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ORIGINAL ARTICLE

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Mechanisms of Adherence of a Probiotic *Lactobacillus* Strain during and after *in vivo* Assessment in Ulcerative Colitis Patients

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In a pilot-scale, open-label study to determine the ability of well-characterized probiotic *Lactobacillus salivarius* UCC118 cells to adhere to human epithelial cells *in situ*, the bacterial strain was administered to ulcerative colitis patients at approximately 10^9 CFU/day for 12 days. Microbiological analysis of biopsy specimens demonstrated that the ingested bacteria effectively adhered to both inflamed and non-inflamed mucosa of the large bowel in significant numbers. In previous reports, we have described the ability of the lactobacilli to adhere to enterocytic epithelial cells *in vitro*. In this study, we found that the bacteria adhered at higher levels to differentiated rather than undifferentiated epithelial monolayers; and that stationary phase lactobacilli were found to adhere to eukaryotic HT-29 and Caco-2 epithelial cells at greater levels than log phase bacterial cells. Pretreatment of the *Lactobacillus* cells with proteolytic enzymes abolished attachment, indicating the potential involvement of surface/exposed protein(s) as bacterial adhesin(s). SDS-PAGE (denaturing) techniques determined that the proteolytic treatment resulted in degradation of a cell wall-associated protein of approximately 84 kDa. The proteinaceous factor was purified by both anion-exchange chromatography and by gel extraction after SDS-PAGE electrophoresis, and under *in vitro* assay conditions proved capable of adherence and significant inhibition of bacterial attachment to enterocytic epithelial cells. *Key words:* probiotic, *Lactobacillus*, adhesin, cell-borne, proteinaceous.

INTRODUCTION

In a number of intestinal disease states altered microflora, impaired gut barrier and/or intestinal inflammation offer a rationale for the effective therapeutic use of probiotic microorganisms (1). Such 'probiotics', defined as living microorganisms, which upon ingestion in certain numbers, exert health affects beyond inherent basic nutrition (2), have been assessed in human disorders such as small bowel bacterial overgrowth (3), colorectal cancer (4), diarrhoea in children (5), traveller's diarrhoea (6) and antibiotic/*Clostridium difficile*-associated diarrhoea (7, 8).

Most commonly, the effects of probiotics require the consumption of large numbers of cells which then transit, or persist within, the distinct physiological and chemical environments of the gut. Influencing factors vary from acid conditions in the stomach to an alkaline pH in the small bowel, presence of bile juices, pancreatic secretions and an active mucosal immune system (1). Proposed mechanisms through which the ingested probiotic microbes may subsequently benefit their host include the production of antimicrobial factors, competition for nutrients, degradation of toxins or eukaryotic toxin receptors and immunomodulation (1). However, of the main criteria for selecting probiotic strains, adherence to intestinal epithelia is thought to be paramount (9, 10). Indeed, adhesion to epithelial cells and/or mucus appears to mediate colonization of the gastrointestinal tract by lactobacilli and may be a prerequisite for competitive exclusion of enteropathogenic bacteria (11, 12) and immunomodulation of the host (13–16).

To understand the interactions between bacterial cells and the surrounding environment and, thereby, the probiotic traits described above, investigators have begun to clarify the taxonomy, physiology and genetic properties of probiotic bacteria (17, 18). These studies have implicated a number of factors in the attachment of probiotic bacterial cells to epithelial cells. Such factors include: passive entrapment of the bacterial cells by fimbrial cell matrix material (19), bacterial cell surface-associated lipoteichoic acid (20), proteinaceous extracellular adhesins (21) and

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bacterial cell surface-associated proteinaceous factors (22, 23).

In addition, studies in patients with Crohn's disease (24) and ulcerative colitis (25, 26) have begun to expand the scientific rationale upon which the concept of bacteriotherapy of inflammatory bowel disease is based (1, 27). Of particular relevance to this study, Gionchetti et al. (26) reported a study of 40 patients with chronic pouchitis, a long-term complication that can occur following ileal pouch-anal anastomosis for ulcerative colitis. The results were convincing – as consumption of the combinational probiotic product resulted in 85% of the test cohort maintaining remission throughout the 9-month study period compared with none of the patients in the control limb – and promote the prophylactic use of probiotic mixtures against relapse of the disorder.

In the present study, a dairy-based probiotic product (24, 28–30) was administered to ulcerative colitis patients. Subsequent collection of faecal and biopsy specimens facilitated evaluation using conventional microbiological techniques of probiotic adhesion to both inflamed and non-implicated tissue. Laboratory-based biochemical and microscopy experiments characterized the physiological and biochemical mechanisms mediating the observed adherence of the strain.

EXPERIMENTAL PROCEDURES

In vivo assessment of L. salivarius UCC118 adherence: feeding trial in ulcerative colitis patients

Twelve ulcerative colitis patients aged from 20 to 65 years were recruited to assess the adherence abilities of a spontaneous rifampicin-resistant derivative of *Lactobacillus salivarius* UCC118 (*L. salivarius* UCC118^{Rif}) within the human gastrointestinal tract. These individuals were recruited using the following exclusion criteria: no recent history of antibiotic therapy, no current drug therapy, no chronic viral illness, no known allergies and no participation in other clinical trials. In addition, candidates for the trial were excluded if they were pregnant or suffering from IgA deficiency. Informed consent was obtained from all volunteers, and the ethical committee local to Kuopio, Finland approved the study. The trial subjects consumed a fermented milk product (100 g) containing viable *L. salivarius* UCC118^{Rif} (10^9 CFU/day) each day for 12 days, and were followed up for a further period of 14 days. All candidates were asked to refrain from consumption of other known probiotic products for the duration of the study.

Production of the fermented milk product (to good manufacturing practice standards) involved inoculating pasteurized cows' milk with a spontaneous rifampicin-resistant derivative of *L. salivarius* UCC118 (*L. salivarius* UCC118^{Rif}) and a commercial strain of *Streptococcus thermophilus*. Before inoculation into milk, the *Lactobacillus* strain was grown in liquid de Mann, Rogosa and Sharpe (MRS) medium (Oxoid, UK) overnight at 37°C, centrifuged and washed twice in sterile PBS. The inoculated milk was incubated at 37°C until *Lactobacillus* numbers reached 10^8 CFU/g. The product was distributed to Finnish patients with *Lactobacillus* UCC118 levels of 10^7 CFU/g.

Biopsies and excreted faecal specimens were obtained on days 0, 7, 12, 19 and 26. Microbial analyses were performed on all biopsy specimens within 6 h of collection. Faecal specimens were stored at −20°C for 24–48 h before analysis. Each sample was weighed and resuspended in 10 ml of sterile phosphate-buffered saline (PBS). The biopsy and faecal samples were serially diluted 10-fold in sterile PBS and spread-plated in appropriate dilutions on MRS agar (de Mann Rogosa & Sharpe; Oxoid, Hampshire, UK) supplemented with rifampicin (50 μg/ml). Plates were incubated anaerobically in GasPak™ jars (BBL) with CO_2 generating kits (Anaerocult A™; Merck) for 2–5 days at 37°C. Microbial numbers were calculated as colony forming units per gram (CFU/g) of biopsy material. No colonies were observed on the antibiotic-containing medium when intestinal tissue was assessed prior to probiotic consumption. In addition, selective media were employed for the culture of total lactic acid bacteria, enterococci, coliforms and total anaerobic bacteria, respectively, as follows: MRS agar, Slanetz and Bartley agar, Violet Red Bile agar (VRBA) and plate count agar respectively (all Oxoid, UK or Difco, USA). Microbial numbers were calculated as CFU/g of wet weight faeces.

Following completion of each of the feeding studies, the numbers of bacteria recovered from faeces and biopsies 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 the $p = 0.05$ level.

Assessment of the effects of eukaryotic and prokaryotic growth phase on the adherence of L. salivarius UCC118 to epithelial cells in vitro

HT-29 enterocytic cell lines were cultured as monolayers in DMEM (Dulbecco's modified Eagle's medium; Gibco, UK) supplemented with 10% (v/v) fetal calf serum (Gibco). Cells were grown in 75 cm^2 tissue culture flasks (Costar, USA) at 37°C in a humidified atmosphere containing 5% CO_2. At 95% confluence, the monolayers were passaged by incubating with a 0.25% trypsin solution (Gibco) for 10 min at 37°C. The adhesion of the strains was examined using a modified version of a previously described method (31). Briefly, monolayers of HT-29 cells were prepared on sterile 22 mm^2 glass coverslips, which were placed in tissue culture dishes. Cells were seeded at a concentration of 4×10^4 cells/cm^2 and fed fresh medium every 2 days for a maximum of 10 days. The HT-29 monolayers were washed twice with PBS. Antibiotic-free DMEM (2 ml) and 2 ml of

bacterial suspension (containing approx. 10^9 CFU/ml log phase, stationary phase or bacterial cells inactivated by incubation at 80°C for 10 min) were added to each dish and cells were incubated for 120 min at 37°C in a humidified atmosphere containing 5% CO_2. After incubation the monolayers were washed five times with sterile PBS, fixed with methanol for 3 min, Gram stained and examined microscopically under oil immersion. For each glass coverslip monolayer, the number of adherent bacteria per 20 epithelial cells was counted in 10 microscopic fields. The mean and standard error of adherent bacteria per 20 epithelial cells was calculated. Each adhesion assay was performed in triplicate. In a second confirmatory method, following washing with PBS, adherent bacterial cells were removed by immersing the monolayers in sterile H_2O and vortex mixing. Bacteria were then enumerated by serial dilution and incubation on MRS agar. Each adhesion assay was carried out in triplicate.

When examining adhesion of strain UCC118 to differentiated HT-29 cells in culture, the monolayers were grown up for 10–14 days on the glass coverslips before the adhesion assay was performed, as described above. When assessing bacterial adherence to undifferentiated HT-29 cells, the monolayers were grown for 3–4 days before the assay.

Biochemical treatments of bacterial cells and spent culture supernatants

Lactobacillus UCC118 was propagated in MRS broth (Oxoid) at 37°C for 18 h before adherence assays were performed. Bacterial cells and spent culture supernatant were separated by centrifugation at 3000 *g* for 10 min. The bacterial cells were washed twice in quarter-strength Ringer's solution (Oxoid) and resuspended in an equal volume of MRS broth before the adhesion assay was performed, as described above. In another experiment, the bacterial cells were treated with trypsin (2.5 mg/ml; Sigma T-4665: EC 3.4.21.4) for 60 min at 37°C, centrifuged, washed twice in quarter-strength Ringer's solution and resuspended in MRS before the adhesion assay. In a third experiment, the spent culture supernatant alone was also treated with trypsin under identical conditions. Trypsin was then inactivated by the addition of 2 ml of heat-inactivated (60°C, 30 min) FCS before the supernatant was used to resuspend the previously centrifuged bacterial cells and the adherence assay was completed. To determine the involvement of carbohydrates in *L. salivarius* UCC118 adherence, bacterial cells were pre-incubated with metaperiodate (50 mM; Sigma S-1878) for 30 min at 37°C, centrifuged, washed twice and resuspended as before. Alternatively, the HT-29 monolayers were washed five times with 2 ml of the chelating agent ethylene diamine tetra-acetic acid (EDTA, 20 mM; Sigma E-1644) in PBS following addition of the bacterial cells.

Polyacrylamide gel electrophoresis of cell wall-associated proteins

Strain UCC118 was propagated in MRS broth (50 ml) at 37°C for 18 h. Both untreated and trypsin-treated bacterial cells were washed three times in quarter-strength Ringer's solution before being resuspended in 2 ml TEL solution (100 mM Tris-HCl, pH 8.0, 5 mM EDTA and 1% lysozyme) and incubated for 3 h at 37°C. Supernatant was collected following centrifugation at 3000 *g* for 10 min. The obtained cell extracts were electrophoresed on denaturing polyacrylamide gels (10% SDS) as described previously (32). Selected cell extracts post-FPLC (fast protein liquid chromatography) isolation were also electrophoresed on denaturing polyacrylamide gels (10% SDS) as described previously (32). All images were captured using a digital camera (Finepix model 2600, Fuji, Japan).

FPLC isolation and functional assessment of L. salivarius UCC118 cell-borne factors

Cell extracts (as above) were filter-sterilized and concentrated (× 20) by filtration through 'Centricon' spin columns with a 50-kDa cut-off (Amicon, USA). Concentrates were dialysed against 50 mM Tris-HCl, 0.05% (w/v) NaN_3, 0.15 M NaCl (pH 7.0) for 12 h and proteins were separated on a 'Hiload' Superdex 75 preparation grade gel filtration column (26/60 cm; Pharmacia Biotech AB, S-75182 Uppsala, Sweden) equilibrated with the same buffer. Bound proteins were eluted with a gradient between 0 and 500 mM NaCl in the same buffer. Eight-ml fractions were collected using the FPLC Gradifrac™ system (Pharmacia). Each fraction was assessed for protein content by measuring the optical density (OD) at 280 nm using a Beckman DU640 spectrophotometer and cuvettes of 1 cm path-length. Fractions found to contain proteinaceous material were assessed for the ability to influence the adherence of *L. salivarius* UCC118 to HT-29 cells when added to the monolayer prior to the adhesion assay as described above.

Assessment, exploiting fluorescent labelling, of in vitro adherence of UCC118-borne cell wall factors to epithelial cells

HT29 cells were cultured in 24-well plates at 37°C in a humidified atmosphere of 5% CO_2, in DMEM, supplemented with 10% iron-supplemented donor calf serum, 50 μg/ml gentamicin, 300 μg/ml L-glutamine and 10 mM HEPES, pH 7.4. The 'peptide' (84 kDa) was labelled with an Alexa 488 fluorescent dye as per the manufacturer's protocol (Molecular Probes, Oregon, USA). The purified fluorescent conjugate (free of any unbound fluorescent dye) was resuspended in a PBS solution and incubated with the HT29 cells for 2 h at 37°C in a humidified atmosphere of 5% CO_2.

Images of the HT29 cells were obtained 2 h after exposure (at 100 × magnification) using an inverted fluorescence microscope (Nikon TE2000-S) equipped with standard FITC filters. The peptide–Alexa 488 fluorescent conjugate PBS solution was removed from the wells of the plate and washed five times with 2 ml of PBS, with images of the HT29 cells being taken at each step.

RESULTS

Enumeration of ingested L. salivarius UCC118 following transit through the gut of ulcerative colitis patients and evaluation of adhesion to intestinal tissue

Each of the 12 recruited patients completed the study, without obvious adverse reactions. Prior to initiation of the probiotic feeding regime (day 0), there were no rifampicin-resistant bacteria detected in the faecal or biopsy specimens obtained from the patients. However, immediately following cessation of feeding (day 12), all of the individuals administered the probiotic product were found to have excreted lactobacilli resistant to the antibiotic at levels of $4.0 \times 10^3/6.3 \times 10^5$ CFU/biopsy or g wet weight faeces, respectively (Table I). These bacteria were confirmed to be *L. salivarius* UCC118 ^{Rif} on the basis of the ability to inhibit the growth of the *B. coagulans* 1761 indicator strain on solid media, previously described as being due to the excretion of a potent bacteriocin-like antimicrobial agent (33). This also confirmed general compliance with the feeding regimen. Furthermore, it was evident that *Lactobacillus* UCC118 adhered to different anatomical regions of the large bowel and, significantly, to both inflamed and non-inflamed mucosa of the gastrointestinal tract. Overall, after cessation of feeding the ingested *L. salivarius* UCC118 strain was found to represent approximately 1–2% of total recoverable lactobacilli from both biopsy and faecal samples, representing 10^3–10^5 CFU. Further microbiological analysis of faecal and biopsy samples during the 14-day follow-up period demonstrated that the UCC118 strain continued to be excreted (2×10^2 CFU/g wet weight faeces on day 19) and remained associated with intestinal mucosa (10^2 CFU on day 19) (Table I), albeit for a relatively short period. There were no significant differences observed in the numbers of lactobacilli, bifidobacteria, enterococci, bacteroides or coliforms excreted or associated with biopsy tissue as a result of the probiotic consumption (data not shown).

Table I

*Levels of L. salivarius UCC118 recovered from biopsy tissue and excreted faecal specimens collected from ulcerative colitis patients (n = 12) throughout the feeding study**

Days on which specimens were collected†	Average levels of *L. salivarius* UCC118 detected from biopsy tissue (CFU)	Average levels of *L. salivarius* UCC118 detected from excreted faeces (CFU/g wet weight)
0	0	0
7	2.0×10^2	2.5×10^5
12	4.0×10^3	6.3×10^5
19	1.0×10^2	2.0×10^2
26	ND	ND

ND, not detected.

*The trial subjects consumed a fermented milk product (100 g) containing viable *L. salivarius* UCC118^{Rif} (10^9 CFU/day) each day for 12 days, and were followed up for a further period of 14 days.

†Microbial analyses were performed on all biopsy specimens within 6 h of collection, and on all faecal samples within 48 h following frozen storage. Specimens were weighed and resuspended in sterile phosphate-buffered saline (PBS) before serial dilution and incubation on MRS agar supplemented with rifampicin (50 μg/ml).

Characterization of factors mediating attachment of L. salivarius UCC118 to epithelial cells

As expected, *Lactobacillus* UCC118 was found to adhere at significantly higher levels to differentiated versus undifferentiated HT-29 monolayers (Fig. 1a). In addition, the adhesive properties of *Lactobacillus* UCC118 were significantly greater for stationary phase cells compared with those in logarimetric phase (Fig. 1b). Heat-inactivated

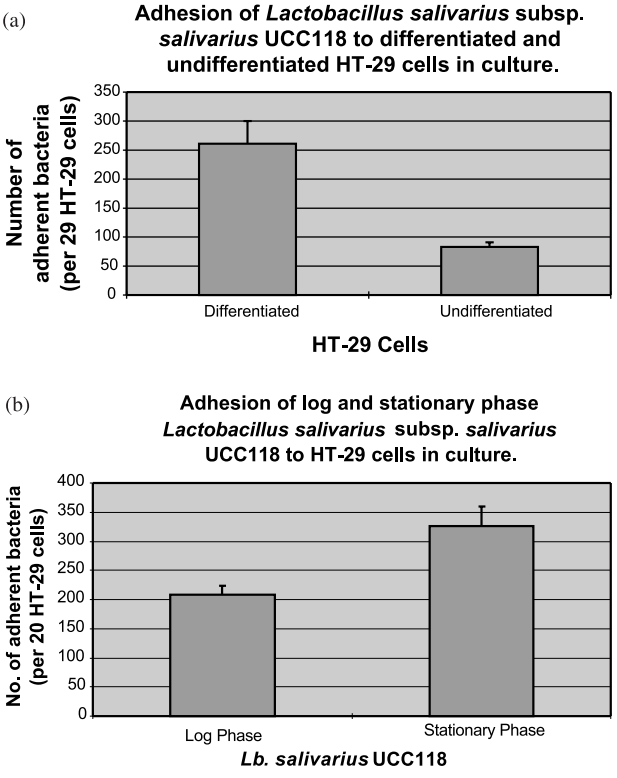


Fig. 1. Adherence of probiotic *L. salivarius* UCC118 cells to HT-29 epithelial cell monolayer. (a) It is evident that the bacterial cells adhere at a greater level when introduced onto differentiated epithelial cells. (b) The *L. salivarius* UCC118 cells adhere at a greater level when introduced into the assay during the stationary phase of bacterial growth.

UCC118 cells were also capable of attaching to the eukaryotic cells, indicating that adhesion of the probiotic strain is not intrinsically associated with ongoing biological activity (data not shown).

Washing *Lactobacillus* UCC118 cells with sterile quarter-strength Ringer's solution had no obvious effect on the ability of the lactobacilli to adhere to the HT-29 monolayers (Fig. 2). However, pre-incubation of the *Lactobacillus* cells with trypsin resulted in a significant reduction in bacterial adhesion while treatment of the culture supernatant (as described) did not (Fig. 2), suggesting that a cell-associated proteinaceous factor(s) mediates the attachment of the probiotic strain to epithelial cells. Metaperiodate treatment (to determine the involvement of carbohydrate moieties in *Lactobacillus* UCC118 adherence) and EDTA treatment (to determine calcium involvement) did not significantly impair adhesion of the bacterial cells (Fig. 2).

Determination of the nature of Lactobacillus UCC118 cell wall-associated factors

The cell wall-associated proteins of both control and trypsin-treated *Lactobacillus* UCC118 cells were isolated using a treatment designed to prevent gross contamination of the preparation by intracellular material. The resulting preparations, containing a putative cell wall-borne adhesion factor(s), were then separated using SDS-PAGE. The resulting profiles (Fig. 3) were found to differ by the absence in the trypsin-treated sample of two specific protein bands of approximately 100 kDa and 84 kDa, presumably due to proteolytic degradation (data not shown).

Partial purification and functional characterization of the Lactobacillus UCC118 cell-borne adhesin

The proteinaceous material associated with the *Lactobacillus* UCC118 cell wall was isolated by passage through a gel filtration column. The protein content of each of the collected fractions was determined and resulted in the identification of six obvious peaks (Fig. 4a). Each of these fractions, in addition to a protein-free fraction, was pre-incubated on HT-29 cells prior to the completion of an assay evaluating the adherence of *Lactobacillus* UCC118 cells to the eukaryotic cell line. Of the seven fractions tested, only one (fraction 18), demonstrated an ability to significantly influence the attachment of the introduced lactobacilli to the HT-29 cells (Fig. 4b). The observed reduction in the levels of recovered *Lactobacillus* UCC118 cells by approximately 60% indicated the presence in fraction 18 of a factor capable – possibly through competitive occupation of available sites of adherence – of impairing the attachment of *Lactobacillus* UCC118 cells (Fig. 4b).

The content of fraction 18 was subjected to SDS-PAGE and a single band of approximately 84 kDa was observed (Fig. 5a). The 84-kDa band was extracted from the SDS gel and labelled with a commercially available fluorescent tag (a control peptide of 100 kDa was similarly extracted and labelled and incorporated into the study as a control) that enabled detection of the test peptide (but not the control) attached to HT-29 cells after incubation and washing, indicating that the 84-kDa peptide is capable of adherence to eukaryotic cells (Fig. 5b).

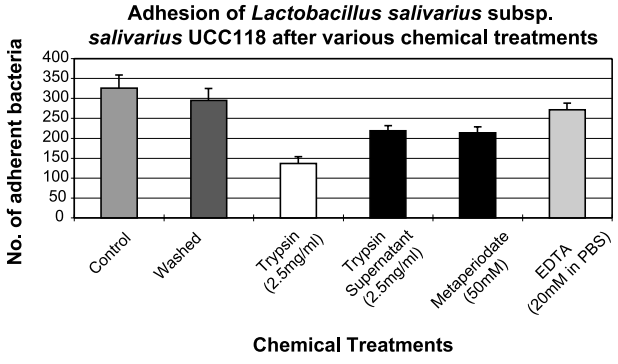


Fig. 2. Adherence of probiotic *L. salivarius* UCC118 cells to HT-29 epithelial cell monolayer. The adherence of the bacterial cells is mediated by the presence of a proteinaceous, cell-associated factor. This trait can be negatively influenced by treatment of the bacterial cells with trypsin.

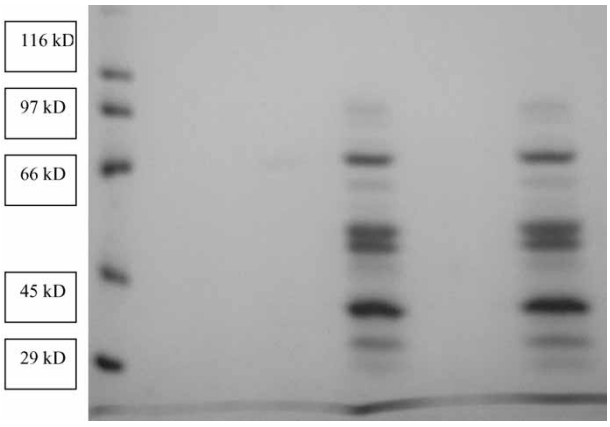


Fig. 3. Gel electrophoresis (SDS-PAGE) of *L. salivarius* UCC118 proteinaceous, cell-associated factors. The two lanes with several bands are the profiles obtained following two separate extractions.

DISCUSSION

The human gastrointestinal environment is a complex, interactive system involving many microbial species, ingested dietary components and, perhaps most importantly, the host itself. Measurement of ingested probiotic microbes from faecal material and biopsy tissue is problematic if the administered bacterial strains do not possess specific characteristics that facilitate their differentiation from indigenous, closely related, strains (34, 35). For the purposes of this study, a previously selected rifampicin-resistant

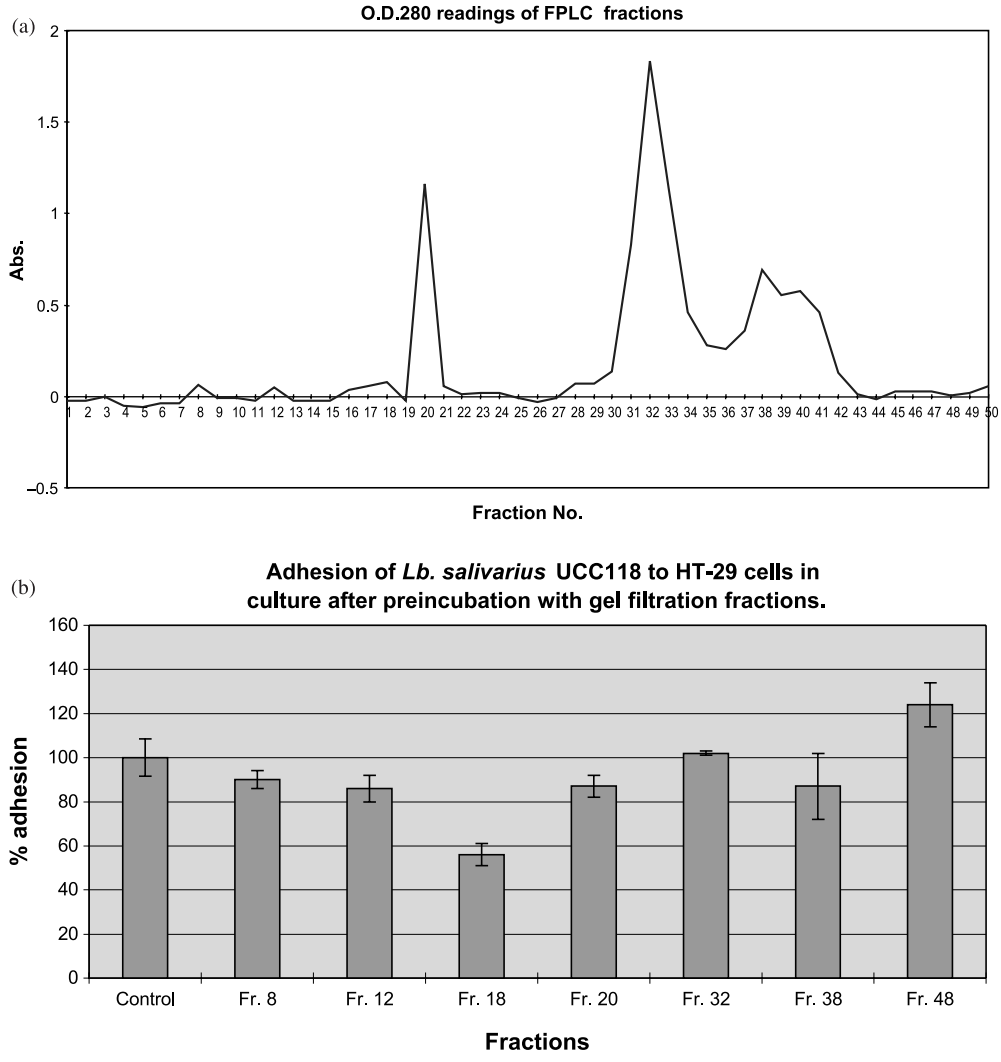


Fig. 4. (a) Purification of proteinaceous, cell-associated factors from *L. salivarius* UCC118 by gel filtration chromatography. Analysis of the protein content of the collected FPLC fractions shows obvious peaks at fractions 8, 12, 18, 20, 32 and 38. (b) Adherence of *L. salivarius* UCC118 cells to HT-29 epithelial cell monolayer. It is apparent that the adherence of the bacterial cells is significantly reduced when fraction 18 is added prior to the bacterial cells ($p = 0.049$). FPLC fractions 8, 12, 20, 32, 38 and 48 do not significantly affect adherence of *L. salivarius* UCC118 cells. Error bars represent standard deviation.

derivative of *L. salivarius* UCC118 (30) was used to facilitate enumeration of the strain from collected samples, and as a defined method of distinguishing the administered strain from indigenous lactobacilli. A confirmatory condition-specific assay was also employed based on the ability of the strain to produce a potent bacteriocin-like antimicrobial agent (33).

As in previous studies completed in healthy volunteers (36) and mild-moderately active Crohn's disease patients (24), the probiotic bacteria were administered to the ulcerative colitis patients as components of a fermented milk product. There has been unresolved speculation as to whether the health benefits associated with probiotic consumption are dependent on the microorganisms becoming long-term residents in the gut. However, in this study the introduced lactobacilli were detected at levels comparable to those observed in the previously reported studies (24, 36), and they remained recoverable, at least at low levels, for a period of 7 days after cessation of consumption. This suggests that continual ingestion of beneficial microbes will probably be required for most individuals if probiotics in their current form are to be exploited for enhancement of health.

To evaluate whether the ingested *L. salivarius* UCC118 cells simply transited the lumen of the digestive tract without any direct engagement with the host or became adherent to the mucosa, biopsy specimens were collected at each of the time points. Analysis of the data showed that the probiotic lactobacilli were capable of attaching to the mucosa (both inflamed and healthy) at detectable levels,

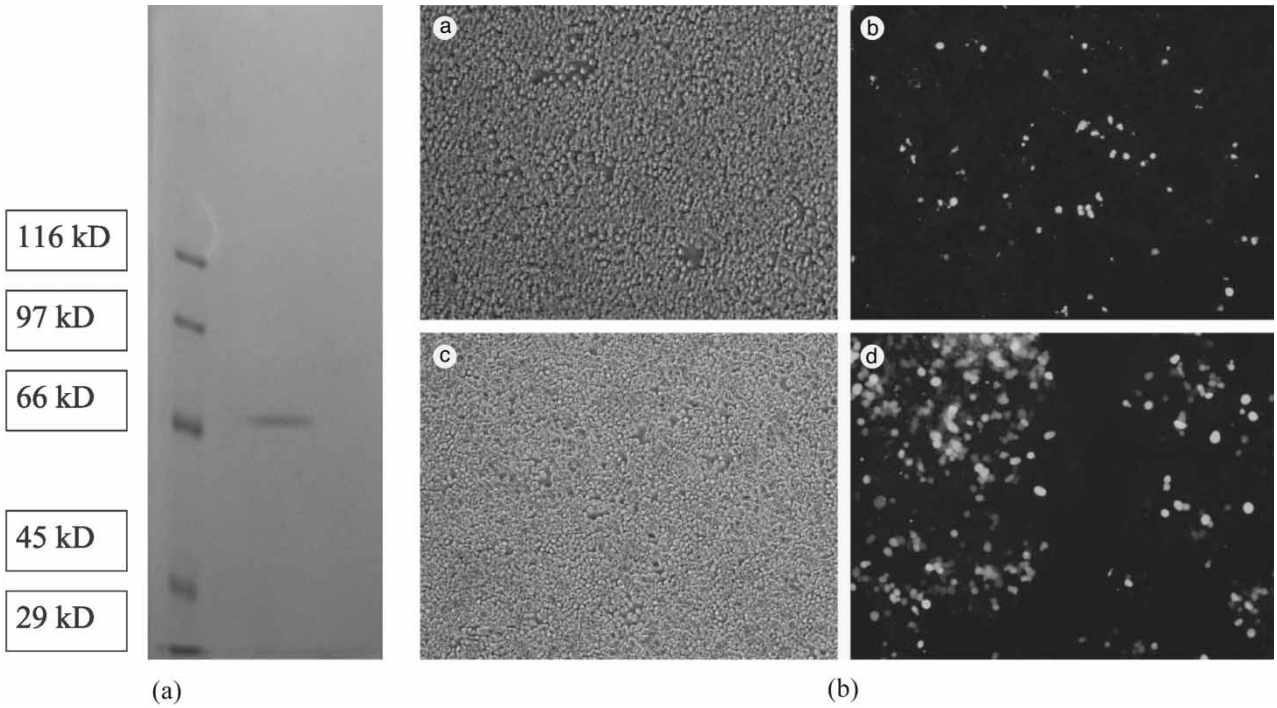


Fig. 5. (a) Gel electrophoresis (SDS-PAGE) of a *Lactobacillus* UCC118 proteinaceous, cell-associated factor present in FPLC fraction 18. This protein band corresponds with the protein band having an approximate molecular weight of 84 kDa seen in Fig. 3. (b) Bright-field and fluorescent microscopy assessing the adherence of the 84-kDa peptide to HT-29 cells. a) Bright-field view (100 ×) of eukaryotic cells after the introduction of fluorescently labelled control peptide (100 kDa); b) identical field viewed (100 ×) using GFP filter; c) bright-field view (100 ×) of eukaryotic cells after the introduction of the fluorescently labelled 84-kDa peptide; d) identical field viewed (100 ×) using GFP filter.

indicating that this strain may be suitable for development as a vehicle for targeted delivery of therapeutic agents (including proteins, enzymes and cytokines). No significant changes were observed in any of the selected bacterial species assayed for using both faecal and biopsy tissue specimens at each of the time points.

These observations prompted investigation of the adherence trait observed with this strain *in vivo*. To assess probiotic adherence, *in vitro* adhesion assays have been developed which are thought to reflect the ability of consumed bacteria to persist within the intestinal tract (13–15). Blum et al. (10) described an evaluation of methodologies recognizing that results obtained by different laboratories using identical probiotic strains but varying experimental conditions can fluctuate widely. The report presents a comparative assessment of three well-characterized probiotic bacterial strains under rigidly defined conditions; concluding that differentiated enterocytes (e.g. HT-29) and intestinal mucus are suitable for preliminary comparison of probiotic strains. In the present study, following the guidelines suggested by Blum et al. (10) it was observed that the *Lactobacillus* UCC118 cells adhered at a significantly higher level to differentiated rather than undifferentiated monolayers, while stationary phase *Lactobacillus* UCC118 cells exhibited a better capacity for adherence than log phase cells; possibly due to the proliferation or alteration of adhesion components/factors as the cells age. Chemical treatment of the probiotic lactic acid bacteria indicated that adhesion of the lactobacilli was mediated by a cell-borne trypsin-sensitive moiety.

Development of an extraction method based on a combination of Tris-HCl, EDTA and lysozyme facilitated the isolation of proteinaceous factors from the *Lactobacillus* UCC118 cell wall, and confirmation of the involvement of a distinct cell wall component through functional assays based on competitive occupation of adhesion sites and fluorescent microscopy.

In summary, this study provided evidence for the transit, and adherence to intestinal mucosa, of probiotic *Lactobacillus* UCC118 following ingestion by ulcerative colitis patients. In itself, this feeding study facilitated optimization by the Irish and Finnish research groups of standard operating procedures for the manufacture and distribution of probiotic products; experience that would prove invaluable at the initiation of large-scale clinical studies in Spanish, French, Irish and Finnish inflammatory bowel disease patients to be completed as part of the PROEU-HEALTH cluster funded by the European Commission (PROGID: QLK1-2000-00563). In addition, determination of the factors mediating the observed adherence abilities of the strain may, in time, provide the mechanisms through which molecular therapeutics are delivered *in situ*.

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