Gut Microbes 2:2, 120-123; March/April 2011; © 2011 Landes Bioscience

Is microencapsulation the future of probiotic preparations?

The increased efficacy of gastro-protected probiotics

Mario Del Piano,[1,*] Stefania Carmagnola,[1] Marco Ballarè,[1] Massimo Sartori,[1] Marco Orsello,[1] Marco Balzarini,[1] Michela Pagliarulo,[1] Roberto Tari,[1] Andrea Anderloni,[1] Gian Paolo Strozzi,[2] Luca Mogna,[3] Filomena Sforza[4] and Lucio Capurso[5]

[1]Gastroenterology Unit; Maggiore della Carità Hospital; [2]Probiotical SpA; [3]Biolab Research Srl; [4]Private Hospital "I Cedri"; Fara Novarese; [5]Centro Ricerca Clinica; San Filippo Neri Hospital; Roma, Italy

In a recent publication we assessed the kinetics of intestinal colonization by microencapsulated probiotic bacteria in comparison with the same strains given in an uncoated form. It is well known, in fact, that microencapsulation of probiotics with specific materials is able to confer a significant resistance to gastric juice, thus protecting the cells during the gastric and duodenal transit and enhancing the probiotic efficacy of supplementation. This was the first study comparing the colonization time of the same probiotic strains administered in coated and uncoated form. Here, we discuss additional in vitro data of resistance of these bacterial strains to gastric juice, human bile and pancreatic secretion and correlate this data with the results of in vivo gut colonization.

Key words: probiotic strain, microencapsulation, gastric juice, intestinal colonization, gastroduodenal transit, stability

Submitted: 11/12/10

Revised: 04/07/11

Accepted: 04/08/11

DOI: 10.4161/gmic.2.2.15784

*Correspondence to:
Mario Del Piano; Email: mario.delpiano@alice.it

Addendum to: Piano MD, Carmagnola S, Andorno S, Pagliarulo M, Tari R, Mogna L, et al. Evaluation of the intestinal colonization by microencapsulated probiotic bacteria in comparison with the same uncoated strains. J Clin Gastroenterol 2010; 44:42–6; PMID: 20697290; DOI: 10.1097/MCG.0b013e3181ed0e71.

We recently showed that the administration of microencapsulated probiotic bacteria is five times more efficient than the same uncoated strains as regards gut colonization and amount of bacteria detected in the feces (J Clin Gastroenterol 2010; 44:42–6).

This was the first study published on this topic, even if there is good knowledge about the increased gastroresistance of probiotics if administered in specific microencapsulated forms or included as active ingredients in gastroresistant formulations.[1-3]

There are other publications showing the kinetics of increase of some populations of bacteria in the feces when the subjects are given a specific probiotic formulation for a certain period of time, generally not longer than 2 weeks. However, in these studies, the probiotics have always been administered in an uncoated form.[4-6]

To date, there is good evidence that the majority of probiotic bacteria administered per os are able to reach the gut and integrate into the microbiota, thus exerting different beneficial actions. In any case, there are many more studies showing general benefits of probiotics rather than reporting a quantitative kinetics of intestinal colonization by such bacteria.

It is well known that probiotics need to colonize the gut in order to exert their positive actions on human health.[7] Specific components of the intestinal microflora, such as Lactobacilli and Bifidobacteria, have been associated with beneficial effects on the host, including promotion of gut maturation and integrity, antagonism against pathogens and modulation of the immune system.[8] Probiotics used as dead cells can only modulate the gut immune system (GALT) in some ways,[9] even if the qualitative and quantitative interaction with innate immune cells may be quite different from the same live strains, especially regarding the induction of specific cytokines (data not published).

It is also well known that before reaching the gut, probiotic microorganisms have to pass through the stomach and the duodenum, which represent a very harsh environment.

As a result, an unavoidable reduction in the number of viable cells occurs during transit. In order to be effective and confer

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	5.53 ± 0.23	*	5.47 ± 0.20	*	0.85
Bifidobacteria	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.

health benefits to the host, probiotics must be able to survive passage through the stomach and upper intestine and be present in a sufficient amount to impact the colon microenvironment. This means that they must tolerate the acidic and protease-rich conditions of the stomach and survive and grow in the presence of bile acids. This feature is strongly strain-dependent, but on average 10 to 25% of the ingested cells are able to survive and reach the gut, thus exerting their probiotic benefits.[10]

The Gastric juice is generally the strongest barrier for probiotics, whereas bile salts and pancreatic secretion together are responsible for no more than 35 to 40% mortality of the cells which survived stomach transit.

Even if all microrganisms are very sensitive to human biological gastrointestinal fluids, some are able to survive and colonize the gut. The transit may be facilitated by food matrices, especially those which reduce gastric acidity.

It is possible to evaluate the resistance of probiotic strains in vitro using simulated or real human juices and secretions. These in vitro evaluations may represent a reliable model to predict the amount of cells which could be delivered to the human gut after intake per os.

In a recent study by Del Piano et al.[12] seven *Lactobacillus plantarum* probiotic strains were tested for resistance to both simulated gastric juice and human gastric juice withdrawn on an empty stomach from healthy individuals. It was noted that less than 20% of the bacteria survived after an hour of exposure to simulated gastric juice, while human gastric juice allowed a survival rate between 15% and 45%.

Another recent study[13] demonstrated that many probiotic strains are clearly less sensitive to human bile than to bovine bile, while sensitivity of probiotics to real human or simulated pancreatic secretion is very comparable.[14]

Even if for most strains the amount of viable cells which are able to pass through the stomach and the duodenum is sufficient to guarantee a probiotic effect, there are some strategies that could be used to significantly improve the effectiveness of probiotics.

Microencapsulation of bacteria with a gastroresistant material may be applied to accelerate and amplify the onset of the beneficial effects. Microencapsulation is the process by which small particles or droplets are surrounded by a coating to produce microcapsules.[15] The concept of microencapsulation allows the functional core ingredient (in this case the probiotic cells) to be separated from its environment by a protective coating. Separation of the functional core ingredient from its environment continues until the release of the functional ingredient is desired (post-stomach for the probiotic).[16]

Microencapsulation of probiotics could be realized using polisaccharidic or lipid-based coatings. In the first case, coated strains could be successfully added to aqueous solutions, such as fruit juices, still beverages, or yogurts, while a lipidic coating is more suitable for addition of probiotics to oils, creams, cheeses or other lypophilic matrices.

Probiotic strains administered in this study, uncoated or microincapsulated in a lipidic matrix, were kindly manufactured and provided by Probiotical, Novara, Italy.

The subjects enrolled in the study were divided into two groups, one group

Table 2. In vitro quantification of the survival of *Lactobacillus plantarum* LP01 and *Bifidobacterium breve* BR03 to human gastroduodenal biological fluids

Probiotic strain	Parameters evaluated	Survival Rate (%)			
	Contact time in minutes	5'	30'	60'	
***Bifidobacterium breve* BR03 DSM 16604**	Human gastric juice	92	34	27	
	Simulated gastric juice	96	40	9	
	Simulated pancreatic secretion	91	42	20	
	Human bile (in the medium)				35
	Bile salts (in the medium)				10
***Lactobacillus plantarum* LP01 LMG P-21021**	Human gastric juice	85	56	45	
	Simulated gastric juice	94	32	25	
	Simulated pancreatic secretion	85	81	76	
	Human bile (in the medium)				97
	Bile salts (in the medium)				63

(21 subjects) received a mix of probiotic strains *Lactobacillus plantarum* LP01 (LMG P-21021) and *Bifidobacterium breve* BR03 (DSM 16604) in an uncoated form, while the other group (23 subjects) was given the same strains microencapsulated with a gastroresistant material. A 3-week wash-out phase was included at the end of the first period of probiotic treatment. At the end of the wash-out period, the groups were crossed and received the other active probiotic formulation for the same period. *L. plantarum* LP01 and *B. breve* BR03 strains have been previously demonstrated to be very useful in the reduction of intestinal discomfort and bloating typically associated with Irritable Bowel Syndrome.[17]

For both treatment periods the ratio between the number of viable cells of uncoated strains and the number of microencapsulated bacteria was always 5:1 (10 billion CFU uncoated strains : 2 billion CFU microencapsulated strains).

Statistically significant increases of Lactobacilli and Bifidobacteria in feces in both groups at the end of each treatment of this double blind, randomized, crossover study demonstrated similar kinetics of intestinal colonization by microincapsulated bacteria compared to uncoated strains given at an amount five times higher.

Table 1 briefly summarizes these results (Lactobacilli and Bifidobacteria in the feces are expressed as means ± standard errors of the mean). Paired t-test statistical analysis was used to compare the results.

Comparison of the kinetics of colonization suggests that the microencapsulated bacteria at one-fifth concentration (2 billion CFU/day) colonizes the gut better than uncoated strains at 10 billion CFU/day, even if differences are not statistically significant.

Our group has previously assessed the survival of many probiotic strains in gastric juice, pancreatic secretion and bile salts. A comparison between simulations and real biological fluids was also performed, highlighting the fact that simulated gastric juice is not comparable to human gastric juice and bovine bile secretion are not comparable to human bile.

For this reason, the survival of *L. plantarum* LP01 and *B. breve* BR03 strains during gastro-duodenal transit was assessed using real human gastric juice and bile salts,[12,13] while for pancreatic secretion, a simulated pancreatic solution was used.[14] The composition of this simulation has been previously described by Charteris WP, et al. (addition of pancreatin 1 g/l in 0.5% NaCl solution, then adjustment of pH to 8 using 0.1 N NaOH).[18] The resistance to gastric juice and pancreatic secretion was measured after 5, 30 and 60 minutes. The impact of bile salts was evaluated by their addition to the cultural media of the strains at a concentration of 0.3%.

Table 2 reports the survival data in vitro of uncoated LP01 and BR03 strains.

Data reported in **Table 2** show that only about 15 to 25% of the number of ingested cells is able to reach the gut if the strains are administered in an uncoated form.

If probiotic strains are administered in a microencapsulated form, the survival in gastric juice is almost totally complete, as the coating material does not dissolve in the acidic environment of the stomach. Dissolution of the coating material starts only in the gut where the pH is alkaline.

In light of this evidence, it is possible to reliably estimate that at least 90% of microencapsulated cells are able to survive passage through the stomach and the duodenum.

Microencapsulation of probiotic strains with a gastro-resistant coating should not only be regarded as a strategy to improve survival of strains after oral intake, thus enhancing their probiotic effects, but also as a tool to improve shelf-life stability of the strains in different finished product matrices. This is especially important if the water content of the product is high.

Many studies conducted by our group (internal data, not published yet) have demonstrated improved stability of microencapsulated bacteria in comparison with the same uncoated strains in different food supplements or functional foods. For certain products (e.g., some oils, lipidic creams used as fillings, powder formulations with a high water content), the use of uncoated strains would not be feasible due to inadequate stability.

In conclusion, the present study highlights the possibility to use a lower concentration of strains if delivered in lipidic microincapsulated form able to confer a strong gastroresistance to the cells. This type of microincapsulated probiotic also confers a significant improvment in stability of various final product applications.

Financial Support

The original study was financed by Probiotical SpA. No additional external source of funding contributed to the realization of the study.

Conflict of Interest

Paolo Strozzi is an external consultant for Probiotical SpA. Luca Mogna is an employee at Biolab Research Srl.

References

1. Chávarri M, Marañón I, Ares R, Ibáñez FC, Marzo F, Villarán M, et al. Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. Int J Food Microbiol 2010; 142:185-9.
2. Ding WK, Shah NP. An improved method of microencapsulation of probiotic bacteria for their stability in acidic and bile conditions during storage. J Food Sci 2009; 74:53-61.
3. Ding WK, Shah NP. Acid, bile and heat tolerance of free and microencapsulated probiotic bacteria. J Food Sci 2007; 72:446-50.
4. Fujimoto J, Matsuki T, Sasamoto M, Tomii Y, Watanabe K. Identification and quantification of *Lactobacillus casei* strain Shirota in human feces with strain-specific primers derived from randomly amplified polymorphic DNA. Int J Food Microbiol 2008; 126:210-5.
5. He T, Priebe MG, Zhong Y, Huang C, Harmsen HJ, Raangs GC, et al. Effects of yogurt and bifidobacteria supplementation on the colonic microbiota in lactose-intolerant subjects. J Appl Microbiol 2008; 104:595-604.
6. Strozzi GP, Mogna L. Quantification of folic acid in human feces after administration of Bifidobacterium probiotic strains. J Clin Gastroenterol 2008; 42:179-84.
7. Guarner F, Schaafsma GJ. Probiotics. Int J Food Microbiol 1998; 39:237-8.
8. Schiffrin EJ, Blum S. Interactions between the microbiota and the intestinal mucosa. Eur J Clin Nutr 2002; 56:60-4.
9. Adams CA. The probiotic paradox: live and dead cells are biological response modifiers. Nutr Res Rev 2010; 23:37-46.
10. Del Piano M, Morelli L, Strozzi GP, Allesina S, Barba M, Deidda F, et al. Probiotics: From research to consumer. Dig Liver Dis 2006; 38:248-55.
11. Floch MB. Bile salts, intestinal microflora and enterohepatic circulation. Dig Liver Dis 2002; 34:54-7.
12. Del Piano M, Ballarè M, Anderloni A, Carmagnola S, Montino F, Garello E, et al. In vitro sensitivity or probiotics to human gastric juice. Dig Liver Dis 2006; 38:134.
13. Del Piano M, Ballarè M, Anderloni A, Carmagnola S, Montino F, Garello E, et al. In vitro sensitivity of probiotics to human bile. Dig Liver Dis 2006; 38:130.
14. Del Piano M, Strozzi P, Barba M, Allesina S, Deidda F, Lorenzini P, et al. In vitro sensitivity of probiotics to human pancreatic juice. J Clin Gastroenterol 2008; 42:170-3.
15. Gibbs BF, Kermasha S, Alli I, Mulligan CN. Encapsulation in the food industry: A review. Int J Food Sci Nutr 1999; 50:213-24.
16. Vidhyalakshmi R, Bhakyaraj R, Subhasree RS. Encapsulation "The Future of Probiotics"-A Review. Adv Biol Research 2009; 3:96-103.
17. Saggioro A. Probiotics in the treatment of Irritable Bowel Syndrome. J Clin Gastroenterol 2004; 38:104-6. Erratum in: J Clin Gastroenterol 2005; 39:261.
18. Charteris WP, Kelly PM, Morelli L, Collins JK. Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic Lactobacillus and Bifidobacterium species in the upper human gastrointestinal tract. J Appl Microbiol 1998; 84:759-68.