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Revisión

*Lactobacillus Paracasei subsp. Paracasei* F19: a farmacogenomic and clinical update

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Abstract

Introduction: Many reports in literature have underlined particular features of *Lactobacillus paracasei* subsp *paracasei* F19, however a critical review of main clinical outcomes has not been performed so far.

Objectives: This review summarizes the most relevant reports, in terms of clinical benefits, of *Lactobacillus paracasei* subsp *paracasei* F19 administration reviewing it’s historical background and outlining new interesting perspectives in clinical practice.

Methods: We searched Pubmed/Medline using the terms “*Lactobacillus paracasei* subsp *paracasei* F19”. All clinical and experimental articles on the use of *Lactobacillus paracasei* subsp *paracasei* F19 were included.

Results and discussion: The genetic stability of F19, the most relevant clinical claim, renders it’s administration reliable and effective in immunocompromised people. Adequate concentrations of this strain support a dose/effect strategy ranging between NF B host macrophage activation to pathogenic bacteria overgrowth control as well as to fine interaction with the gut nerve endings. Moreover preliminary results from our lab support the formulation of F19 encapsulated with lyophilized HA in patients with IBD due to both an increased mucus-strain adherence and a possible enhanced strain proliferation and maintenance.

Conclusions: Further experiments are required to overcome the lack of informations about this new formulation for IBD management.


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Key words: *Lactobacillus paracasei* subsp *paracasei* F19. Lactic acid bacteria. Probiotic.

(LACTOBACILLUS PARACASEI SYBSP. PARACASEI F19; UNA ACTUALIZACIÓN DE FARMACOGENOMIC Y CLÍNICA)

Resumen

Introducción: Muchas notificaciones en la bibliografía han puesto de manifiesto las características particulares del *Lactobacillus paracasei*, subespecie *paracasei* F19; sin embargo, la revisión crítica de los principales resultados clínicos aún no se ha realizado.

Objetivos: Esta revisión resume los artículos más relevantes, en términos de beneficios clínicos, de la administración del *Lactobacillus paracasei*, subespecie *paracasei* F19, revisando su historia y recalando nuevas perspectivas interesantes sobre su uso en la práctica clínica.

Métodos: realizamos una búsqueda en Pubmed/Medline usando los términos “*Lactobacillus paracasei* subsp *paracasei* F19”. Se incluyeron todos los artículos experimentales que empleasen el *Lactobacillus paracasei*, subespecie *paracasei* F19.

Resultados y discusión: La estabilidad genética de F19, la característica clínica más relevante, hace que su administración sea fiable y eficaz en personas inmunosuprimidas. Las concentraciones adecuadas de esta cepa apoyan una estrategia de dosis/effecto que varía entre la activación de los macrófagos del hospedador hasta un control del sobrecrecimiento de bacterias patógenas, así como una adecuada interacción con las terminaciones nerviosas intestinales. Además, los resultados preliminares de nuestro laboratorio apoyan la formulación encapsulada de *Lactobacillus F19* con AH liofilizada en pacientes con EII debido a una mayor adherencia de la cepa a la mucosa y un posible favorecimiento de la proliferación y mantenimiento de la cepa.

Conclusiones: Se necesitan experimentos adicionales para paliar el déficit de información acerca de esta nueva formulación para el tratamiento de la EII.


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Abbreviations

L.: Lactobacillus.  
LAB: lactic acid bacteria.  
F19: L. paracasei subsp paracasei F19.  
H. pylori: Helicobacter pylori.  
P. pentosaceus: Pediococcus pentosaceus.  
L. mesenteroides: Leuconostoc mesenteroides.  
C. difficile: Clostridium difficile.  
B: Bifidobacterium.  
ECC: enteroinvasive Escherichia coli.  
HA: hyaluronic acid.  
E. coli: Escherichia coli.  
P. aeruginosa: Pseudomonas aeruginosa.

Introduction

The Lactobacillus casei (L. casei) group is mainly composed of lactic acid bacteria (LAB) such as Lactobacillus zeae (L. zeae), L. casei, Lactobacillus paracasei (L. paracasei) and Lactobacillus rhamnosus (L. rhamnosus) widely used in dairy products and lactic beverages and whose major end product of sugar fermentation is lactic acid. These bacteria are also gram-positive, nonsporing, catalase-negative, devoid of cytochromes and of nonaerobic habit but are aerotolerant, unpleasant, acid-tolerant and strictly fermentative. Studies on the electrostatic cell surface properties revealed a pH-dependent configuration with electrophoretic mobility progressively decreasing for lower pH values for the Lactobacillus casei subsp. casei (L. casei subsp. casei) and Lactobacillus paracasei subsp. paracasei (L. paracasei subsp. paracasei) strains until the isoelectric point (IEP = 4). The pH variations might be linked to cell wall chemical composition with adhesion mechanism during gastric transit.

The taxonomic position and nomenclature of the L. casei group had been long time debated. In the past L. casei group had been one species divided into five subspecies: the L. casei subspecies casei, alactosus, pseudoplanterum, tolerans and rhamnosus, but in 1989 Collins et al. introduced a reclassification by introducing two new species: L. paracasei and L. rhamnosus and tranferring subspecies L. casei subspecies casei to the species L. casei without any subspecies. L. paracasei comprised two subspecies: the subspecies Lactobacillus including L. casei subspecies alactosus and pseudoplanterum and the subspecies tolerans from the L. casei subspecies with the same name. L. paracasei subsp paracasei F19 (F19) belongs to the homofermentative family of lactic acid bacteria which convert almost quantitatively glucose to lactic acid. It showed the ability to bind gastric and bovine mucin, collagen I and III and fibronectin and to express high surface hydrophobicity. The survival to pH 2.5 for 1 h and 20% bile for 2 h exposure, the bacteriocin(s) production, the proteolytic activity as well as the ability the ability to transcribe NF B to the nucleus of macrophages have made this lactobacillus a reasonable candidate for a probiotic product development.

State of the art

F19 was isolated, for the first time, from the deep colonic mucus layer of patients without gastrointestinal disease who were admitted to the Sabbatsberg Hospital of Stockholm in 2002. Then it was included in the multicentre European project PROBDEMO, which involved volunteers from Finland and Sweden, where its gastric survival ability was assessed and where strains closely related to F19 were observed in the intestinal tract of a small percentage of volunteers.

Within the PROBDEMO project human pilot studies, based on the F19 strain, have been conducted. One involved 61 healthy infants and 30 elderly that were randomly assigned to receive or gelatin capsules containing 1×10^6 CFU of F19 in corn starch or corn starch only for 12 weeks. Fecal samples from infants treatment group (n = 30), collected before, during (at 2-3 weeks) and 2 weeks after administration, revealed an increase of F19 in 6/30 and a reduction of 17% of Clostridium difficile (C. difficile) population. However the elderly treatment group (n = 13) did not evidence substantial changes in terms of both microbiological and, as for the infants, Helicobacter pylori (H. pylori) eradication. The overall results indicated the ability of F19 to survive through the gastrointestinal tract and in 8-20% to be enclosed in the microflora for several weeks as a part of the normal microflora. Another trial, still based on the assessment of gastric survival and mucosal adhesion of F19, indicated that it was both in the lumen and adhered to the mucosa of the colon (0.9×10^6 CFU) following 12 day consumption in 5 individuals. Potential side-effects of probiotic consumption, such as intestinal discomfort, increased flatulence and changes in stool consistency and frequency, were monitored. All studies reported no adverse effects.

A questionable issue that has been extensively studied for the F19 is the genetic stability assessment, especially in order to guarantee consumers of the quality of probiotic. The stability of three extrachromosomal elements (plasmids) within F19 was carefully checked in each step of the industrial reproduction process and their genetic profile was compared with those determined in the same strain 6 years earlier. At the end of the study no selection of cured derivatives was detected and all plasmids resulted unaltered.

A limited effect of F19 on resistant isolates during treatment with penicillin and quinolones was also observed. In particular, 20 patients (divided in 2 groups) treated with either penicillin or ciprofloxacin (or norfloxacin) were randomized to receive a placebo or a probiotic product made of powdered milk (10 g) and freeze-dried F19 (10^4 CFU/ml). Fecal samples,
collected before treatment on day 10 and 1 month after the start of the treatment, revealed that resistance in enterococci was not affected by penicillin administration while quinolone resistance increased during quinolone treatment. Moreover F19 was recovered in three samples from 10 patients in penicillin-treated group ($2.1 \times 10^7-5.3 \times 10^8$ CFU) and in three samples of eight patients ($2.1 \times 10^1-6 \times 10^8$ CFU) of the quinolone-treated group on day 10. Further, one patient of the quinolone-treated group still harboured the probiotic strain on day 30 ($2.1 \times 10^8$ CFU).

The effectiveness of the contemporary oral administration of F19 (Genefilus F19, Siffra Farmaceutici, Florence, Italy) in association with vaginal suppositories containing L. acidophilus has been was tested by Delia et al. 60 healthy women (18-40 years) with suspect or confirmed diagnosis of bacterial vaginosis were randomized to receive either vaginal suppositories containing Lactobacillus acidophilus (L. acidophilus) (Calagin, Siffra Farmaceutici, Florence, Italy) or the same vaginal suppositories plus oral administration of Genefilus F19 ($12.5 \times 10^9$ CFU per sachet). The patients were examined at the end of therapy (3 months) and 3 months after the end of treatment. A significant reduction of vaginal pH, an improvement of sniff test as well as of the subjective symptomatology were observed in both groups at end of therapy and still decreased during the 3 months follow-up. However the Genefilus F19-treated group had a meaningful reduction of vaginal pH and of sniff test at the end of therapy and a maintenance of positive effect also after 3 months.

Also in the host immunomodulation, a claim of many probiotics involved in normal immune function development, has been analyzed. Based on previous clinical studies, a symbiotic combination of $10^{11}$ CFU of Pediococcus pentosaceus (P. pentosaceus) 5-33:3, Leuconostoc mesenteroides (L. mesenteroides) 32-77:1, F19; L. plantarum 2,362 plus inulin, oat bran, pectin, and resistant starch (Synbiotic 2000 Forte, Medipharm, Sweden) was randomly administered for 15 days (1 sachet of 12 g) whereas the placebo consisted of an identical dose of maltodextrin (Caloreen, Nestle, UK) on 65 critically ill patients.

Analysis of infections, systemic inflammatory response syndrome, severe sepsis, and mortality, were performed. Results indicated that symbiotic-treated patients significantly reduced the rate of infections ($P = 0.01$), systemic inflammatory response syndrome, severe sepsis ($P = 0.02$), and mortality; further, hospitalization within the intensive care unit ($P = 0.01$) and days under mechanical ventilation were significantly reduced with respect to placebo ($P = 0.001$).

The role of F19 in this mix has been peculiar, it was the strongest inducer of Th1 and repressor of Th2 cytokines and, along with Lactobacillus plantarum (L. plantarum), was effective to eliminate C. difficile strains. On the other hand it is worth noting that the PRONUT study evidenced that Synbiotic 2000 Forte did not improve severe acute malnutrition outcomes in 399 Malawian children.

On the basis of microbiological evidence it has been also observed that F19 did not modify CD4 T-cells growth, responsible for normal immunomediates response, on the contrary this population was stimulated by L. paracasei subsp. paracasei B21060.

By means of the gene array technology it has been possible to map the host global gene expression profile changes caused by both F19 and L. acidophilus NCFB 1748 administration ($5 \times 10^9$ CFU/ml). Differences in gene transcription were evaluated in the distal ileum of normal microflora and germ-free mice. In the normal microflora mice both strains caused concerted enhancement in a cluster of genes involved in immune response (such as Igh-5; Ms4a1; Clu; Cxcl13), some belonging to B cell receptor-signalling (Cd79a; Ms4a1; Cd19; Blk; Cd79b; Ptprc), some implicated in phagocytosis (Mfgf8; Coro1a), in complement function (C3, C4), in the architectural organization of B cells within lymphoid structures (Ltb; Cxcl13) and some implicated in protection against mucosal damage in inflammatory bowel disease (Serpina1) suggesting also a possible role of Lactobacilli in reducing the severity of inflammatory bowel disease. Although no qualitative differences on the expression profile of immune response-related genes were detected the mean signal increase was higher in mice fed with L. acidophilus NCFB 1748. In mice raised under germ-free conditions immune stimulatory effects were not observed obviously due to gut lymphoid tissue incompetence. Moreover, in germ-free mice fed with Lactobacilli an increased signal for adiponectin and adipisin (or factor D) in combination with reduced expression of resistin like β was observed.

West and coworkers determined the impact of F19 during weaning on infections and IgG antibody responses to routine vaccines in 179 infants. 89 infants were fed cereals with F19 (treatment group) whereas 90 without F19 (placebo) from 4 to 13 months of age. All of them were immunized with diphtheria and tetanus toxoid and a cellular pertussis, polio and Hib-conjugate vaccines at 5½ and 12 months of age and the number of days with infections, antibiotic prescriptions and antibody concentrations to all vaccines before and after the second and third doses were assessed. Both groups did not differ in the days number with infectious symptoms but in days with antibiotic prescriptions (fewer in the treatment group $p = 0.044$); moreover F19 feeding ($1 \times 10^6$ CFU/ml) enhanced anti-diphtheria concentrations when adjusting for breastfeeding duration and colonization ($p = 0.024$). An interaction of the intervention and colonization with F19 on anti-tetanus toxoid concentrations occurred during the course of vaccination ($p = 0.035$). No effect exerted by F19 was observed on anti-HibPS (Polio + Hib) concentrations on infants breastfed <, > or = 6 months. Nevertheless F19 feeding increased the capacity to raise immune responses to protein antigens...
especially in infants breastfed < 6 months but did not prevent infections.

One year later the same group evaluated the effects of F19 feeding on the incidence of eczema and Th1/Th2 balance during weaning. From 4 to 13 months of age 89 infants were fed cereals with F19 (1 × 10^7 CFU) whereas 90 were only fed cereals. As a proxy for immune balance it was used the IFN-γ/IL4 mRNA expression levels in polyclonally stimulated peripheral blood T cells. The cumulative incidence of eczema at 13 months was 11% and 22% in the probiotic and placebo groups, respectively (p < 0.05) and the IFN-γ/IL4 mRNA ratio was higher in the probiotic compared with the placebo group (p < 0.05). Conversely, no differences between groups in serum concentrations of total or specific IgE were observed.

F19 has been confirmed to be efficacious and well tolerated in patients with IBS presenting with diarrhoea or with constipation. In particular, 100 IBS patients both with diarrhoea (n = 52) and constipation (n = 48) were administered Genefilus F19 at a dose of one sachet (12 × 10^6 CFU, combined with 5 mg glucose-oligosaccharides plus vitamins B1, B5 and B6), dissolved in water, twice daily for 14 days. Moreover the content of F19 was evaluated in the stool of 20 patients before and after treatment. 2 weeks after the end of the treatment 94% of patients with IBS with diarrhoea had no more abdominal pain and 88% had no more diarrhoea; on the other hand, abdominal pain and constipation were no longer present in 87% and 83% of patients with IBS with constipation respectively; 95% of the overall population considerably improved or eliminated abdominal distension. Microbiological evaluations of patients stool revealed a marked increase of F19 load following the treatment, with a minimum of 2, to a maximum of 10, CFU/g faeces. The same trend, although with less evidence, was observed by Simrén et al. (2009). 74 IBS patients were randomized to receive a daily treatment with either milk fermented with the yoghurt bacteria and containing F19, L. acidophilus La5 and Bifidobacterium lactis (B. lactis) Bb12, 5 × 10^9 CFU/ml, (n = 37; Cultura; active) or simply acidified milk without these bacteria (n = 37; control) for 8 weeks. Responders were 14/37 (38%) patients in the treatment group and 10/37 (27%) patients in the control group (P = 0.3). IBS symptom severity improved significantly Both groups had an improvement of IBS symptom severity during the treatment period, in particular during the first 2 weeks.

Based on the evidence that part of the pathogenesis in chronic fatigue syndrome of the host might be due to Disturbances in intestinal microbial ecology and in the immune system, Sullivan et al. (2009) evaluated the effect of F19, L. acidophilus NCFB 1748 and B. lactis Bb12, 10^10 CFU/ml, (Cultura Dofilus Natural Yogurt, Arla Foods, Stockholm, Sweden) on fatigue and physical activity in 15 chronic fatigue syndrome (CFS) patients. The rationale was that lactic acid producing bacteria have been shown to prevent and alleviate gastrointestinal disturbances and to normalize the cytokine production and that such regulatory mechanism may be useful for CFS suffering patients. After 4 week of probiotic intake neurocognitive functions were improved in 6/15 patients, while no significant changes in fatigue and physical activity scores as well as in the gastrointestinal microflora were observed.

It has been observed that gut microbiota modulation, by means of probiotics intake, could be used also during obesity intervention strategies. In particular, F19 supplementation, was shown to increase the levels of lipoprotein lipase inhibitor ANGPTL4 (involved in the triglyceride deposition control into adipocytes) and peroxisome proliferator activated receptors (PPARγ and PPARα) specific targets for the treatment of type 2 diabetes and dyslipidemia. ANGPTL4 inhibited lipoprotein lipase action decreasing fat storage. To investigate the effects of F19 supplementation SPF C57B/6J mice were used due to their propensity for weight gain. After 10 week treatment the serum analysis revealed that free fatty acids were not affected by the presence of F19, while the triglyceride load of the lipoprotein VLDL showed a slight but significant increase although cholesterol levels remained unchanged; on the other hand circulating ANGPTL4 levels were up-regulated and magnetic resonance imaging showed a significantly reduced body fat profile.

Recently, Nardone et al. (2010) have proposed the use of F19 as protective agent in a rat model with induced oxidative and metabolic hepatic injury (30 min ischemia and 60 min reperfusion). More in detail, 27 Wistar rats were fed a standard diet and 27 rats a methionine/choline deficient diet for 8 weeks before the ischemia/reperfusion procedure; within each group 7 rats were sham-operated to determine baseline conditions, 10 rats underwent I/R of the liver whereas 10 rats underwent I/R after dietary supplementation with F19 (3 × 10^10 CFU) for 8 weeks. After I/R rats fed a standard diet showed a decrease in sinusoid perfusion (P < 0.001), a severe liver inflammation, necrosis, an increase of tissue levels of MDA (P < 0.001), TNF-α (P < 0.001), IL-1 (P < 0.001), IL-6 (P < 0.001) as well as of serum levels of transaminase (P < 0.001) and LPS (P < 0.001) with respect to sham-operated rats. A decrease in Bacteroides, Bifidobacterium, and Lactobacillus spp (P < 0.01, P < 0.001, and P < 0.001, respectively) and an increase in Enterococcus and Enterobacteriaceae (P < 0.01 and P < 0.001, respectively) on intestinal mucosa was also observed. F19 supplementation significantly reduced the harmful effects of I/R on the liver and on gut microbiota in both groups of rats, however in methionine/choline deficient-fed rats, where the severity of liver and gut microbiota alterations were greater, a slightly less effect was observed.

Annibale et al. (2011) have successfully proposed the use of Genefilus F19 along with a high-fibre diet, for abdominal bloating and prolonged abdominal pain reduction in symptomatic uncomplicated diverticular
A recent study has highlighted the potential use of F19 in NEC Bell’s stage 2 (the most common acquired acute gastrointestinal illness in the neonatal period that affects about 5% of infants with birthweight ≤1,500 g and that is characterized by abdominal distension, bloody stools and *pneumatosis intestinalis*) in order to prevent the clinical progression to stage 3. 32 infants with birth weight 600 to 1500 g were randomly assigned to receive either a 5 ml probiotic supplement (n = 18; F19; 6 × 10^9 CFU/ml) or standard medical treatment (n = 14). F19 supplementation was associated with lower progression to stage 3 (P < 0.05), lower mortality rate and shorter hospital stay (P < 0.05). Moreover none of probiotic-treated patients presented either sepsis or intestinal complications such as diarrhea.

The hypothesis that enteric glial cells might participate in host–bacteria cross-talk has been evaluated by Turco et al. (2013)\(^4\). Primary cultures of human enteric glial cells have been exposed both to live and heat-killed pathogenic enteroinvasive *Escherichia coli* (EEC) and probiotic (F19; 3.4 × 10^9 CFU/ml) bacteria. Results indicated that EEC activated enteric glial cells inducing the cFos and MHC II expression. After 6h exposure TLR1, TLR3 and TLR4 mRNA expression was significantly up-regulated by both EEC and F-19 (p < 0.01) with respect to the basal level. On the other hand, EEC induced a higher TLR3 expression (p < 0.01) and a significantly lower expression of TLR5 and TLR7 (p < 0.01) with respect to F19. After 24 hours exposure TLR7, TLR9 and TLR5 mRNA expression was significantly up-regulated only by F19 (p < 0.01) with respect to the basal level. Moreover TLR2 expression was significantly up-regulated by both EEC and F19 (p < 0.01) with respect to the basal level, however TLR3 expression was significantly up-regulated only by EEC (p < 0.01) and conversely TLR7, TLR9 and TLR5 mRNA expression was significantly up-regulated only by F19 (p < 0.01) with respect to the basal level. Notably, EEC induced a significantly higher expression of TLR2, TLR3, TLR7 and TLR9 (p < 0.01) and a lower TLR5 expression (p < 0.01) with respect to F19.

When enteric glial cells were challenged for 6 h with either heat-inactivated EEC or F19, TLR2, TLR7 and TLR9 expression was virtually undetectable with respect to the basal level while TLR5 expression was significantly down-regulated by heat-inactivated EEC (p < 0.01). After 24 h challenge, heat-inactivated EEC significantly up-regulated TLR3 expression (p < 0.01) with respect to the basal level whereas both heat-inactivated EEC and F19 up-regulated TLR4 expression (p < 0.01).

Interestingly, immunofluorescence analysis showed that TLR2 was mainly detected in the cytoplasm and in the plasma membrane of enteric glial cells while TLR3 and TLR4 were mainly cytosolic and nuclear. Moreover, western blot analysis of enteric glial cells showed that EEC, but not F19, induced nuclear translocation of NF Bp50 protein (p < 0.05) with respect to the basal level as well as TLR2, TLR3 and TLR4 agonists (p < 0.05). Conversely, when enteric glial cells were treated with the specific MyD88-blocking peptide, only the TLR3 agonist significantly increased NFκBp50 expression (p < 0.05) with respect to the basal level while TLR2 and TLR4 agonists failed to induce NFκBp50 nuclear translocation. Finally, after 24 h exposure to both viable EEC and F19, S100B protein expression (and consequently NO release) was significantly higher in response to the first one (p < 0.01) compared to basal conditions. This study emphasizes both the aspect that enteric glial cells express TLR (involved in the innate immune system response mechanism)\(^4\) and their role in discriminating between pathogens and probiotics by modulating TLR expression.

More recently Palumbo et al. (personal communication) have further characterized the enteric glial cells - F19 interaction evaluating the effects of mediators released by these cells after probiotic challenge by means of conditioned media analysis\(^4\). In particular, conditioned media from probiotic stimulated cells showed increased lactase activity as compared to the untreated ones (1.15 ± 0.17 and 1.29 ± 0.19 fold increase vs control p < 0.05). However, a decreased lactase activity was observed when enteric glial cells were treated with pathogens (0.85 ± 0.23 fold decrease vs control p < 0.05).

**F19: in vitro study of a potential new prebiotic enhancing activity**

The concept of a possible combined administration of F19 with some new prebiotic enhancer, induced our research group to focus on the lactobacilli interaction with hyaluronic acid (HA), a large linear glycosaminoglycan which is mostly present within extracellular matrix\(^5\), that in previous microbiological and virological investigations had shown some definite properties in controlling the pathogenic bacteria and viruses growth\(^6\).
The role of HA on in vitro growth rate of F19 was investigated by means of an innovative technique. The procedure consisted in introducing, in 10 sterile vials already filled with an eugonic broth, $10^7$ CFU/ml of F19 plus a decreasing concentration of HA (from 1 to 0.125 mg ml$^{-1}$) and placing these in a bacterial culture (HB&L$^{TM}$; Alifax, Padova, Italy). HB&L$^{TM}$ was used for the real time detection of bacteria growth curves. Such analyzer is a new combination of a turbidimeter (based on the dynamic light scattering mechanism), with Mc Farland Monitor and an incubator within the same device and, for the first time, successfully used to accelerate studies on lactobacillus strain growth investigations. Each sample is analyzed by two laser beams which have their own photodetector, one more sensible (placed at 30° with respect to the beam source) and one more specific (placed at 90° with respect to the beam source).

With this investigation diagnostic device we demonstrated that HA concentration, from 1 to 0.125 mg ml$^{-1}$, resulted in an increased bacterial strains growth within 24 hours (fig. 1). Observations might suggest a possible protective role of low doses of HA towards F19, supporting its in vivo proliferation and engraftment after oral administration.

Although speculative, a possible role of HA on the bacterial growth and proliferation might be ascribed to the ability of some strains of utilizing HA as a carbon source.

**Discussion**

The issue of a single lactobacillus administration compared with a pool in the probiotic therapy scenario has been debated long time without a definite indication: the concept that links each single microbiologic agent to a well defined biochemical or genetic interaction within the host environment is a clear cut premise to achieve the goal of an effective medical treatment with some sort of pharmacological approach in microbiology.

Very often the production and market of a mixed lactobacilli combination in nutraceutical products did not previously take into account the in vitro interaction between each other strain, and the impact of a stranger microbiological task force intrusion into the gut environment, doesn’t guarantee at all a quicker integration or a better balance of different pathogenic versus saprophytic populations.

The bioavailability of a single strain formulation, like in the case of F19 in adequate concentration, supports a dose/effect strategy ranging between NFkB...
macrophage activation plus immunity host modula-
tion, and the success over pathogenic bacteria over-
growth moreover the F19 fine interaction with the gut
nerve endings, as it has been demonstrated by the in
vitro experiment of Turco et al. achieves convincing
explanation of the clinical healthy effect on IBD symp-
toms during nutraceutical treatment with this lacto-
 bacillus. Other experimental investigations opened the
hypothesis that F19 displays some metabolic activating
properties in the lipid imbalance and in the liver
impaired functions.

Last, but not least, the genetic stability of the strain is
a first class safety clinical claim, especially when
probiotic therapy is required in very weak, immuno-
compromised people, with altered mucous barrier and
with the real risk of lymphohematogenous spread of
the bacteria into the bloodstream.

The term bacterial translocation, coined by Berg and
Garlington50, has been defined as the passage of both vi-
able and non-viable microbes and microbial products
(endotoxins) from the intestinal lumen through the
epithelial mucosa into the mesenteric lymph nodes and
then to other organs. It might be related to a decrease in
microbes turnover rather than an increase in their
transepithelial penetration and those which seem to
translocate most readily hold the ability to both survive
in the bloodstream and to resist leucocyte digestion and
phagocytic killing (e.g. Salmonella species). On the
contrary, normal enteric species are easily killed after
phagocytosis (with the only exception of Escherichia
coli (E. coli), other enterobacteriacesae, Pseudomonas
aeruginosa (P. aeruginosa), enterococci and some
streptococci5, surviving only under circumstances in
which host defences are impaired. Recent findings have
ascribed the ability of translocate of these specific
strains to a better adherence and facilitated attachment
to the mucus-epithelium layer with respect to nonpatho-
genic strains51. Although intestinal anaerobic bacteria
(such as Lactobacilli) exceed aerobic bacteria by 100:1
to 1000:1 and act as an insulating layer over the mucous
on the mucosal surface, limiting colonization and over-
growth of other potentially invasive microbes52, their
translocation has been reported only in extreme circum-
stances such as athymic51, lethally irradiated4 or
severely burned rodents53 and in all these conditions
there are breaks in enteric integrity and the bacterial
translocation appears to be in direct proportion to the
degree of tissue damage. The fact that the aerobic
Gram-negative bacilli can translocate even across histo-
logically intact intestinal epithelium52 (through morpho-
logically intact enterocytes55) and that selective elimi-
nation of anaerobic bacteria facilitates intestinal
accumulation and translocation of facultative bacteria,
has led to the assumption that bacterial overgrowth
might be one of the main factors (reduced gastric
acidity56, impaired gastrointestinal motility and, conse-
quentially, prolonged intestinal transit time57 promoting
bacterial translocation. However bacterial translocation
can occur in the normal host, shown by recovery of
viable intestinal bacteria from mesenteric lymph nodes
in a small proportion of healthy animals and humans58-59
and is a probably normal and essential process, regu-
late local and systemic immunity and tolerance to the
innumerable antigens that make contact with the in-testinal epithelium60. Usually, enteric bacteria translo-
cation by oral antibiotics displacement remains
confined to the satellite mesenteric lymph nodes and do
not appear to extend a persistent infection state, more-
over when the antibiotic is discontinued the caecal
population of enteric organisms returns to normal
levels54-55. In this context, however, immunosuppression
can activate the translocating bacteria to spread system-
ically, ultimately resulting in lethal sepsis61. Moreover,
the lower part of the gut, which contains a large number
of microbes, has been suggested to hold a more efficient
capacity for killing translocated bacteria with respect to
the upper part; in particular the colon, which has been
demonstrated to have higher electrical resistance and
lower permeability to the passive movement of ions62.

The lactobacilli translocation is a not uncommon rele-
vant event that might rise pathogenic complications and
even septic death: the genetic stability of F19, confirmed
in more than 20 years of clinical use, not only is
reassuring that the up date risk of spontaneous muta-
tion is not consistent, but even in case of hematogenous
spread of F19, it will maintain its proper immunomodu-
minating activity; thus rendering the invaded host more
active in the cell-mediated defence against the septic
agents, but also it can be easily destroyed by the stan-
dard antibiotic dosages, being it’s antibiogram still
unchanged since 20 years.

A final comment about the F19 high daily concentra-
during the oral intake: being F19 acid-bile resistant
it’s transit through the proximal gastrointestinal tract
leaves a great number of lactobacilli viable for the
colonic harboring and able to survive in the colonic
environment for at least three months after 4 weeks of
oral intake. This means a prolonged therapeutic effect
and a better temporary integration in the host micro-
bacteria related to the length of F19 administration with
the confidence that the high lactobacilli count pro dose
is without untoward effects (bloating, constipation or
other common symptoms observed during probiotic
administration, were not detected during F19 oral
intake inducing an excellent compliance by the users.

Summarizing, based on recent achievements, F19,
provided of genetical stability, actively interacts with
gut epithelium and immune system, correlating with
both gene sequences and genes whose down-regulation
may be the cause of gastrointestinal pathologies.

Our present preclinical investigation addresses us to
perform a next pilot study to administer F19 encapsu-
lated with lyophilized HA in patients with IBD, in
comparison with the existing formulation, in order to
evaluate further symptomatic benefits due to the gut
mucosa-lactobacillus interaction with a better mucous-
germm adherence, and possible enhanced F19 prolifera-
tion and bioavailability into the gut lumen.
As matter of fact, an other our previous unpublished study on the HA administration by enemas on a group of patients with ulcerative colitis under Pentasa treatment showed a remarkable symptomatic benefit on the number of stool-mucous discharges, bloating and pain.

We have thus a rationale to suppose that further benefit will be achieved by the synergy between probiotic and glycosaminoglycan administration. Furthermore F19 as a single therapeutic agent we’ll more easily identify further specific benefits not only in the bowel inflammatory and motion control, but also in other indications related to it’s metabolism in the commensal environment such as obesity and steatosis, during glyco-lipidic imbalance of diabetes, potential treatment.

References


