Lactobacillus paracasei subsp. *paracasei* F19: Survival, Ecology and Safety in the Human Intestinal Tract—A Survey of Feeding Studies within the PROBDEMO Project

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Lactobacillus paracasei F19 is an emerging probiotic strain that shows considerable promise for use in functional foods for intestinal health. In a multicentre European research project, human feeding trials provided an insight into the ability of this strain to survive gastric transit and transiently colonize the human intestinal tract. Analysis of the faecal microbiota in healthy human volunteers showed that a proportion of the subjects carried a strain indistinguishable from *L. paracasei* F19 naturally within their intestines. When consumed in different foods, *L. paracasei* F19 survived gastric transit in healthy infants, adults, and elderly subjects. The bacterium transiently colonized both the colonic lumen and mucosa. Molecular analysis of faecal and colonic biopsy samples from children fed the probiotic showed that *L. paracasei* F19 did not perturb the population dynamics of other major populations of bacteria in the intestinal microbiota. This strain was well tolerated by young children, healthy adults, adults with milk-hypersensitivity and elderly subjects infected with *Helicobacter pylori*. *Key words*: F19, *Lactobacillus*, microbiota, paracasei, probiotic, survival.

INTRODUCTION

Probiotics are live microorganisms included in foods to provide health benefits to consumers by positively contributing to the composition and activity of their intestinal microbiota (1). A number of bacteria from the genera *Lactobacillus* and *Bifidobacterium* have been identified as having characteristics necessary for probiotic action. These properties include technological parameters, survival during passage through the upper gastrointestinal tract, transient persistence in the intestinal tract, and proven safety for human consumption (2–4). Health benefits to humans are strain specific, with different probiotic organisms providing varying effects on health parameters such as immunomodulation and protection against intestinal infections (5).

Lactobacillus paracasei ssp. *paracasei* isolate F19 (*Lactobacillus* F19) is an emerging probiotic strain that is readily amenable to commercial manufacture and which retains viability and functionality in dairy products while providing good flavour and organoleptic properties (6). This strain has shown considerable promise during *in vitro* trials assessing its potential to survive gastric transit and to persist in the colonic environment (unpublished data). As part of a multicentre European project named PROBDEMO (described in (7)), *L. paracasei* was included in human pilot studies to assess the ability of probiotics to survive intestinal transit and to examine their influence on the native microbiota of consumers. Subjects in the trials were also monitored for any deleterious effects resulting from probiotic consumption. The current paper outlines the results of trials involving *Lactobacillus* F19, focusing on its survival, ecology and safety in the human intestinal tract.

HUMAN FEEDING STUDIES

Four pilot feeding studies assessing *Lactobacillus* F19 were conducted within the PROBDEMO project, involving volunteers from Finland and Sweden (Table I). A variety of

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Table I

Human pilot studies involving Lactobacillus F19 conducted within the PROBDEMO project

	Subjects	Probiotic delivery	Trial design
Trial A	61 healthy infants 1–1.5 years old (Sweden) (12)	Gelatin capsules containing 1×10^{10} CFU *Lactobacillus* F19 in corn starch, or placebo (corn starch only)	Randomized, double-blind, placebo controlled design. Two capsules per day for 3 weeks. Analysis of faecal samples for *Lactobacillus* F19 and other microflora components. DGGE analysis of faecal flora from five control and five treatment subjects
Trial B	Five healthy adults (Finland)	Fermented milk containing 1×10^{8} CFU/g each of *Lactobacillus* F19, *L. acidophilus*, and *B. longum*	Two daily doses of 200 ml of fermented milk for 12 days. Faecal samples and taken initially and after 12 days of administration. Biopsy samples from the colonic mucosa taken after 12 days of administration. Analysis of *Lactobacillus* F19 and total lactobacilli
Trial C	Five healthy adults and four milk-hypersensitive adults (Finland)	Non-fermented milk containing 1×10^{6} CFU/ml of *Lactobacillus* F19 (added to the milk as a freeze dried powder)	Single-blind, placebo controlled with crossover, two daily doses of 200 ml of milk for 1 week. Analysis of *Lactobacillus* F19 in faecal samples and other microflora components
Trial D	30 elderly subjects (> 65 years) seropositive to *H. pylori* (Sweden)	Treatment–fermented milk containing mesophilic starter culture and 5×10^{8} CFU/g of *Lactobacillus* F19. Placebo control–fermented milk containing mesophilic starter culture only	Randomized, double-blind, placebo controlled design. Consumption of 2 daily doses of 150 ml of fermented milk for 12 weeks. Analysis for *Lactobacillus* F19 and other microflora components in faecal samples

target groups were tested, ranging in age from infants to the elderly, and including both healthy subjects and individuals with mild health disorders. The probiotic delivery systems included capsules and milk containing freeze-dried *Lactobacillus* F19, and milk and yoghurt fermented by mesophilic or yoghurt cultures including *Lactobacillus* F19. In each case, samples of the product were tested to ensure delivery of viable and functionally active probiotic bacteria to the volunteers throughout the trials.

Prevalence of Lactobacillus F19 in the native intestinal microbiota of humans

The intestinal microbiota of humans is complex, containing more than 400 different species of bacteria and possibly many thousands of strains (8, 9). Modern molecular techniques such as PCR and restriction fragment length polymorphism (RFLP) analysis allow researchers to identify individual strains within the intestinal microbiota. *Lactobacillus* F19 was originally isolated from the small intestine of a human subject. This strain was not produced commercially at the time of the trials described in this report, and therefore, the subjects were not previously exposed to this strain in probiotic foods. Despite this, strains indistinguishable from *Lactobacillus* F19 using RAPD (randomly amplified polymorphic DNA) analysis were identified in the initial baseline sample (pre-administration) of one of the individuals in Trial B, and in three samples from one individual in the control group in Trial D. Therefore, *Lactobacillus* F19, or at least very closely related strains, are present in the native intestinal microbiota of a small proportion of individuals from Nordic countries. This enables further confidence in the strain's safety and its ability to persist in the intestinal tract of humans.

Earlier research investigating the diversity of strains of lactobacilli and bifidobacteria within humans failed to find common strains among 10 people, suggesting that individuals may harbour distinct populations of these bacteria within their intestinal tracts (10). In contrast, the PRODEMO studies suggest that strains closely related to *Lactobacillus* F19 are present in the intestinal tract of a small percentage of the population in northern Europe.

Survival of Lactobacillus F19 in the human intestinal tract

If a probiotic bacterium is to beneficially contribute to the activity of the intestinal microbiota it must successfully survive transit through the harsh gastric environment, and then tolerate bile released into the small intestine that can also reduce bacterial viability. Trials conducted using a sophisticated *in vitro* model of the intestinal tract at TNO in the Netherlands indicated that *Lactobacillus* F19 can be expected to survive intestinal transit (11). In order to confirm intestinal transit survival in humans, analysis of faecal samples was conducted prior to and following probiotic ingestion.

RAPD analysis using validated primers for the specific detection of *Lactobacillus* F19 in intestinal samples was developed in order to identify this strain among the myriad of strains present in the intestinal microbiota. In each of the four trials described in Table I, faecal samples were cultured for total lactobacilli using Rogosa agar and then colonies resembling *Lactobacillus* F19 were analysed using RAPD to confirm their identity. In all of the age groups tested, from 1-year-old infants to subjects >85 years of age, *Lactobacillus* F19 could be identified in faecal samples following ingestion. During ingestion of the probiotic, *Lactobacillus* F19 was the numerically dominant *Lactobacillus* isolated in faecal samples (Table II). This confirmed that *Lactobacillus* F19 survived intestinal transit and could transiently colonize the intestinal tract of humans. When monitoring of faecal microbiota was continued following cessation of probiotic consumption in Trials A and D, six infants were colonized by *Lactobacillus* F19 2 weeks after consumption, and two elderly subjects remained colonized after 8 weeks (12, 13). This shows that *Lactobacillus* F19 can colonize the intestinal tract of some individuals for relatively long periods following the cessation of probiotic intake.

Adhesion to the intestinal mucosa is considered a desirable characteristic for potential probiotic bacteria, possibly aiding colonization and probiotic action including immunomodulation (14, 15). In Trial B, samples that represented the colonic lumen (faecal) and the colonic mucosa (biopsy) were collected in order to determine the intestinal location of the bacteria. Biopsy samples were washed prior to microbiological analysis so that only bacteria adhering to the mucosa were enumerated. Although *in vitro* studies using mucus from the intestinal mucosa have indicated that adhesion to intestinal mucus by *Lactobacillus* F19 was moderate to poor compared with other intestinal bacteria tested (16, 17), the results of Trial B (Table II) indicate that *Lactobacillus* F19 was both in the lumen and adhering to the mucosa of the colon following consumption. In contrast to most faecal samples, *Lactobacillus* F19 only dominated the *Lactobacillus* community on the mucosa in two of five volunteers. This may reflect colonization resistance by the indigenous mucosal microbiota of some individuals, or may be the normal distribution of *Lactobacillus* F19 within its niché in the colon. Mucosal biopsies taken from the ascending, transverse, and descending colon contained similar levels of *Lactobacillus* F19 (data not shown), demonstrating that it can colonize the length of the colon.

Trials involving adhesive and non-adhesive isogenic mutants of *Lactobacillus crispatus* showed that after ingestion by humans, the adhesive strain colonized the intestinal mucosa (biopsy samples) and persisted longer in the intestinal tract (18). The ability of *Lactobacillus* F19 to adhere to intestinal mucosa may provide it too with an advantage in colonizing the human intestinal tract.

Effects on other intestinal bacteria

One of the proposed benefits of probiotics is to prevent overgrowth or colonization by deleterious microorganisms within the intestinal tract following perturbations to the microbial ecosystem. It follows that it is undesirable for an exogenous probiotic to disturb the microbial balance in the intestinal tract of healthy individuals. Techniques such as PCR using universal bacterial primers, coupled with denaturing gradient gel electrophoresis (DGGE), allow visualization of the major groups of bacteria within the intestinal microbiota (19). Samples from Trial A (five from different individuals in the placebo group and five from the treatment group) were analysed before and after feeding. The subjects were young children, an age group for whom it is known that the composition of the intestinal microbiota is still developing and is relatively unstable (20). Some changes were observed in the microbiota composition in the individuals tested in Trial A (Fig. 1). However, none correlated with the consumption of *Lactobacillus* F19, indicating that this probiotic does not disturb the balance of major population groups within the intestinal microbiota. A band for *Lactobacillus* F19 was not visible on the gel suggesting that, while *Lactobacillus* F19 dominated the luminal *Lactobacillus* population during treatment, it constituted less than 1% of the total bacterial

Table II

Numbers of total lactobacilli and the probiotic strain Lactobacillus F19 in faecal and colonic mucosal samples from five healthy adult humans prior to and following 12 days of consumption of the probiotic in yoghurt at approximately 4×10^{10} CFU/day

Sample	Total lactobacilli average CFU/g ($\pm$SD)	*Lactobacillus* F19[a] average CFU/g ($\pm$SD)
Faeces prior to administration of the probiotic	$2.2\ (\pm3.5)\times10^{6}$	None detected in four individuals. 1.2×10^{4} in one individual
Faeces after 12 days of consumption of the probiotic	$2.0\ (\pm1.3)\times10^{6}$	$1.3\ (\pm1.5)\times10^{6}$
Colonic mucosal biopsy after 12 days of consumption of the probiotic	$0.9\ (\pm1.7)\times10^{5}$[b]	$0.9\ (\pm1.7)\times10^{4}$[b]

[a] Strain specific RAPD PCR used to identify *Lactobacillus* F19 from colonies with a *L. paracasei* appearance.
[b] CFU/biopsy samples (colonic mucosal section approximately 3 mm in diameter).

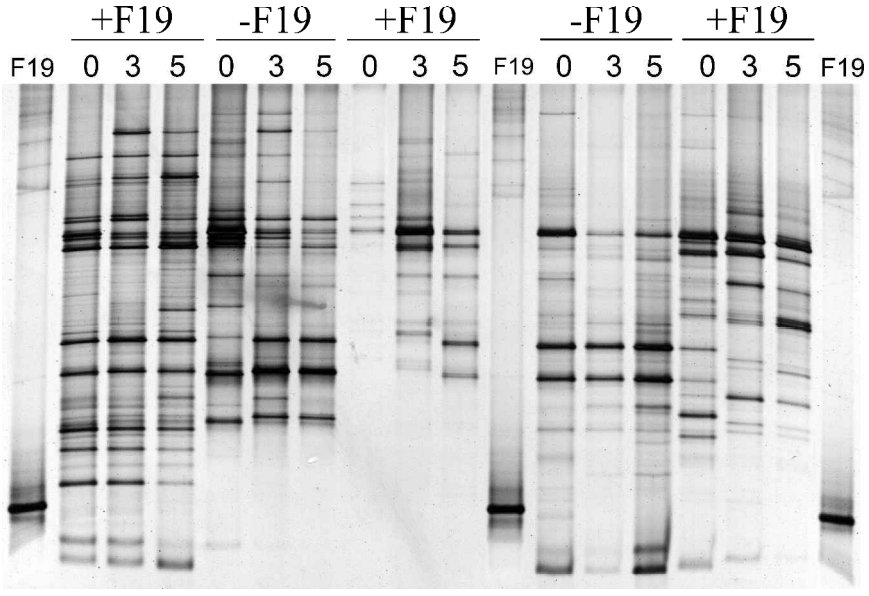

Fig. 1. DGGE profiles of PCR products obtained using primers 968-GCf and 1401r on total DNA isolated from faecal samples. The samples of individual children in Trial A, fed with *L. paracasei* F19 (F19) or placebo (P) were taken pre (0) and post-administration (3 and 5 weeks). The DGGE pattern of *L. paracasei* F19 is indicated (courtesy of GHJ Heilig, EE Vaughan, ADL Akkermans, WM de Vos).

population in the intestinal tract. Recently, specific PCR-DGGE has been developed for intestinal bifidobacteria and the *Lactobacillus* group (21, 22). Analysis of the specific *Lactobacillus* population in faecal samples of children indicated that it is rather unstable, like the dominant community (22). Furthermore, using this approach the *Lactobacillus* F19 strain was detected throughout the feeding period of children (Trial A), and again provided support for the natural presence of the *Lactobacillus* F19 strain within the intestinal community.

SAFETY OF *Lactobacillus* F19

Included in each of the trials were observations monitoring of potential side-effects of probiotic consumption. These included intestinal discomfort, increased flatulence, and changes in stool consistency and frequency. The trials included healthy subjects, adults with verified milk-hypersensitivity, and elderly people infected with *Helicobacter pylori*. No adverse effects of probiotic administration were observed in any of the pilot studies. The probiotic was well tolerated by all individuals (a total of 85 subjects consumed the probiotic) including those in Trial D, in which the probiotic was consumed daily for 3 months without adverse effects. The fact that no side-effects were detected in subjects ranging in age from 1 to >85 years, and in healthy individuals and subjects suffering mild illnesses (milk-hypersensitivity and *H. pylori* infection) suggests that *Lactobacillus* F19 is a safe microbial food supplement. Adding weight to this argument are the strain's origin from the intestinal tract of a healthy human and its natural prevalence in a healthy human population.

CONCLUSIONS

Lactobacillus F19 is an emerging probiotic with good technological characteristics. It now has a proven ability to survive gastric transit and to persist in the colonic environment of humans. This was achieved in subjects over a wide range of ages from the very young (1 year) to the very old (>85 years), and with four different food delivery matrices.

It appears that *Lactobacillus* F19 is indigenous to the intestinal tract of a portion of the population in Finland in Sweden. This, combined with the absence of deleterious effects during the human feeding trials, even in subjects with underlying disorders, suggests that the strain is safe for use as a human probiotic. The way forward is open for further clinical testing of this strain to assess its efficacy in contributing to improved human health. As for all probiotics, identifying health benefits stemming from ingestion of *Lactobacillus* F19 against specific intestinal disorders, and determining their mechanisms of action are the pending research challenges.

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