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► To cite this version:

C. Murphy, S. Murphy, F. O'Brien, M. O'Donoghue, T. Boileau, et al.. Metabolic activity of probiotics – oxalate degradation. *Veterinary Microbiology*, 2009, 136 (1-2), pp.100. 10.1016/j.vetmic.2008.10.005 . hal-00532521

HAL Id: hal-00532521

<https://hal.science/hal-00532521>

Submitted on 4 Nov 2010

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Accepted Manuscript

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PII: S0378-1135(08)00481-1
DOI: doi:10.1016/j.vetmic.2008.10.005
Reference: VETMIC 4234

To appear in: *VETMIC*

Received date: 16-5-2008
Revised date: 2-10-2008
Accepted date: 6-10-2008

Please cite this article as: Murphy, C., Murphy, S., O'Brien, F., O'Donoghue, M., Boileau, T., Sunvold, G., Reinhart, G., Kiely, B., Shanahan, F., O'Mahony, L., Metabolic activity of probiotics – oxalate degradation, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2008.10.005

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Metabolic activity of probiotics – oxalate degradation

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Keywords: Hyperoxaluria, Oxalic acid, Probiotics

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Abstract

Urinary tract stones are an important clinical problem in human and veterinary medicine. Hyperoxaluria is the single strongest promoter of kidney stone formation. The aims of the present study were to, (a) evaluate oxalate degradation by a range of *Bifidobacteria* species and *Lactobacillus* species isolated from the canine and feline gastrointestinal tract *in vitro* and, (b) to determine the impact of oxalate degradation by selected strains *in vivo*. The bacteria were grown in oxalate-containing media and their ability to degrade oxalate *in vitro* was determined using reverse-phased HPLC. *Bifidobacteria* species and *Lactobacillus* species that degraded oxalate *in vitro* and survived gastric transit were selected for further examination. The selected probiotics were fed to rats for 4 weeks. Urine was collected at week's 0, 2 and 4 and oxalate levels determined by HPLC. *In vitro* degradation was detected for 11/18 of the *Lactobacillus* species. In contrast, the capacity to degrade oxalate was not detected for any of the 13 *Bifidobacterium* species tested. *Lactobacillus animalis* 223C, *Lactobacillus murinus* 1222, *Lactobacillus animalis* 5323 and *Lactobacillus murinus* 3133 were selected for further investigation in a rat model. Urinary oxalate levels were significantly reduced ($p<0.05$) in animals fed *L. animalis* 5323 and *L. animalis* 223C but were unaltered when fed *L. murinus* 1222, *L. murinus* 3133 or placebo. Probiotic organisms vary widely in their capacity to degrade oxalate. *In vitro* degradation does not uniformly translate to an impact *in vivo*. The results have therapeutic implications and may influence the choice of probiotic, particularly in the setting of enteric hyperoxaluria.

1. Introduction

Hyperoxaluria complicated by renal tract stones is an important clinical problem in humans, particularly those with enteric hyperoxaluria secondary to conditions such as Crohn's disease (Kumar et al., 2004). In veterinary medicine, domestic animals, such as cats and dogs, are particularly prone to oxalate stones. Currently, there is no successful medical dissolution protocol, and renal stones must be removed or disrupted by physical methods. Epidemiological studies over the last decade have associated a decrease in struvite calculi with an increase in calcium oxalate renal stone formation (Hesse et al., 1998; Lekcharoensuk et al., 2001). Acidification of commercial diets to maintain urine pH between 6.0 and 6.4 reduces struvite crystal formation but increases the risk of calcium oxalate formation in companion animals (Buffington and Chew, 1996). Oxalic acid and its salts are widely distributed in dry commercially prepared dog food (Hodgkinson, 1977; Stevenson et al., 2003). Increased dietary oxalate results in increased urinary oxalate and calcium oxalate relative supersaturation in healthy adult dogs (Stevenson et al., 2003).

While some components of the enteric bacterial flora, (such as *Oxalobacter formigenes*) have oxalate degrading capacity, these organisms are not uniformly present in all animals (Allison et al., 1986; Sidhu et al., 2001). However, dietary supplementation with probiotics has emerged as a potential strategy for increasing the degradation of dietary oxalate (Campieri et al., 2001; Weese et al., 2004). Therefore, the purpose of our study was to screen a range of *Lactobacillus* species and *Bifidobacteria* species derived from the feline and canine gastrointestinal tract for oxalate degradation capacity *in vitro* and then to determine the impact of feeding such strains on urinary oxalate excretion *in vivo*.

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70 **2. Materials and methods**71 *2.1 Probiotic stain isolation*

72 The small intestine, caecum or colon of cats and dogs were dissected post mortem
 73 and the removed tissue washed in Ringers solution (Oxoid, Basingstoke, Hampshire, UK)
 74 to remove loosely adherent bacteria. The tissue was vortexed and homogenised in
 75 Ringers solution to select adherent bacteria. The supernatants from the wash and vortex
 76 steps were plated on de Man, Rogosa, Sharpe (MRS) agar (Oxoid, Basingstoke,
 77 Hampshire, UK) supplemented with 20 µg/ml vancomycin (Sigma-Aldrich Chemie, St.
 78 Louis, MO, USA) and Wilkins Chalgren Agar (Oxoid, Basingstoke, Hampshire, UK)
 79 supplemented with 50 µg/ml mupirocin (Oxoid, Basingstoke, Hampshire, UK) for
 80 *Lactobacillus* species and *Bifidobacteria* species, respectively. The plates were incubated
 81 at 37°C in an anaerobic environment for 72 h. Isolated colonies were re-streaked to
 82 ensure purity. Isolates from MRS agar + vancomycin plates were re-streaked on MRS
 83 agar and isolates from Wilkins Chalgren Agar + mupirocin were re-streaked on
 84 Reinforced Clostridia Agar (RCA: Oxoid, Basingstoke, Hampshire, UK) supplemented
 85 with 0.05% (v/v) L-cysteine hydrochloride (Sigma-Aldrich Chemie, St. Louis, MO,
 86 USA) for the purification of *Lactobacillus* species and *Bifidobacteria* species,
 87 respectively. Following purification, single strain cultures were identified on the basis of
 88 colony morphology, gram reaction, catalase activity and the Fructose-6-phosphate
 89 phosphoketolase assay. Gram-positive, catalase negative rods were genetically
 90 characterised using primers specific for the 16 S intergenic spacer region and
 91 *Lactobacillus* species and *Bifidobacteria* species isolates were further examined.

Lactobacillus species strains were routinely cultured in MRS broth at 37°C in an anaerobic environment for 24 h. *Bifidobacteria* species isolates were routinely cultured in MRS broth supplemented with 0.05% (v/v) L-cysteine hydrochloride and incubated at 37°C in an anaerobic environment for 48 h. *Lactobacillus* species and *Bifidobacteria* species stocks are maintained in 40% glycerol at -80° C (Alimentary Health Ltd., National University of Ireland, Cork, Ireland).

2.2 Assaying *Lactobacillus* and *Bifidobacteria* isolates for growth in ammonium oxalate media and determining oxalate-degrading capability

The procedure for the determination of oxalate-degrading capacity of probiotic isolates was based on the method previously described by Campieri et al. (2001). Briefly, 5 ml of filtered sterilised ammonium oxalate solution [20 mM/l ammonium oxalate and 40 g/l dextrose (Roquette, Lestrem, France)] was added to 5 ml of base media (Protease peptone 20 g/l, yeast extract 10 g/l, Tween 80 2 ml/l, KH₂PO₄ 4 g/l, NA acetate 10 g/l, di-Ammonium-hydrogen-citrate 4 g/l, MgSO₄·7H₂O 0.1 g/l and MnSO₄ 0.1 g/l). All reagents were supplied by either Sigma-Aldrich (St. Louis, MO, USA) or BDH Laboratory supplies, Poole, UK; unless otherwise stated. Culture broths were inoculated at 2% into base media and base media containing 20 mM ammonium oxalate. The base media was supplemented with 0.05% (v/v) L-cysteine hydrochloride when inoculating *Bifidobacteria* species and all cultures were incubated anaerobically at 37°C for 48 h. A media control (ammonium oxalate base media) was prepared as above, but without the inoculation of bacteria. Optical density (600 nm) and plate counts (colony forming units/ml) were performed to determine growth of each strain in ammonium oxalate base

media, which was compared to growth in base media. Ammonium oxalate base media cultures and the media control were centrifuged at 3000 rpm for 10 min and the supernatants filter sterilised using 0.45 μ M filters (Sartorius AG, Goettingen, Germany). The culture filtrates were stored at 4°C until plate counts were recorded and HPLC analysis was performed on strains that grew in 20 mM ammonium oxalate base media.

2.3 Chemicals and materials for HPLC

All chemicals were of spectral or analytical grade. Unless otherwise stated, all chemicals employed were obtained from Sigma-Aldrich (St. Louis, MO, USA) or BDH Laboratory supplies, Poole, UK. HPLC grade water (Reagecon, Shannon, Ireland) was utilised throughout the experiments. The procedure for the determination of oxalic acid in samples by HPLC was based on the method previously described by Khaskhali et al. (1996). The mobile phase was composed of 0.25% potassium dihydrogen phosphate and 0.0025 M tetrabutylammonium hydrogensulphate, buffered at pH 2.0 with orthophosphoric acid. The mobile phase was filtered through a 0.2 μ m nylon membrane. Aqueous oxalic acid standards were prepared in the range 0.02-20 mM. These solutions were stable for 3 months at 4°C.

2.4 Apparatus and chromatographic conditions

Chromatographic analysis was performed using a Spectraseries 100 (Thermoseparation Products, Minnesota, USA) with a chromjet integrator, UV detector and a Synergi Hydro-RP column, 4 μ m, 250 x 4.6 mm I.D. (Phenomenex, Cheshire, UK). The analytic column was routinely cleaned by rinsing the column with: 94% water/5%

acetonitrile, tetrahydrofluran, 95% acetonitrile/5% water and mobile phase for 20 min each. The column was purged by pumping the mobile phase at 4 ml/min for 3 min and equilibrated by pumping the mobile phase to waste. The detector wavelength was fixed at 210 nm. The total cycle time was 35 min with 20 µl injections from each sample. At the end of each run, acetonitrile: HPLC-grade water (65:35) was pumped through the column for 15 min prior to storage.

2.5 Preparation of filtrate samples

20 mM, 15 mM, 10 mM, 5 mM and 2 mM ammonium oxalate standards were prepared from 200 mM ammonium oxalate stock solution. All filtrates and standards were diluted 1:50 in mobile phase and analysed using HPLC.

2.6 Survival in a low pH environment.

Probiotic strains must be capable of resisting the effects of a low pH environment. Bacterial cells were harvested from overnight cultures, washed twice in phosphate buffer (pH 6.5) and resuspended in the MRS broth adjusted with 1 N HCl to pH 2.5. The cells were incubated anaerobically @ 37°C and their survival measured at intervals of 0, 30, 60, 120, 180, 240 and 360 min using the plate count method.

2.7 Resistance to bile salts

Resistance to bile was examined using MRS agar plates supplemented with 0.5, 1.0 and 5.0 % (w/v) porcine bile (Sigma-Aldrich Chemie, St. Louis, MO, USA). *Lactobacillus* species probiotics were inoculated into MRS broth and incubated at 37°C

under anaerobic conditions for 24 h. Strains were spot inoculated (10µl) onto the various concentrations of porcine bile plates and incubated at 37°C under anaerobic conditions for 48 h. The growth rate on porcine bile plates were compared to the growth rate on MRS agar plates and recorded.

2.8 Tolerance to freeze drying process and stability

The probiotic strains were grown overnight in MRS broth, centrifuged and resuspended in cryoprotectant (18% reconstituted skim milk, 2 % sucrose). The mixtures were then frozen at -20°C for 24 Hrs and then freeze dried for another 24 Hrs. The mixtures were freeze-dried at a vacuum pressure of 133×10^{-3} mBar with a condenser temperature of -53°C. All strains were examined for stability to freeze-drying and their shelf life at room temperature was assessed for one month post-processing by MRS plate counting techniques.

2.9 Generation of spontaneous rifampicin-resistant variants of isolated probiotics

Selected probiotics were streaked onto MRS agar for *Lactobacillus* species isolates and RCA supplemented with 0.05% L-cysteine hydrochloride for *Bifidobacteria* species isolates. All isolates were incubated at 37°C in an anaerobic environment for 48 h. Isolates were sub-cultured onto appropriate agar plates containing 100 µg/ml rifampicin and incubated at 37°C in an anaerobic environment for 72 h. Spontaneous rifampicin resistant variants (Rif^R) were stocked in 40% glycerol (Sigma-Aldrich Chemie, St. Louis, MO, USA), stored at -80 °C and checked for their continuous resistance to 100 µg/ml rifampicin by restreaking onto appropriate agar plates containing

100 µg/ml rifampicin and incubated at 37°C in an anaerobic environment for 48 h.

Growth curves of isolates and Rif^R isolates were performed to ensure the growth rate was not altered.

2.10 *In vivo gastric transit of selected probiotic isolates*

15 female Spague-Dawley rats of similar age and weight were enrolled in the study. Freeze dried Rif^R probiotic powders were resuspended in an appropriate volume of water to ensure a dose of $\sim 9.8 \times 10^9$ colony-forming units (cfu) for *L. animalis* 223C, *L. murinus* 1222, *L. animalis* 5323 and *L. murinus* 3133 or 0 cfu control freeze dried product for the placebo group. The resuspended powders were administered, ad libitum, for 6 days (n=3 animals per group). Rats were weighted daily and the volume of probiotic consumed was calculated daily. Rat faecal pellets were collected prior to feeding (Day 0) and on Days 1, 3 and 6 (post probiotic feeding). All faecal pellets were weighed and resuspended in 1 ml Ringers (Oxoid, Basingstoke, Hampshire, UK). The colony forming units/g was determined by plating onto MRS agar containing 100 µg/ml rifampicin, in order to facilitate uncomplicated identification of the freeze dried Rif^R probiotics from all other Lactobacilli.

2.11 *In vivo urinary oxalate levels using selected probiotics*

30 female Sprague-Dawley rats of similar age and weight were enrolled in the study. Freeze dried probiotic powders were resuspended in an appropriate volume of water to ensure a dose of $\sim 2 \times 10^9$ cfu for *L. animalis* 223C, *L. murinus* 1222, *L. animalis* 5323 and *L. murinus* 3133 or 0 cfu control freeze dried product for the placebo group.

The resuspended powders were administered, ad libitum, for 4 weeks (n=6 animals per group) Rats were weighed weekly and urine samples were obtained on Weeks 0, 2 and 4 by placing the animals in metabolic cages for a 24 h period.

2.12 Preparation of urine samples

10 ml of a 24 hour sample was obtained from the metabolic cage and placed in polyethylene bottles to which 10 ml of 6 *M* hydrochloric acid was added as a preservative. Deproteinisation of the samples was performed at ambient temperature by mixing a homogeneous urine sample (10 ml) from each collection with 0.5 g crystalline sulfosalicylic acid and after 10 min filtering the mixture through a 0.45 µm Minisart filter (Khaskhali et al., 1996).

2.13 Statistical analysis

Statistical analysis of the in vitro results was performed using a paired student t-tests. Changes in rat urinary oxalate excretion levels over time were assessed using a one-way analysis of variance (ANOVA) with replicates.

3. Results

3.1 *In vitro* growth and oxalate degradation by probiotics of canine and feline origin.

Thirteen *Bifidobacteria* species and 18 *Lactobacillus* species were included in the *in vitro* assessment, which were identified using 16S intergenic spacer sequencing. **These strains included 11 *B. longum* strains (feline-derived), 1 *B. globosum* strain (canine-derived), 1 *B. animalis* strain (canine-derived), 1 *L. acidophilus* strain (feline-derived), 5 *L. reuteri* strains (feline-derived), 8 *L. animalis* strains (7 canine-derived & 1 feline-derived), 1 *L. salivarius* strain (canine-derived) and 3 *L. murinus* strains (canine-derived).** All selected isolates grew in the presence of 20 mM ammonium oxalate illustrating that oxalate at this concentration is not toxic to LAB. The average cfu/ml of isolates, grown in the presence of 20 mM ammonium oxalate, was 2.3×10^8 cfu/ml. This was comparable to growth of isolates in base media. Supernatants from isolates were subsequently analysed using HPLC. A media control (base media + 20 mM ammonium oxalate) was included in order to provide a 20 mM ammonium oxalate standard.

The ability of Lactic Acid Bacteria (LAB) to degrade oxalate was strain dependant. No oxalate degradation was detected for any of the *Bifidobacterium* species isolates when compared to the 20 mM ammonium oxalate media control (Fig. 1). Oxalate degradation was detected for 11/18 (61%) of the *Lactobacillus* species when compared to the ammonium oxalate media control (Fig. 2). *L. acidophilus*, *L. reuteri* and *L. salivarius* isolates did not demonstrate oxalate degradation, but *L. animalis* and *L. murinus* isolates demonstrated significant oxalate degradation. Two representative isolates from *L. animalis* and two representative isolates from the *L. murinus* group were selected for further examination in an *in vivo* rat model. Mean rate of *in vitro* oxalate

degradation for the selected strains was 0.15 mM/h (*L. animalis* 223C – **feline isolate**), 0.15 mM/h (*L. murinus* 1222 – **canine isolate**), 0.14 mM/h (*L. animalis* 5323 – **canine isolate**) and 0.09 mM/h (*L. murinus* 3133 – **canine isolate**).

3.2 Assessment of gastric transit of probiotic bacteria in vitro

Prior to reaching the intestinal tract, probiotic bacteria must first survive transit through the stomach, which involves survival to stomach and bile acids. The survival of selected strains to a low pH environment was assessed by adding approximately 10^8 cfu/ml of *L. animalis* 223C, *L. murinus* 1222, *L. animalis* 5323 and *L. murinus* 3133 to acidified MRS broth, pH 2.5. The results indicate that all selected probiotic strains have the potential to successfully transit the human stomach, as strains were viable after 360 minutes in a low pH environment and the loss of viability was <1.5 logs (Fig 3).

The survival of probiotic strains upon exposure to deconjugated porcine bile was examined using MRS agar plates supplemented with various concentrations of bile. All selected strains survive up to 5.0 % bile acid (Table 1).

3.3 Stability of bacterial strains following the freeze-drying process

The putative probiotic strains were examined for their stability, following the freeze-drying process, for 1 month at room temperature. *L. animalis* 223C, *L. murinus* 1222, *L. animalis* 5323 and *L. murinus* 3133 remained at high numbers post freeze-drying and demonstrated no loss of activity during storage at room temperature (Fig 4).

3.4 In vivo gastric transit of selected probiotic isolates

Changes in rat weight were monitored daily during the gastric transit feeding trial. No significant changes in body weight were detected for the duration of the trial. The volume of Rif^R probiotic consumed ad libitum was recorded and the dose of Rif^R probiotic consumed was calculated based on the dose of freeze-dried probiotic supplied (Table 2). The average dose of probiotic consumed/day was 9.8×10^9 CFU. The consumed probiotics survived gastric transit in this rat model (Fig 5). Prior to feeding probiotics (Day 0), no Rif^R probiotics were detected on culture plates. This baseline ensures the selectivity of the agar plates containing 100 µg/ml rifampicin. The Rif^R probiotics were detected in faeces from all mice in the probiotic group within 1 day of feeding. During the 6 day feeding study, the Rif^R probiotics were recovered at approximately 4.6×10^9 bacteria per gram of faeces. Rif^R probiotics were not cultivated from any of the rats in the placebo group. The amount of Rif^R probiotic consumed/day is equivalent to the gastric transit of the probiotics/day. No significant difference was observed between groups fed different probiotics or between transit levels on Day 1, 3 or 6.

3.5 *In vivo* oxalate degradation of selected probiotics in a rat model.

Sprague-Dawley rats (n=6/group) received 2×10^9 cfu/day of *L. animalis* 223C, *L. murinus* 1222, *L. animalis* 5323 and *L. murinus* 3133 or placebo. During the study, 24 h urine specimens were obtained on Week 0, Week 2 and Week 4 by placing the rats in metabolic cages. **The mean urinary output per rat was 14.3mls over the 24 hours (range 10.5 – 21.2mls).** Rat weights were monitored for the duration of the study, and demonstrated no significant difference when compared to the placebo control (Table 3).

Fig. 6 illustrates the trial results with urinary oxalate levels expressed as μM oxalate over a 24 hour period. Urinary oxalate levels remained constant in the first group of rats (not receiving a probiotic supplement). In contrast, rats consuming the probiotic strains *L. animalis* 223C and *L. animalis* 5323 had decreased urinary oxalate excretion. Rats consuming *L. murinus* 1222 and *L. murinus* 3133 did not have decreased urinary oxalate excretion.

4. Discussion

The results of this study show that **some strains of *Lactobacillus*** but not *Bifidobacteria* species degrade oxalate *in vitro* and reduce urinary oxalate excretion *in vivo*. Several *L. animalis* and *L. murinus* isolates degrade ammonium oxalate *in vitro* while four strains were selected for inclusion in the animal study, 2 representatives from the *L. animalis* group (*L. animalis* 223C and *L. animalis* 5323) and 2 representatives from the *L. murinus* group (*L. murinus* 1222 and *L. murinus* 3133). Both *L. animalis* strains (*L. animalis* 223C and *L. animalis* 5323) reduced oxalate excretion in rats. All 4 selected strains survived gastric transit.

Previous studies have demonstrated oxalate degradation by *O. formigenes*, a gram negative, anaerobic bacterium that inhabits the gastrointestinal tracts of humans and mammals (Allison et al., 1986; Dawson et al., 1980). The presence of *O. formigenes* has been shown to reverse hyperoxaluria in a rat model and reduce urinary oxalate excretion in humans (Duncan et al., 2002; Sidhu et al., 2001). It has been suggested that the absence of *O. formigenes* in the gastrointestinal tract correlates with the number of recurrences of oxalate stone disease (Sidhu et al., 1999). However, the establishment of

O. formigenes in a rat model was transient and the faecal population of *O. formigenes* declined below the detectable limit once rats were placed on a normal diet (Sidhu et al., 2001). Difficult isolation and transient colonisation of *O. formigenes* have resulted in investigators screening for alternative oxalate-degrading bacteria in the intestine, such as LAB (Campieri et al., 2001; Hokama et al., 2000; Hokama et al., 2005). *P. rettgeri* and *E. faecalis* appear to have a mechanism of oxalate degradation similar to *O. formigenes*, but they were unable to maintain their oxalate degrading ability when subcultured into nutrient rich medium (Hokama et al., 2000; Hokama et al., 2005). We have shown, using *in vitro* and *in vivo* models, that certain probiotics offer a therapeutic strategy to reducing urinary oxalate excretion.

All four candidate strains tested degraded oxalate *in vitro*, but only two of these strains degraded oxalate *in vivo*. It is unlikely that the inability of *L. murinus* 1222 and *L. murinus* 3133 to degrade oxalate *in vivo* could be attributed to the physiological aspects of the intestinal tract (gastric acidity, peristalsis, bile acids etc.) and the anti-microbial defence mechanisms (adhesion, colonisation, nutrient competition etc.), as all four strains transited the gut in equivalent amounts. Rather, the *L. animalis* and *L. murinus* strains may interact with the host in a strain specific manner such as that demonstrated for probiotic adherence to intestinal tissue and mucus (Ouweland et al., 1999). In addition, the utilisation of oxalate as a substrate for *L. murinus* *in vivo* may not be allowable at a genetic level due to phenomena such as quorum sensing. This highlights the importance of carefully selecting strains using *in vitro* characteristics, in addition to using animal models to observe the biological impact *in vivo*. **It is unlikely that the original source of these strains has a significant impact on the excretion of oxalate in the rat studies**

as one of the successful strains was canine-derived (*L. animalis* 5323) while the other was feline-derived (*L. animalis* 223C).

Our results suggest considerable variability in the ability of probiotics to degrade oxalate, both *in vitro* and *in vivo*. We detected oxalate degradation for 61% of the *Lactobacillus* species examined *in vitro*. In contrast, *Bifidobacterium* species appears not to possess the mechanism of oxalate degradation demonstrated by *Lactobacillus* spp when examined *in vitro*. Weese et al. (2004) also reported considerable variation in oxalate degradation by different probiotics *in vitro*. They reported a mean oxalate degradation of 17.7 % for 37 LAB, but they did not further identify the strains. Campieri et al. (2001) previously reported variable *in vitro* oxalate degradation with *L. acidophilus*, *L. plantarum*, *L. brevis*, *Streptococcus thermophilus* and *B. infantis*. They demonstrated little or no oxalate degradation in *L. plantarum* and *L. brevis*, but *L. acidophilus*, *S. thermophilus* and *B. infantis* degraded oxalate. However, the level of *in vitro* oxalate degradation was low, with degradation of 5.26% of 10 mM/l ammonium oxalate and 2.18% of 20 mM/l ammonium oxalate and *in vivo* degradation was assessed in a mixture of freeze-dried LAB (*L. acidophilus*, *L. plantarum*, *L. brevis*, *S. thermophilus*, *B. infantis*). Why only some probiotics strains degrade oxalate remains unclear, fuelling a desire to better understand the mechanism of oxalate degradation in probiotics. *O. formigenes* has two oxalate degrading enzymes, oxalyl-coenzyme A decarboxylase (65 kDa) and formyl-coenzyme A transferase (48 kDa) (Kodoma et al., 2002). While these oxalate degrading enzymes have been found in *Providencia rettgeri* and *Enterococcus faecalis*, it is unknown if these enzymes have been found in LAB (Hokama et al. 2005; Hokama et al. 2000).

The detected oxalate degradation in this study appears to be interspecies dependent, with *L. animalis* and *L. murinus* degrading oxalate *in vitro* and *L. acidophilus*, *L. reuteri* and *L. salivarius* demonstrating no oxalate degradation *in vitro*. Indeed, only *L. animalis* strains and not *L. murinus* strains degraded oxalate *in vivo*. Other studies have demonstrated considerable interspecies variation in metabolic activity; in particular the ability to produce the health-promoting fatty acid conjugated linoleic acid (CLA) from free linoleic acid (Coakley et al., 2003). They demonstrated considerable interspecies variation, with *B. breve* and *B. dentium* being the most efficient CLA producers.

5. Conclusion

We have highlighted the metabolic potential of probiotics by examining one specific metabolite, but mining the gut microbiota for further health promoting effects is a viable option for future dietary management strategies of specific metabolic symptoms or dysfunction. Future studies should also consider the development of an effective oxalate degrading synbiotic (probiotic + prebiotic) by tailoring a prebiotic towards the specific organism and investigating this combination using *in vitro* and *in vivo* studies (Weese et al., 2004). Given that all rats tolerated the probiotic treatment well and strains *L. animalis* 223C and *L. animalis* 5323 in particular demonstrated superior oxalate degradative capability, these strains are being further investigated as a probiotic food supplement for the prevention and treatment of hyperoxaluria and renal stone formation.

Acknowledgements

The authors are supported in part by Science Foundation Ireland in the form of a centre grant (Alimentary Pharmabiotic Centre), by the Health Research Board (HRB) of

Ireland, the Higher Education Authority (HEA) of Ireland, and the European Union
(PROGID QLK-2000-00563).

Disclosures

Alimentary Health is a multi-departmental university campus-based research
company, which investigates host-flora interactions. The content of this article was
neither influenced nor constrained by this fact.

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Table 1. Resistance of putative probiotic strains to porcine bile acids. Probiotic strains were streaked onto MRS agar supplemented with porcine bile at 0.5, 1.0 and 5.0% (w/v). Plates are incubated @ 37°C under anaerobic conditions and growth was recorded after 24-48 h. **Survival is illustrated as the mean percent of control (n=3; mean +/- SD).**

Table 2. Quantity of freeze-dried probiotic consumed ad libitum/day by each group (n=3). The average dose of probiotics consumed /day was 9.8×10^9 CFU. **Doses are illustrated as the mean dose/group +/- SD.**

Table 3. Animal weights for the placebo and test groups are illustrated over the 4 week feeding study. Body weight was not significantly influenced (compared to placebo) by feeding probiotics to the animals. Results are illustrated as mean (grams) per group (n=6) +/- SD.

Fig. 1. Lack of ammonium oxalate degradation by strains of *Bifidobacterium* species was observed *in vitro*. No significant difference ($p > 0.05$) was observed when compared to the ammonium oxalate media control. The species examined were 11 *B. longum*, 1 *B. globosum* and 1 *B. animalis*. **Results are expressed as mean \pm SD.**

Fig. 2. Degradation of ammonium oxalate by strains of *Lactobacillus* species *in vitro*. No significant difference ($p > 0.05$) was observed for 7 of the strains (*L. acidophilus*, *L. reuteri*, *L. salivarius*). 11/18 strains (*L. animalis*, *L. murinus*) demonstrated significant oxalate degradation ($p < 0.05$) when compared to the ammonium oxalate media control. The detected oxalate degradation appears to be species dependent, with *L. animalis* and *L. murinus* degrading oxalate and *L. acidophilus*, *L. reuteri* and *L. salivarius* demonstrating no oxalate degradation *in vitro*. **Results are expressed as mean \pm SD.**

* $p < 0.05$ versus control

Fig. 3. Survival of selected probiotics in a low pH environment. Bacterial cells (approximately 10^8 cfu/ml) are resuspended into MRS broth adjusted with 1 N HCl to pH 2.5. Survival was measured at intervals of 0, 30, 60, 120, 180 and 360 min using the plate count method. **Results are expressed as mean \pm SD.**

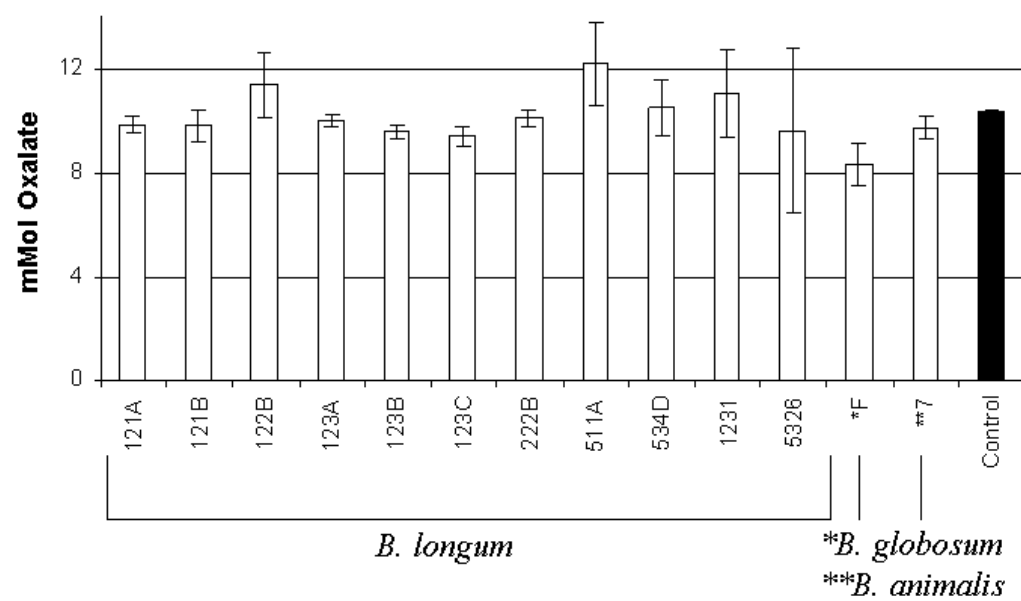
Fig. 4. Stability of putative probiotic strains during storage for 1 month at room temperature. Selected probiotic strains were examined for their stability to freeze-drying and their shelf life at room temperature for one month was assessed following the process

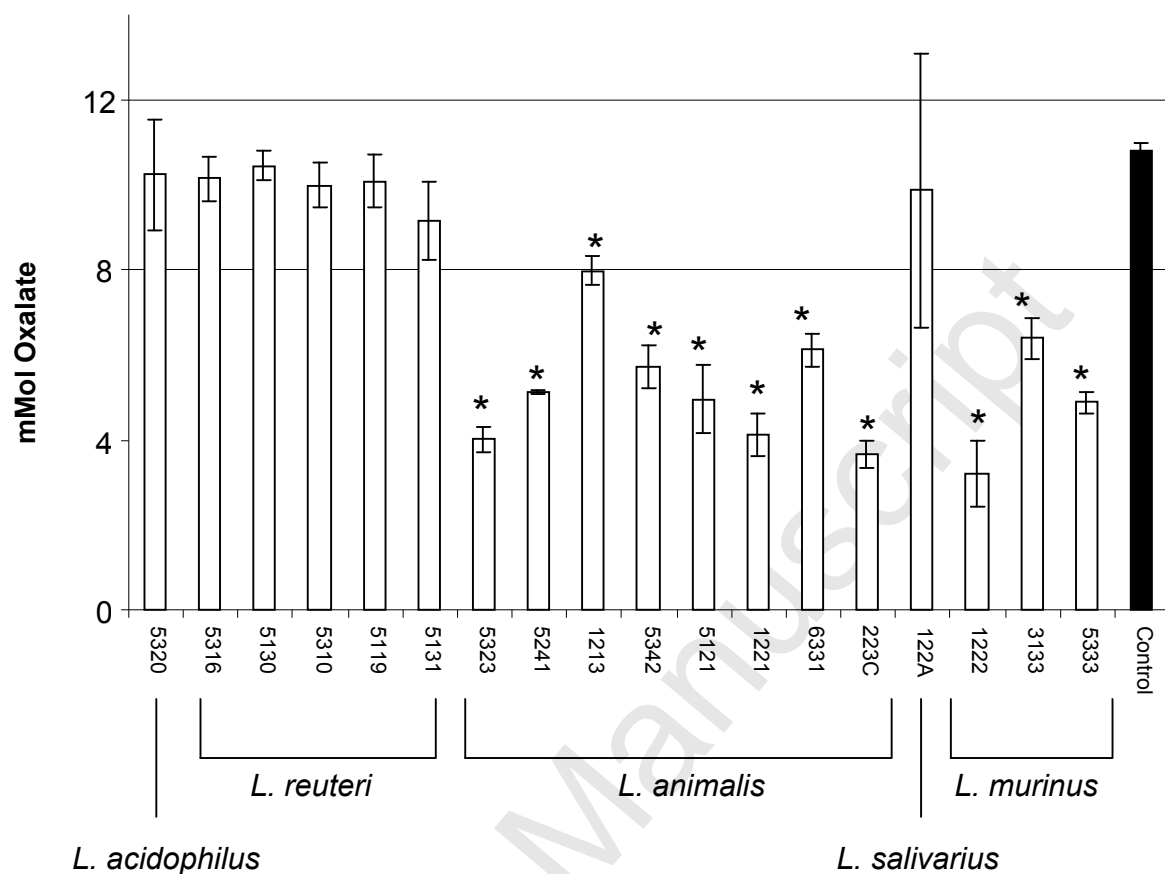
using the plate count method on MRS agar (n=2). **Results are expressed as mean +/- SD.**

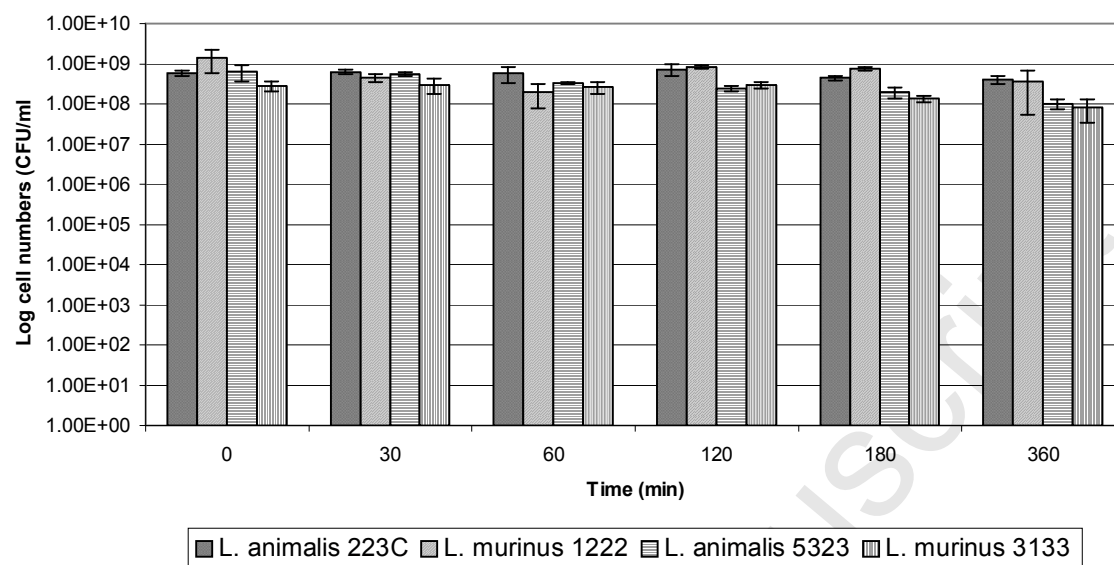
Fig. 5. Gastric transit of Rif^R freeze-dried probiotics. Freeze-dried Rif^R probiotics were administered, ad libitum, at a dose of 9.8×10^9 CFU/dose to Sprague Dawley rats (n=3/group). No Rif^R probiotics were detected on Day 0, which was prior to feeding and confirms the selection of the Rif^R probiotics post feeding. Rif^R probiotics were detected on Days, 1, 3 and 6 (post feeding) with no significant difference ($p > 0.05$) observed between groups fed probiotic or between the transit on Days 1, 3 and 6. **Results are expressed as mean +/- SD.**

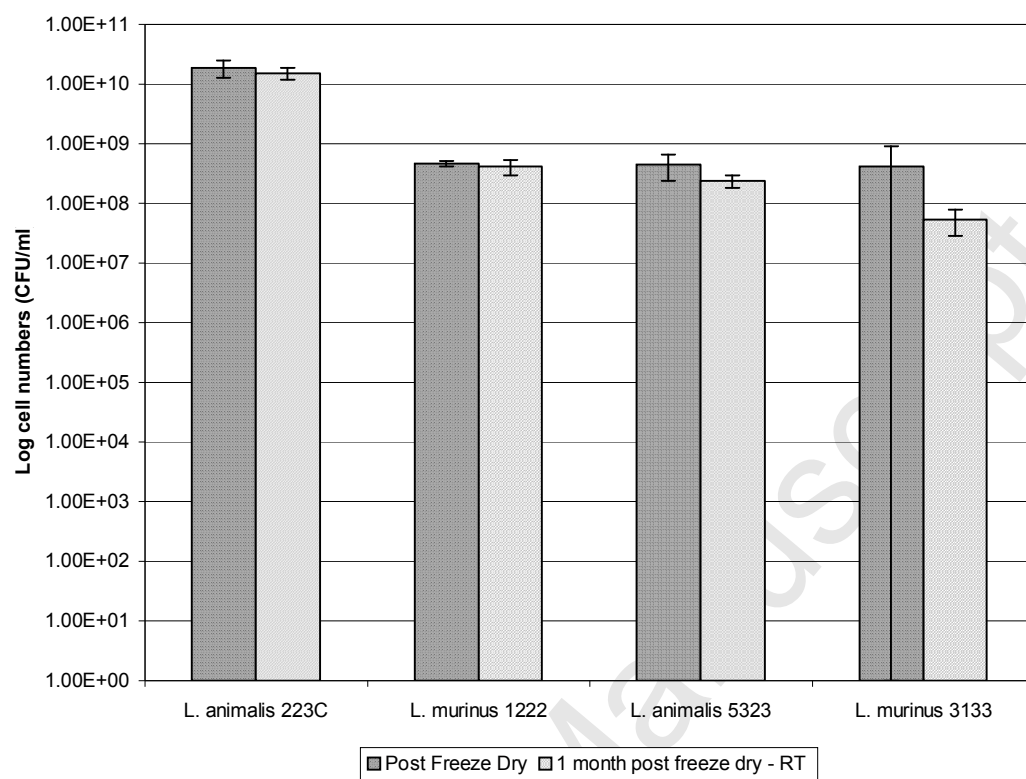
Fig. 6. Reduction of urine oxalate concentration by different strains of LAB *in vivo*. Comparison of urine oxalate concentration ($\mu\text{M}/24$ hours) of rats before (Week 0) and after probiotic or placebo treatment (n=6/group) revealed that *L. animalis* 223C and *L. animalis* 5323 significantly reduced oxalate concentration when compared to placebo. **Results are expressed as mean +/- SD.**

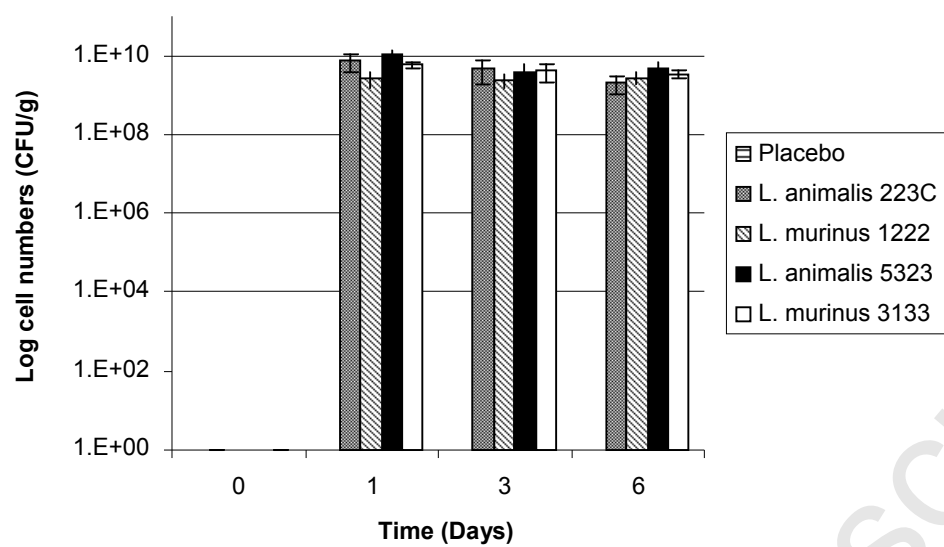
* $p < 0.05$ versus placebo

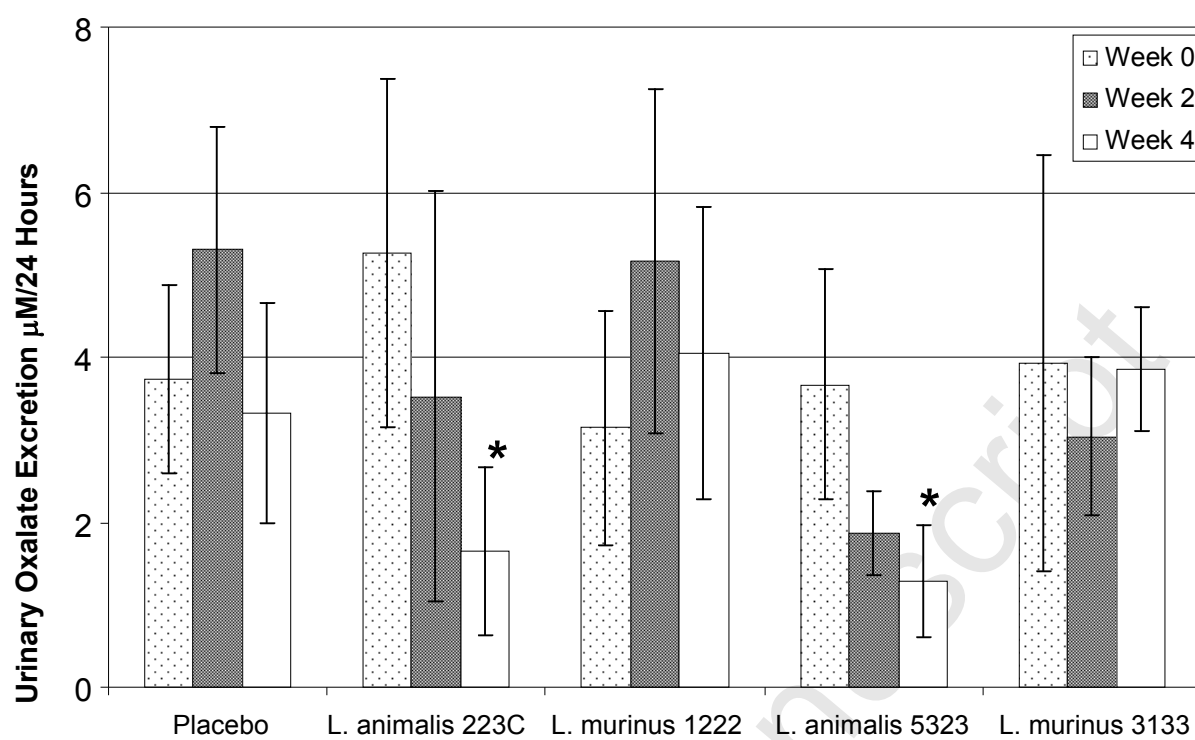












Strain ID	Percentage survival in porcine bile \pm standard deviation		
	0.5% Bile	1.0% Bile	5.0% Bile
<i>L. animalis</i> 223C	66.6 \pm 0.00	66.6 \pm 0.00	49.95 \pm 23.55
<i>L. murinus</i> 1222	100 \pm 0.00	83.3 \pm 23.55	66.6 \pm 0.00
<i>L. animalis</i> 5323	100 \pm 0.00	66.6 \pm 0.00	49.95 \pm 23.55
<i>L. murinus</i> 3133	66.6 \pm 0.00	49.95 \pm 23.55	49.95 \pm 23.55

Group	Freeze dried probiotic (CFU/ml)	Volume consumed ad libitum/day (ml)	Probiotic dose/day (CFU, n=3)
Placebo	0	19.00 ± 3.21	0
<i>L. animalis</i> 223C	6.8 x 10 ⁸	19.67 ± 1.67	1.29 x 10 ¹⁰ ± 1.43 x 10 ⁸
<i>L. murinus</i> 1222	3.4 x 10 ⁸	20.67 ± 2.19	7.03 x 10 ⁹ ± 2.90 x 10 ⁹
<i>L. animalis</i> 5323	7.8 x 10 ⁸	17.67 ± 3.71	1.38 x 10 ¹⁰ ± 1.61 x 10 ⁸
<i>L. murinus</i> 3133	3.1 x 10 ⁸	17.67 ± 0.58	5.48 x 10 ⁹ ± 9.29 x 10 ⁷

	Weight (grams) \pm SD		
Group	Week 0	Week 2	Week 4
Placebo	129 \pm 5	140 \pm 4	156 \pm 4
<i>L. animalis</i> 223C	136 \pm 9	157 \pm 11	169 \pm 12
<i>L. murinus</i> 1222	137 \pm 11	147 \pm 10	163 \pm 13
<i>L. animalis</i> 5323	131 \pm 6	148 \pm 10	162 \pm 8
<i>L. murinus</i> 3133	125 \pm 8	139 \pm 9	155 \pm 7