

In vivo assessment of potential probiotic *Lactobacillus salivarius* strains: evaluation of their establishment, persistence, and localisation in the murine gastrointestinal tract

Lisa Murphy^{1,2}, Colum Dunne^{1,2}, Barry Kiely², Fergus Shanahan³, Gerald C. O'Sullivan⁴ and J. Kevin Collins^{1,3}

¹Department of Microbiology, ²National Food Biotechnology Centre, ³Department of Medicine, University College, Cork, ⁴Department of Surgery, Mercy Hospital, Cork, Ireland

Correspondence to: Professor J. Kevin Collins, Department of Microbiology, University College Cork, Cork, Ireland. Tel: +353 (0)21 902392; Fax: +353 (0)21 903101; E-mail:nfbc@ucc.ie

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The enteric flora comprise approximately 95% of the total number of cells in the human body. Numerous studies have investigated potentially beneficial members of this microbial community due to their ability to elicit immune responses while also protecting against microbial pathogens. We have previously reported on the isolation and identification, from surgically-resected segments of the human gastrointestinal tract (GIT), of potential probiotic lactic acid bacteria (LAB). These bacterial strains exhibit potentially beneficial probiotic traits *in vitro* such as bile tolerance in the absence of deconjugation; gastric acid resistance; and adherence to epithelial cell lines. The objective of this study was to administer two strains of the previously-isolated LAB to mice over a period of 7 or 14 days in order to assess their ability to establish themselves within specific regions of the GIT. Throughout this feeding period, and for 4 days following cessation of feeding, the numbers of total culturable lactobacilli and of the administered LAB present in faeces were monitored. Spontaneous rifampicin resistant derivatives (50 µg/ml) of *Lactobacillus salivarius* subsp. *salivarius* UCC1(LM5) and *Lb. salivarius* subsp. *salivarius* UCC118(LM2) were generated to facilitate enumeration of the strains in GIT and faecal samples. Each potential probiotic strain was individually administered to Balb/c mice at a daily concentration of approximately 4.0×10^9 CFU. After 1 day of feeding, strains UCC1(LM5) and UCC118(LM2) were recovered from murine faeces at Log₁₀ 6.95 (1.18) CFU/g and Log₁₀ 6.33 (0.37) CFU/g, respectively. Interestingly, UCC118(LM2), which was originally isolated from the ileal-caecal region of the human GIT, was found to have become established in the corresponding region of the murine GIT regardless of the length of the feeding period. UCC118(LM2) was also found to persist in faeces for a period of up to 3 days following cessation of feeding. Administration of UCC1(LM5) and UCC118(LM2) did not result in any significant changes in the levels of indigenous bacteria culturable from faeces. In conclusion, human isolate *Lb. salivarius* subsp. *salivarius* UCC118(LM2) was found to effectively colonise, and survive transit through, the murine gastrointestinal tract. *Lactobacillus salivarius* subsp. *salivarius* UCC118 has been deposited at The National Collections of Industrial and Marine Bacteria (NCIMB) and accorded the accession number NCIMB40829. **Key words:** Probiotics, *Lb. salivarius*, *in vivo*, feeding trials, mouse, gastrointestinal tract, colonisation, transit.

INTRODUCTION

The gastrointestinal tract (GIT) is a complex ecosystem host to a diverse and highly evolved microbial community. The GIT provides a varied physiological environment for the micro-organisms which inhabit it, varying from acid conditions in the stomach to an alkaline pH in the small bowel. Micro-organisms are further subjected to the influences of bile juices and pancreatic secretions. In addition, the GIT is host to an active mucosal system with gut-associated lymphatic tissue (GALT) accounting for 80% of all immunoglobulin producing cells in the human body (1). The enteric flora, including greater than 500 bacterial species, comprise approximately 95% of the total number of cells in the human body and contribute significantly to

the host's resistance to infectious disease. Furthermore, changes in the composition of the intestinal flora are often associated with disease and may, in some cases, be their cause (2). While relatively early studies demonstrated the presence of bifidobacteria and lactobacilli in the faeces of breast-fed infants (3), a more recent study in which rectal and oral mucosa was sampled from 42 healthy volunteers demonstrated the presence of 17 defined lactobacilli clusters, most of which were found on mucosa from both sites (4). The largest taxa were *Lb. plantarum*, *Lb. rhamnosus* and *Lb. paracasei* isolated from 52%, 26% and 17% of the individuals, respectively. These results suggest that lactobacilli may be major colonisers of the human gastrointestinal mucosa (4).

Cocktails of various micro-organisms, particularly species of *Lactobacillus* and *Streptococcus*, have traditionally been used in fermented dairy products to promote human health. However, it was Metchnikoff in 1907 who first implied that ingested bacteria, in the form of yoghurt and other fermented foods, could beneficially affect the normal gut flora (5, 6). Currently, probiotics (defined as “live microbial food supplements which beneficially affect the host by improving the intestinal microbial balance” (7), or more broadly, as “living micro-organisms, which upon ingestion in certain numbers, exert health affects beyond inherent basic nutrition” (8)) are being employed successfully to improve the quality of animal feed provided to domestic animals (9–11). In the development of probiotic foods intended for human consumption, strains of LAB such as *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* have been most commonly used, due primarily to the perception that they are desirable members of the intestinal microflora (10, 12). However, while a number of probiotic bacteria of human origin are now being exploited commercially (e.g., *Lactobacillus rhamnosus* GG (13), *L. casei* Shirota (14), and *L. acidophilus* LA-1 (15)), many consumers, consumer organisations, and members of the scientific community are sceptical of such products and their publicised probiotic claims. The dairy-food industry is therefore under considerable pressure to scientifically validate these and new probiotic food products (16). Evidence is now being provided to support the proposed benefits of fermented products which demonstrates that LAB have both therapeutic and prophylactic properties. Indeed, several prospective studies have demonstrated the efficacy of LAB administration for both prophylactic and therapeutic use against diarrhoea in premature infants (17); new-borns (18); children (19); and the elderly (20); and in the therapy of antibiotic-related and traveller’s diarrhoea (21, 22). One of the most studied probiotic strains, *Lactobacillus rhamnosus* GG, has been shown to exert many beneficial effects including the prevention of antibiotic associated diarrhoea, flatulence, stomach and abdominal pain (21). *Lb. rhamnosus* GG has also been applied in the area of oral bacterial therapy in children presenting with both rotavirus gastro-enteritis and gastro-enteritis of unknown cause (23). Results from such studies indicate that *Lb. rhamnosus* GG may reduce duration of symptoms of mild diarrhoea and, in addition, may reduce viral excretion.

Due to the fact that the human GIT is a complex and hostile environment, it appears unlikely that a single probiotic bacterial strain will be capable of significantly influencing the microbial ecology of the host. However, for any micro-organism to effectively fulfil a prophylactic role, it must be capable of surviving and colonising this environment at least transiently. For instance, while *Lb. rhamnosus* GG is easily enumerated, it shows limited persistence in faeces after termination of feeding and was found to

cause no significant changes in lactobacilli, enterics, anaerobes or facultative bacteria over a five week study (24). Recently, however, Alander et al. (25) have shown that *Lb. rhamnosus* GG is recoverable from colonic biopsies obtained from humans administered the probiotic strain, indicating that faecal enumeration is not truly reflective of what occurs in the human gut.

In previous reports we have described the strategies used for the isolation of LAB strains from healthy human adults (26–28). For the purpose of this study, we selected two strains of *Lb. salivarius* subsp. *salivarius*, UCC1(LM5) and UCC118(LM2), which as was suggested by the participants involved in the lactic acid bacteria industrial platform (LABIP) workshop on probiotics (8), are of human origin and exhibit non-pathogenic behaviour; resistance to gastric acidity and bile toxicity; and production of potent anti-bacterial compounds effective against a wide variety of pathogens including *Bacillus*, *Listeria*, and *Staphylococcus* species. In addition, both *Lactobacillus* strains demonstrated the ability *in vitro* to adhere to the human intestinal cell-lines HT-29 and Caco-2, which have been extensively utilised in attempts to elucidate the mechanisms mediating the adherence of lactic acid bacteria to epithelial cells. In particular, the observed adherence of *Lactobacillus salivarius* UCC118 compared well with that of the well-characterised adherent strain *Lactobacillus rhamnosus* GG.

The LABIP workshop participants, amongst others, have further stated that *in vitro* assays or animal studies are useful in the preselection of (probiotic) bacterial strains (8, 10, 28). Therefore, the objectives of this study completed in mice focused upon (i) effective delivery of the probiotic micro-organisms to the GIT; (ii) evaluation of the ability of the strains to survive transit through, and possibly colonise, the murine GIT; (iii) accepting the complexity of the hostile GIT and faecal environments, development of a method of enumerating the introduced bacterial strains using conventional microbiological techniques; and (iv) assessment of their effect on the numbers of indigenous bacteria in the murine GIT and faeces.

MATERIALS AND METHODS

Bacterial strains

Lactobacillus salivarius subsp. *salivarius* strains UCC1(LM5) and UCC118(LM2) were previously isolated from the ileal-caecal region of a healthy adult human. The strains both exhibit beneficial probiotic traits such as bile tolerance in the absence of deconjugation, acid resistance, and *in vitro* adherence to epithelial cell lines (27). The bacterial strains also demonstrate *in vitro* antagonism of certain micro-organisms, including the indicator strain *Bacillus coagulans* 1761 (27). In subsequent studies, spontaneous rifampicin resistant derivatives of strains UCC1(LM5) and UCC118(LM2) were generated by plating cells, previously grown overnight in MRS (de Mann

Rogosa & Sharpe; Oxoid, Hampshire, England) broth and washed in quarter-strength Ringer's solution, on MRS agar (pH 5.5) containing 50 µg/ml rifampicin (Sigma-Aldrich Co. Ltd., Dublin, Ireland) and incubated anaerobically for 2 days at 37°C. The resulting antibiotic resistant derivatives were determined to be otherwise phenotypically identical to the parent strain. This selectable trait enabled the strain to be readily enumerated from GIT tissue specimens and following gut transit on MRS agar (pH 5.5) supplemented with rifampicin (50 µg/ml).

Animals and maintenance

Twelve week old, female mice (Balb/c), weighing between 21–28g were obtained from the biological services unit in University College Cork, Ireland. The pathogen-free animals were housed individually in plastic cages under positive air pressure with filtered air at room temperature and fed 4×10^9 cfu of lactobacilli daily for the duration of the feeding period. The bacterial strains, which were grown overnight in MRS broth and washed in quarter-strength Ringer's solution, were resuspended in skim milk (10%) and administered at a final concentration of 20% in the otherwise sterile drinking water as described by Perdigon et al. (29). Control mice received sterile milk diluted in sterile water and were maintained under identical conditions as the test group.

Enumeration of administered probiotic bacterial strains following transit through the murine GIT

Three independent trials were completed investigating the effects of transit through the murine GIT on numbers of faeces-borne UCC1(LM5) and UCC118(LM2). Each experimental group consisted of 10 mice (5 test, 5 control). On days 0, 1, 5, and 7, faecal samples were collected from each mouse. Samples were weighed and resuspended in 10 ml phosphate buffered saline (PBS). The weighed samples were then serially diluted in sterile PBS and spread-plated on MRS agar (pH 5.5) to enumerate total lactobacilli and MRS agar (pH 5.5) supplemented with rifampicin to specifically enumerate the administered *Lb. salivarius* strains. The plates were then incubated anaerobically in "Gas Pak" jars (BBL, Oxford, U.K.) with CO₂ generating kits (Anaerocult A; Merck KGaA, Darmstadt, Germany) for 48 hours at 37°C. Colonies arising on the media were counted manually and numbers calculated per gram of wet faeces. The persistence of *Lb. salivarius* strain UCC118(LM2) in the faeces of appropriately fed mice following termination of feeding was performed in duplicate. Faecal samples were collected for 4 days after cessation of feeding and bacterial numbers evaluated as described earlier. At each sampling time, a selection of the recovered lactobacilli were randomly chosen (30 colonies per agar plate) and assessed with regard to their Gram stain reaction. Their phenotypes were also assessed biochemically using API50 CHL kits (Bio-Merieux, France)

and, in the case of rifampicin-resistant bacteria (5 colonies per agar plate), their ability to produce antibacterial proteins tested using *B. coagulans* 1761 as the indicator strain as described by Tagg et al. (30). No colonies were observed on the antibiotic-containing medium when faeces from the control mice was assessed.

Evaluation of the effects of administered probiotic bacterial strains on the numbers of specific indigenous bacteria culturable from mouse faeces

The influence exerted by administered *Lb. salivarius* strains UCC1(LM5) or UCC118(LM2) on the microflora of the murine gut was investigated. Each experimental group consisted of 12 mice (6 test, 6 control). On days 0, 7, and 14, faecal samples were collected from each mouse. Faecal samples were weighed, resuspended in 10 ml PBS, serially diluted in sterile PBS, and either pour-plated or spread-plated in appropriate dilutions on appropriate media in duplicate. The following bacterial groups were enumerated: lactobacilli; bifidobacteria; enterococci; bacteroides; and coliforms. The selective media used were: MRS agar (pH 5.5); MRS agar supplemented with 0.2% lithium chloride (BDH Laboratory Supplies, Poole, England), 0.3% sodium propionate (Sigma), 0.05% cysteine hydrochloride (Sigma), and 5% sheep's blood; Slanetz and Bartley agar; Wilkins and Chalgren agar supplemented with anaerobic supplement SR108 and 5% horse blood; and Violet Red Bile Agar (VRBA) (All Oxoid unless otherwise stated). VRBA and Slanetz and Bartley plates were incubated aerobically for 24 h and 48 h at 37°C, respectively. All other plates were incubated anaerobically for 48 h at 37°C.

Enumeration of administered probiotic bacterial strains from specific segments of the murine GIT

The ability of *Lb. salivarius* strain UCC118(LM2) to establish itself within localised regions of the mouse GIT was investigated in two individual experiments. In both studies, two mice previously fed skim milk containing UCC118(LM2) for a period of 7 days were placed on a diet where they consumed a sterile skim milk and water cocktail identical to that administered to the control group. The trials were continued as described previously for a further 7 days after which time the mice were sacrificed and dissected. Segments of the ileal-caecal region and the colon were removed aseptically. The contents were removed and the tissue was washed in sterile PBS. The weight of the tissue was recorded. Tissue samples (in duplicate) were homogenised in PBS, serially diluted in PBS, and plated on MRS agar (pH 5.5) and MRS agar (pH 5.5) supplemented with rifampicin. Plates were incubated as described previously. Resulting rifampicin-resistant colonies were phenotypically assessed as described previously.

Statistical analysis

Following completion of each feeding trial, the numbers of bacteria recovered from mouse faeces and murine GIT segments were averaged and the standard deviations calculated. All values were analysed by ANOVA. Where appropriate, differences were studied using Fisher's LSD test. All analyses were performed at $P = 0.05$ level.

RESULTS

Enumeration of administered *Lactobacillus salivarius* subsp. *salivarius* strains UCC1(LM5) and UCC118(LM2) following transit through the murine GIT

Both *Lb. salivarius* strains UCC1(LM5) and UCC118(LM2) were administered (4×10^9 CFU/day) to Balb/c mice in skim milk diluted with sterile drinking water and enumerated from collected faeces due to their previously-generated resistance to rifampicin. Prior to initiation of the probiotic feeding period (Day 0), there were no rifampicin resistant bacteria detected from mouse faeces. However, immediately following cessation of feeding, all of the LAB-fed mice were found to have excreted rifampicin-resistant lactobacilli, unlike the control mice

which remained devoid of such strains. Following just one day of feeding, the probiotic-fed mice were found to excrete UCC1(LM5) or UCC118(LM2) at levels of approximately Log_{10} 6.95 (1.18) and Log_{10} 6.33 (0.37) CFU/g faeces, respectively (Figs. 1a and 1b). Indeed, throughout the 7-day feeding period the levels of faeces-borne UCC1(LM5) did not decrease below Log_{10} 6.25 (1.65) CFU/g (Fig. 1a). The numbers of UCC118(LM2) detected in faeces collected during this feeding period did not differ significantly from those of UCC1(LM5) (Fig. 1b).

In addition to determining the numbers of administered LAB present in the collected faeces, both prior to and throughout the 7-day feeding period it was observed that the number of total lactobacilli recoverable from control and test mice remained statistically similar (Figs. 1a and 1b). Recovered lactobacilli from the faeces of mice administered strain UCC1(LM5) varied between Log_{10} 8.65 (0.12) CFU/g and Log_{10} 8.82 (0.68) CFU/g compared with levels of between Log_{10} 8.85 (0.37) CFU/g and Log_{10} 8.51 (0.07) CFU/g in control mice (Fig. 1a). Similarly, during feeding of UCC118(LM2), lactobacilli were recovered at between Log_{10} 8.52 (0.56) CFU/g and Log_{10} 8.51 (0.07) CFU/g (Fig. 1b). Overall, it is evident that there is a steady

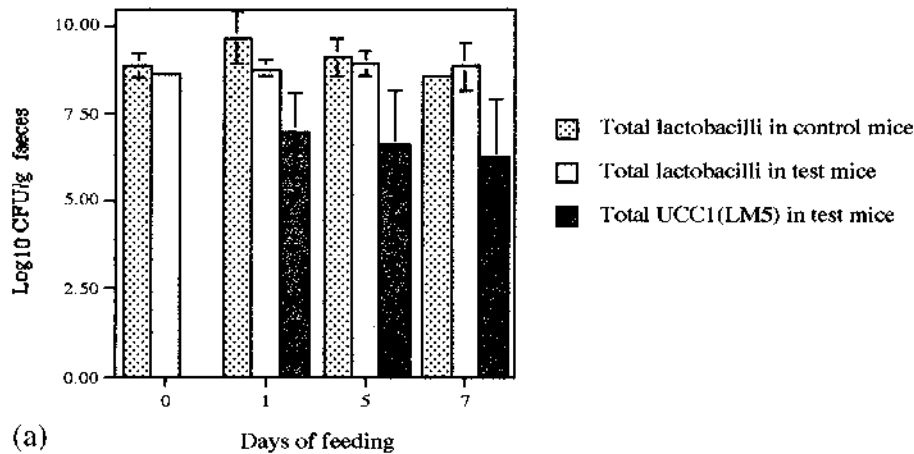


Fig. 1a. Enumeration of total lactobacilli and *Lb. salivarius* subsp. *salivarius* strain UCC1(LM5) in mice during feeding trials. Results are expressed as log_{10} CFU/g faeces and represent the average of three independent experiments performed in duplicate.

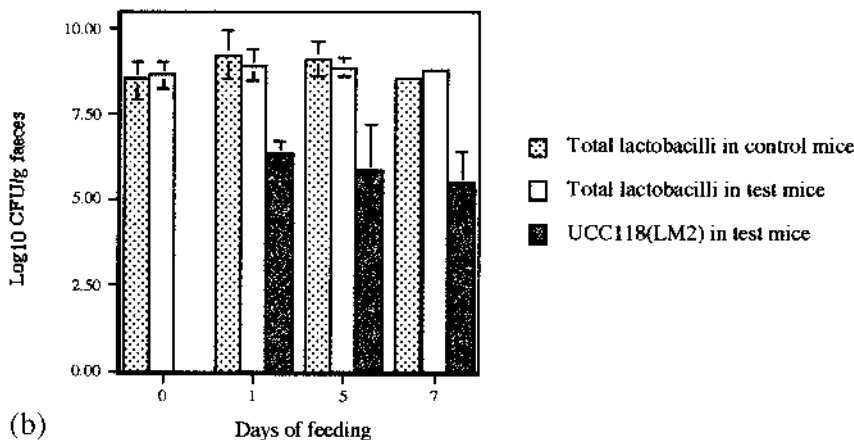


Fig. 1b. Enumeration of total lactobacilli and *Lb. salivarius* subsp. *salivarius* strain UCC118(LM2) in mice during feeding trials. Results are expressed as log_{10} CFU/g faeces and represent the average of three independent experiments performed in duplicate.

Table I

*Examination of the effects of feeding *Lb. salivarius* subsp. *salivarius* UCC1(LM5)¹ on specific culturable microbial populations in mouse faeces*

Bacterial Strains	Log ₁₀ bacterial numbers at time intervals		
	Day 0	Day 7	Day 14
Enterococci (C) ²	7.82 (0.56) ^{4, 5, 6}	8.01 (0.77)	7.71 (0.77)
Enterococci (T) ³	7.45 (0.69)	7.94 (0.65)	7.57 (0.44)
Bifidobacteria (C)	8.50 (0.84)	8.82 (0.38)	8.70 (0.37)
Bifidobacteria (T)	8.39 (0.42)	8.86 (0.33)	8.66 (0.27)
Coliforms (C)	5.57 (0.51)	6.02 (1.04)	6.39 (0.80)
Coliforms (T)	5.79 (0.43)	6.36 (1.37)	6.83 (0.83)
Bacteroides (C)	7.83 (0.52)	8.04 (0.84)	8.19 (0.42)
Bacteroides (T)	7.86 (0.42)	8.50 (0.84)	8.43 (0.32)

¹UCC1(LM5) was administered at a concentration of 4×10^9 CFU/ml.

²C: Control.

³T: Test.

⁴Figures in parentheses represent standard deviation.

⁵There were no statistical differences observed between the numbers of faeces-borne bacteria of any one type cultured from either the control or test mice at each of the sampling times.

⁶There were no statistical differences observed between the numbers of faeces-borne bacteria of any one type cultured from within either the test or control groups throughout the sampling period.

reduction in the numbers of UCC1(LM5) which account for approximately 11% of total culturable lactobacilli on day 1, 7% on day 5, and only 3% on day 7 (Fig. 1a). In similar fashion, UCC118(LM2) accounted for less than 1% of total culturable lactobacilli on days 1, 5 and 7 (Fig. 1b). In summary, while neither of the assayed *Lactobacillus* strains were found to predominate in the collected faeces, UCC1(LM5) accounted for a greater percentage of the total recoverable lactobacilli than UCC118(LM2).

At each sampling time, all of the assessed presumptive lactobacilli colonies recovered on MRS agar (pH 5.5) exhibited Gram stain reactions and API50 CHL (Bio-Merieux, France) profiles consistent with those typical of lactobacilli. In addition, the phenotypes of all of the assessed rifampicin-resistant lactobacilli were observed to be similar to those of the introduced probiotic strains. In all cases, the randomly-selected rifampicin-resistant isolates also demonstrated the ability to inhibit the growth of the *B. coagulans* 1761 indicator strain on solid media.

*Evaluation of the effects of administered *Lb. salivarius* subsp. *salivarius* strains on the numbers of specific indigenous bacteria culturable from mouse faeces*

During the period of feeding, there were no significant differences between the numbers of any of the bacterial groups recovered from the faeces of mice fed either UCC1(LM5) or UCC118(LM2) (Tables I and II). In addition, the levels of bacteria detected in faeces obtained from the control mice were statistically similar to those from the test mice administered either of the probiotic *Lb. salivarius* strains (Tables I and II). There were no statistically significant changes in the numbers of recovered faeces-borne bacteria over the 7-day feeding period (Tables I and II).

*Persistence, following cessation of feeding, of administered *Lb. salivarius* subsp. *salivarius* strain UCC118(LM2) in murine faeces*

Following cessation of feeding, faecal samples from both control and test mice were analysed for the presence of administered strain UCC118(LM2) for a further 4 days. During this period, a steady decrease (Log₁₀ 5.15 (0.79) CFU/g on day 7 decreasing to Log₁₀ 1.45 (2.05) CFU/g on day 10) was observed in the numbers of UCC118(LM2) (Fig. 2). UCC118(LM2) was no longer recoverable ($< 10^1$ CFU/g) from collected faeces four days after termination of feeding (Fig. 2). During this period, the numbers of total faeces-borne lactobacilli varied between Log₁₀ 8.05 (1.00) CFU/g on day 7 and Log₁₀ 1.39 (1.96) CFU/g on day 11 from mice which had consumed UCC118(LM2) (Fig. 2).

*Enumeration of administered probiotic bacterial strain *Lb. salivarius* subsp. *salivarius* strain UCC118(LM2) from specific segments of the murine GIT*

To determine the ability of UCC118(LM2) to establish populations within specific segments of the mouse GIT, gut tissue was removed from mice which had consumed the strain continuously for a period of 14 days and from those mice which had been administered UCC118 for 7 days followed by the implementation of a probiotic-free diet for a further 7 days. Strain UCC118(LM2) was found to effectively colonise the ileal-caecal region of the small intestine of the murine gut (Table III). This strain was originally isolated from the corresponding region of the adult human GIT. UCC118(LM2) was also found to have established itself within the colon of mice which had been

Table II

Examination of the effects of feeding *Lb. salivarius* subsp. *salivarius* UCC118(LM2)¹ on specific culturable microbial populations in mouse faeces

Bacterial Strains	Log ₁₀ of numbers of bacteria/gram of faeces		
	Day 0	Day 7	Day 14
Enterococci (C) ²	7.90 (0.63) ^{4, 5, 6}	7.68 (0.51)	7.52 (0.29)
Enterococci (T) ³	7.82 (0.34)	7.51 (0.35)	8.23 (0.58)
Bifidobacteria (C)	8.03 (0.71)	8.35 (0.39)	8.41 (0.45)
Bifidobacteria (T)	8.22 (0.82)	8.25 (0.43)	8.76 (0.29)
Coliforms (C)	5.52 (1.30)	5.18 (0.49)	6.32 (1.51)
Coliforms (T)	5.18 (0.49)	6.64 (0.74)	7.21 (0.73)
Bacteroides (C)	7.24 (0.79)	7.59 (0.40)	8.18 (0.50)
Bacteroides (T)	7.71 (0.41)	8.03 (0.67)	8.13 (0.40)

¹UCC118(LM2) was administered at a concentration of 4 × 10⁹ CFU/ml.

²C: Control.

³T: Test.

⁴Figures in parentheses represent standard deviation.

⁵There were no statistical differences observed between the numbers of faeces-borne bacteria of any one type cultured from either the control or test mice at each of the sampling times.

⁶There were no statistical differences observed between the numbers of faeces-borne bacteria of any one type cultured from within either the test or control groups throughout the sampling period.

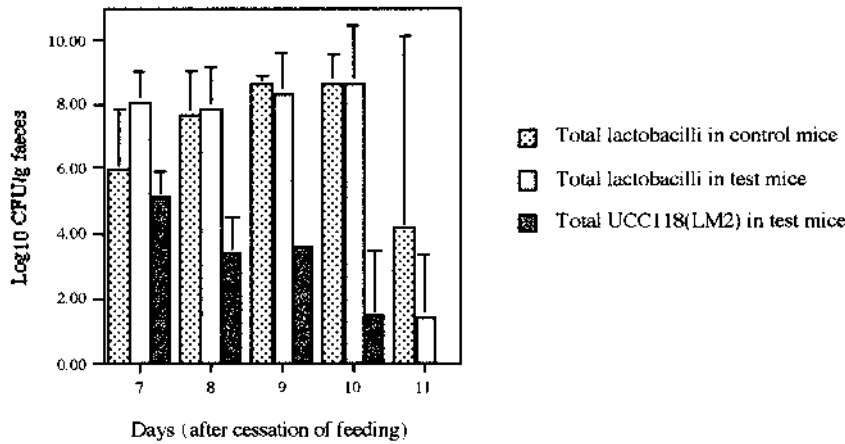


Fig. 2. Enumeration of total lactobacilli and *Lb. salivarius* subsp. *salivarius* strain UCC118(LM2) following cessation of feeding at day 7. Results are expressed as log₁₀ CFU/g faeces and represent the average of two independent experiments performed in duplicate.

Table III

Enumeration of total lactobacilli and administered *Lb. salivarius* subsp. *salivarius* strain UCC118(LM2)¹ from the colon and ileal-caecal regions of the murine GIT

	Colon		Ileal caecal	
	Mice consuming UCC118(LM2) for 14 days	Mice consuming UCC118(LM2) for 7 days	Mice consuming UCC118(LM2) for 14 days	Mice consuming UCC118(LM2) for 7 days
<i>Mouse 1</i>				
Lactobacilli	1.55 × 10 ⁸	2.09 × 10 ⁸	1.55 × 10 ⁸	2.09 × 10 ⁸
UCC118(LM2)	3.30 × 10 ⁴	ND	2.53 × 10 ⁴	5.11 × 10 ⁴
<i>Mouse 2</i>				
Lactobacilli	1.66 × 10 ⁹	1.70 × 10 ⁹	1.66 × 10 ⁹	1.70 × 10 ⁹
UCC118(LM2)	2.05 × 10 ⁵	ND	2.60 × 10 ⁴	6.80 × 10 ⁴

¹UCC118(LM2) was administered at a concentration of 4 × 10⁹ CFU/ml.

fed the probiotic strain over the full period of 14 days, but was not recovered from the colons of mice administered the strain for only 7 days (Table III). At each sampling time, a selection of the recovered rifampicin-resistant lactobacilli were randomly chosen and their phenotypes observed to be similar to those of the introduced probiotic strain.

DISCUSSION

As consumers become increasingly aware of the processes which influence their environment, health, and nutrition, scientific research has focused upon the roles that diet, stress, and modern medical practices (e.g., antibiotics and radiotherapy) may play in influencing human health. As a result, the concept of health promotion through the ingestion of functional foods, including those which act as vehicles for potentially beneficial micro-organisms, has received considerable attention (5, 16, 31). This is primarily due to the increasing incidence of illnesses which may be caused by deficient or compromised microflora, such as GIT infections, constipation, inflammatory bowel disease (IBD)-Crohn's disease and ulcerative colitis, irritable bowel syndrome (IBS), food allergies, antibiotic-induced diarrhoea, cardiovascular disease, and certain cancers (e.g., colorectal cancer) (32). The fermented dairy products containing probiotics that have been successfully manufactured and marketed incorporate strains of *Lactobacillus* and *Bifidobacteria* due to their traditional use in the dairy industry and their "generally regarded as safe" status (26). However, some of the probiotic strains currently employed in the dairy-food industry may not possess desirable traits such as being of human origin, resistance to technological processes (i.e., viability and activity in delivery vehicles), resistance to gastric acidity and bile toxicity, capacity to adhere to gut epithelial tissue, ability to colonise the host GIT, and production of antimicrobial substances (6, 11, 33).

This study describes the behaviour and effects of *Lb. salivarius* subsp. *salivarius* strains UCC1(LM5) and UCC118(LM2), which were originally isolated from the ileal-caecal region of the intestinal tract of a healthy human adult, following their administration to female mice. Previously, specific criteria were employed to determine the suitability of these strains for use as probiotics. These included the ability of the strains to survive in human gastric acid at pH 2.5 and growth at physiological concentrations of human bile (0.03% v/v) (27). Both UCC1(LM5) and UCC118(LM2), originally isolated as crypt-adhering bacteria, were previously confirmed to be strongly adhesive to the human adenocarcinoma cell lines Caco-2 and HT-29 (27).

For the purposes of this study, rifampicin-resistant derivatives of both probiotic strains were generated in order to facilitate enumeration of the strains from faeces and gut samples, and as a defined method of distinguishing the

administered *Lb. salivarius* strains from indigenous lactobacilli. The identity of the rifampicin-resistant isolates as UCC1(LM5) or UCC118(LM2) was further confirmed by assessment of their ability to antagonise the indicator strain *B. coagulans* 1761 *in vitro*. Importantly, examination of the survival of *Lb. salivarius* UCC1 and UCC118 following transit through the murine GIT indicated that rifampicin resistance is a suitable non-genetic method of enumerating introduced bacterial strains, and that skim milk proved an effective delivery vehicle. Indeed, after just one day of feeding, strains UCC1(LM5) and UCC118(LM2) were recovered from collected mouse faeces at \log_{10} 6.95 (1.18) CFU/g and \log_{10} 6.33 (0.37) CFU/g, respectively (Figs. 1a and 1b). In addition, it was observed that having successfully survived passage through the murine gut, both strains maintained their ability to inhibit the growth of *B. coagulans* 1761 *in vitro*.

A further objective of this study was to investigate the length of time administered *Lb. salivarius* UCC118(LM2) would persist in faeces following termination of feeding. UCC118(LM2) was particularly chosen for this study due to its ability to adhere strongly to epithelial cell lines *in vitro* when compared with UCC1(LM5) (Data not shown). It was found that the probiotic strain could be recovered (with no oral supplementation) from faeces for up to three days after cessation of feeding (Fig. 2). UCC118(LM2) was no longer recoverable from faeces after 4 days. These results suggest that *Lb. salivarius* strain UCC118(LM2) is capable of persistence in the mouse gut, at least for a limited period. This observation is supported by the fact that well documented probiotics such as *Lb. rhamnosus* GG have been shown to remain detectable in faeces only temporarily. In particular, a study completed by Goldin et al. (34) demonstrated that 60–80% of individuals consuming *Lb. rhamnosus* GG excreted this strain for 3–4 days, but only 33% of the population after 7 days. Therefore, it appears increasingly likely that daily administration of the preferred strain is necessary for maintenance of high levels of probiotics.

It is not difficult to understand how a single bacterial strain, introduced into an environment such as the human GIT containing greater than 500 bacterial species, may be unable to effectively compete and become established. Fonty et al. (35), suggested that the establishment of an introduced strain is governed, not only by the mode of administration of the strain, but also by the interaction of micro-organisms within the gut environment. Studies involving *Lb. rhamnosus* GG demonstrated that consumption of this strain resulted in an increase in the numbers of total lactobacilli and bifidobacteria over the feeding period (36). However, the increases observed in those specific bacterial populations did not persist beyond cessation of feeding. Throughout this study, the levels of indigenous enterococci, bifidobacteria, coliforms, and *Bacteroides* culturable from mouse faeces were not significantly modified by administration of *Lb. salivarius* UCC1(LM5) or UCC118(LM2) (Ta-

bles I and II). As UCC118 was found to significantly modify the gut microflora (enterococci, lactobacilli, bifidobacteria) of healthy volunteers in an ethically-approved human feeding trial (unpublished data), the results presented in this study suggest that the beneficial effects of probiotics on gut microflora may be species specific.

Alander et al. (25) recently reported the recovery of *Lb. rhamnosus* GG from human colonic biopsies at 7×10^4 CFU/biopsy following 12 days administration of the probiotic. While *Lb. rhamnosus* GG formed a significant proportion of the *Lactobacillus* population recovered from faeces, the strain was not found to dominate the lactobacilli isolated from tissue samples in the same manner. The results of this study further highlight the fact that microbial analysis of excreted faeces alone may be insufficient to allow claims be made regarding the influences exerted by probiotic strains on gastrointestinal microbial ecology. Ethical concerns may, however, limit the extent of colonic biopsies obtained from human subjects. Studying probiotics in animal models may prove an appropriate alternative. In this study, the ability of *Lb. salivarius* strain UCC118(LM2) to colonise the murine gastrointestinal tract was determined throughout the feeding period, immediately following ingestion of the strain for 7 days, and one week after termination of feeding. *Lb. salivarius* strain UCC118(LM2) was found to effectively colonise the ileal-caecal region of mice administered the strain for the full feeding period. However, the probiotic was also recovered from samples of this region obtained from mice fed the strain for 7 days and then placed on a probiotic-free diet for a further 7 days (Table III). Importantly, this region corresponds to the site from which it was first isolated in the healthy adult human gut. UCC118(LM2) was also found in samples removed from the colon of some mice but, interestingly, only those continuously fed the strain for the full 14 day period (Table III). The results presented here suggest that enumeration of probiotics (or other microbes) from excreted faeces may not be an accurate reflection of the microbial situation within the GIT as strains which are not recoverable from faeces may nonetheless persist in tissue.

In summary, this study describes the ability of *Lb. salivarius* subsp. *salivarius* strains UCC1(LM5) and UCC118(LM2) to survive transit through the mouse GIT following delivery in skim milk. These bacteria are shed in high numbers in faeces, retain their biochemical profiles, and maintain their ability to inhibit the growth *in vitro* of an indicator bacterial strain. More importantly, however, *Lb. salivarius* strain UCC118(LM2) proved capable of colonising the region of the murine gastrointestinal tract from which it was originally isolated in a healthy adult human. Future studies involving disease-prone, genetically modified mice and human participants may reveal the true potential of this strain for alleviation or prevention of intestinal disease, possibly through modification of the GIT microbial populations.

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