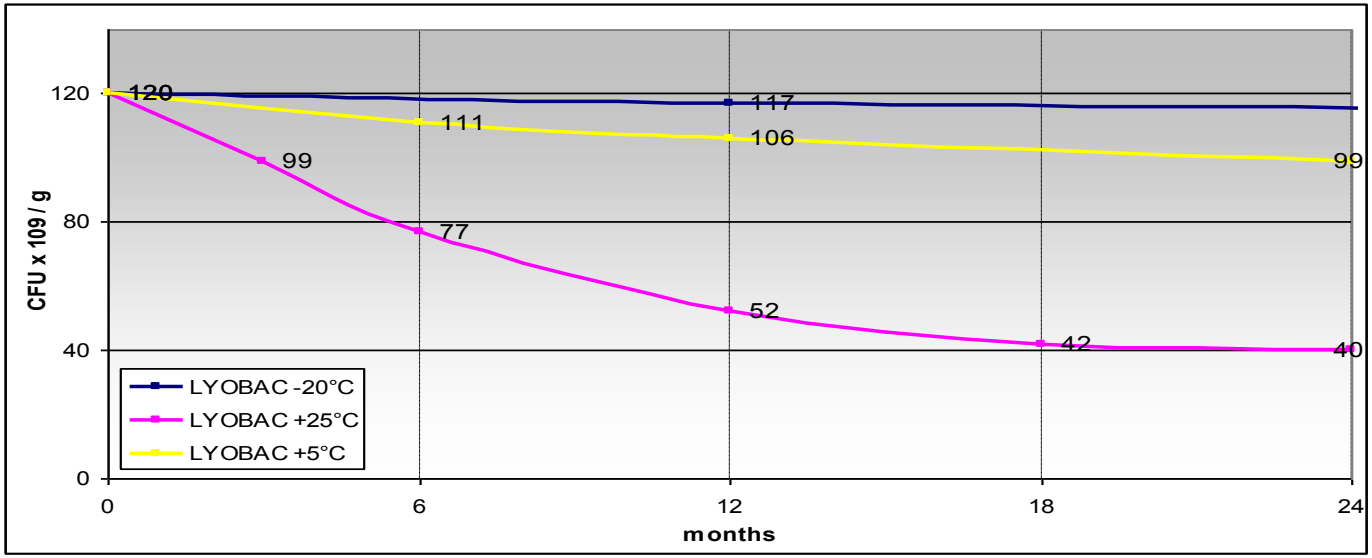
Group A: LA02, LR04, LGG, LR06, BS01 25 MLD/dose non microencapsulated

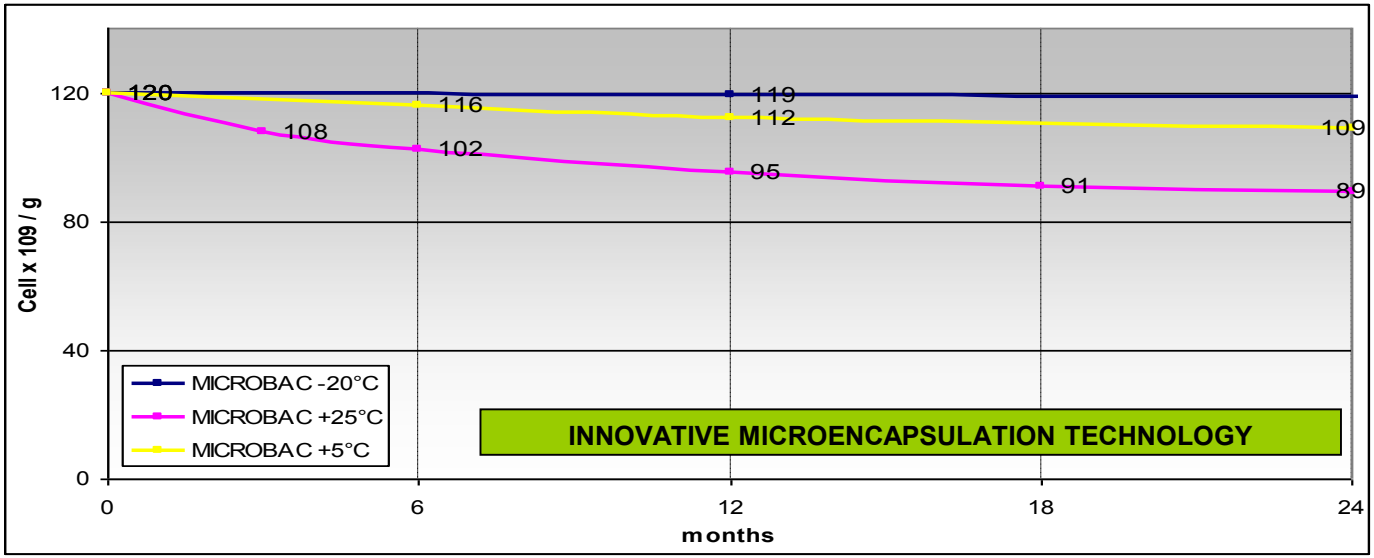
Group B: LA02, LR04, LGG, LR06, BS01 5 MLD/dose microencapsulated

No significant difference between group A and B means that the colonisation cinetics are comparable, that is to say "5 billion microencapsulated strains correspond to 25 billion naked strains".

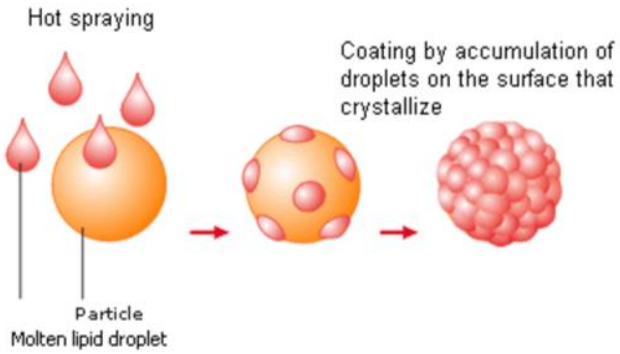
Microencapsulation for a better stability

Data given on average values of various strains





Microencapsulatione: the technology



Typology of microencapsulation: lipid coating

Thanks to Probiotical's patented process, it is possible to cover every single cell with a lipid coating, which confers to them :

- **resistance to humidity**
- **resistance to gastric acidity**

This technology is already used in the pharmaceutical industry for the controlled-release of active substances. However the coating process applicable to pharmaceutical substances is not transferable to a bacterial cell due to the high temperature involved in the process. Probiotical's technology overcomes this difficulty.