Assessment of in vitro oxalate degradation by *Lactobacillus* species cultured from veterinary probiotics

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**OBJECTIVE**  
To culture *Lactobacillus* spp from veterinary probiotics and measure their in vitro oxalate-degrading capacity.

**SAMPLE**  
2 commercial veterinary probiotics containing *Lactobacillus* spp.

**PROCEDURES**  
*Lactobacillus* spp were cultured anaerobically on selective deMan, Rogosa, Sharpe agar medium and subcultured for speciation by 16S rDNA gene sequencing. Isolates were inoculated into broth containing sodium oxalate (5 mg/L) and incubated anaerobically for 72 hours. An oxalate-degrading isolate of *Lactobacillus acidophilus* (American Type Culture Collection [ATCC] 53544) was the positive control sample; sterile broth containing a known quantity of sodium oxalate was the negative control sample. Oxalate concentrations were detected with ion chromatography. Oxalate degradation was assessed with Dunnett tests to detect differences in mean oxalate concentration for each isolate, compared with results for the negative control.

**RESULTS**  
*Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei* or *Lactobacillus zeae* (too closely related to differentiate) were isolated from probiotic 1, and *L. plantarum* was isolated from probiotic 2. Sequencing of the 16S rDNA gene confirmed 100% homology to type species. *Lactobacillus acidophilus* (ATCC 53544) and *L. acidophilus* from probiotic 1 significantly decreased oxalate concentrations by 85.3 and 161.9 mg/L, respectively. *Lactobacillus plantarum* from probiotics 1 and 2 significantly increased oxalate concentrations by 56.1 and 36.1 mg/L, respectively. *Lactobacillus casei* did not alter oxalate concentrations.

**CONCLUSIONS AND CLINICAL RELEVANCE**  
*Lactobacillus acidophilus* isolates significantly reduced oxalate concentrations. In vivo studies are needed to determine whether probiotics containing *L. acidophilus* decrease urine oxalate concentrations and reduce risk of urolith recurrence in dogs with a history of calcium oxalate urolithiasis. (*Am J Vet Res* 2015;76:801–806).

**ABBRERVIATIONS**  
ATCC  American Type Culture Collection  
MRS  deMan, Rogosa, Sharpe

Calcium oxalate urolithiasis in dogs is a common medical condition that is increasing in prevalence. From 1981 to 1985, approximately 6.8% of uroliths analyzed at the Minnesota Urolith Center were composed of calcium oxalate, compared with 42% of uroliths submitted in 2013.1,2 Similar trends are also reported in human medicine, which is not an unexpected finding, given parallel risk factors for calcium oxalate urolithiasis between canine and human populations.3 In mammals, oxalate is derived primarily by dietary intake, although a portion is also produced through endogenous hepatic metabolism of glycine, glyoxylate, and ascorbic acid.4 Oxalate is a terminal metabolite. Elimination is chiefly through intestinal and renal routes and, to a lesser extent, through degradation by oxalate-metabolizing bacteria. Excessive urinary oxalate excretion, also known as hyperoxaluria, leads to urinary calcium oxalate supersaturation, which is a significant risk factor for the development of calcium oxalate uroliths.5 Hyperoxaluria is influenced by numerous factors such as changes in dietary oxalate content, conditions leading to fat malabsorption, and alterations to the number and species of intestinal microorganisms that degrade oxalate.6–10

It is established in both human and veterinary medicine that calcium oxalate uroliths are not amenable to medical dissolution. Therefore, uroliths must be physically removed from the patient for clinical resolution. Current options for urolith removal in veterinary medicine include voiding urohydropropulsion, lithotripsy with basket retrieval, or cystotomy, all of which have limitations such as incomplete removal of...
Oxalobacter formigenes have been isolated from human and canine patients,\(^22,25–27\) and used in the dairy industry to metabolize carbohydrates. However, other studies\(^23,24,28\) have found a reduction of primarily nonpathogenic bacteria that are common in the intestinal tract of several mammalian species, including humans, and enteric colonization has been associated with a reduction in urinary oxalate concentrations.\(^6,12–19\)

Lactic acid bacteria, specifically species of the genera Lactobacillus and Bifidobacterium, have also been evaluated as intestinal oxalate-degrading organisms.\(^20–26\) Lactic acid bacteria are a large, diverse group of primarily nonpathogenic bacteria that are commonly used in the dairy industry to metabolize carbohydrates. Although not considered to be obligate oxalate degraders, several species of lactic acid bacteria have been isolated from human and canine patients,\(^22,25–27\) whereas other studies\(^23,24,28\) have found a reduction in urinary oxalate concentration in rodent and human subjects provided with supplemental lactic acid bacteria.

Currently, several commercially manufactured veterinary probiotics are advertised to contain Lactobacillus spp. These products offer readily available, potential sources of oxalate-degrading bacteria that are formulated for administration to dogs and cats. The objectives of the study reported here were to culture lactic acid bacteria, specifically Lactobacillus spp, from veterinary probiotics and measure their in vitro oxalate-degrading capacity. It was hypothesized that Lactobacillus spp would be cultured from veterinary probiotics and that these isolates would degrade oxalate under in vitro conditions.

**Materials and Methods**

**Sample**

Two commercially available veterinary probiotic products (probiotic 1\(^a\) and probiotic 2\(^b\)) reported to contain multiple species of lactic acid bacteria, specifically Lactobacillus spp, were purchased through an Internet source and stored in accordance with label instructions; lot numbers and expiration dates were recorded for each product. Probiotic composition with guaranteed analysis was obtained from the manufacturer’s label. Both probiotic products contained fructo-oligosaccharides and 5 billion CFUs/capsule, which comprised Lactobacillus acidophilus, Lactobacillus plantarum, Bifidobacterium bifidum, Lactobacillus casei, Lactobacillus brevis, Bifidobacterium longum, and Enterococcus thermophilius for probiotic 1 and L. acidophilus, L. plantarum, B. bifidum, L. casei, Lactobacillus bulgaricus, E. thermophilius, and Enterococcus faecium for probiotic 2. A known oxalate-degrading isolate of L. acidophilus\(^c\) (ATCC 53544; source, human infant rectal swab specimen) was used as the positive control sample. Sterile broth containing a known quantity of sodium oxalate was used as the negative control sample.

**Bacterial isolation and identification**

Lactic acid bacteria were isolated from each probiotic sample as described elsewhere.\(^29\) Briefly, 1 g of each probiotic was added to 9 mL of sterile PBS solution, and 10-fold dilutions were prepared in sterile PBS solution and mixed thoroughly in a vortex device. A 100-μL aliquot of each dilution was plated onto Lactobacillus-selective MRS medium\(^d\) and sheep blood agar for bacterial identification and colony counts. All plates were incubated anaerobically at 37°C for 48 hours. Each morphological colony type was identified and quantified. Colonies representing each morphological type were selected and subcultured for purity and speciation.

Full 16S rDNA sequencing was performed on all isolated colony types for the purpose of species identification. Briefly, bacterial DNA was extracted with a commercial kit\(^e\) in accordance with manufacturer instructions and stored at 4°C until use. For 16S rDNA sequencing, PCR assays were performed by use of a commercial kit\(^f\) with aliquots (0.2 μmol/μL) of forward (5′-AGA GTTTGATCCTTG CTCAG-3′) and reverse (5′-ACG GCT ACC TGT TTA CGA CTT-3′) primers and 2 μL of extracted DNA in a final volume of 50 μL. Amplification was performed as described elsewhere.\(^30,38\) Classic Sanger sequencing was performed by personnel at the University of Minnesota Genomics Center Core Facility. Sequences were assembled and analyzed with a commercial sequence assembly software program.\(^h\) Species identification was performed by comparisons of obtained sequences against known sequences with the aid of bioinformatic search tools.\(^i,j\)

**Selection of isolates for oxalate degradation**

An MRS culture broth\(^h\) was reconstituted in accordance with manufacturer instructions and sterilized at 121°C for 15 minutes. Sodium oxalate solution\(^i\) was prepared (concentration, 5 mg/mL) and filter sterilized through a 0.22-μm filter.\(^j\) The MRS-oxalate solution consisted of 4.75 mL of sterilized MRS broth and 4.75 mL of filter-sterilized sodium oxalate solution. For each of the subcultured bacterial species, 5 individual colonies were selected and inoculated directly into pure MRS broth and cultured anaerobically at 37°C for 24 hours. Aliquots (500 μL) of each isolate were then inoculated into the prepared MRS-oxalate broth and incubated anaerobically at 37°C for 72 hours.
hours (start of incubation was designated as time 0). Replicates of each isolate were subcultured, purified, and identified by full 16S rDNA sequencing during the course of the study to confirm species purity. Negative control samples were prepared as described and inoculated with sterile media. Assays were performed in triplicate for all isolates.

After the incubation period, all samples were centrifuged at 7,018 × g for 20 minutes to pellet the bacteria. Supernatants were filter sterilized (0.22-µm filter) and frozen at −80°C prior to oxalate quantification.

**Colony counts**

After each identified isolate was incubated for 24 hours, 10-fold serial dilutions were made from each isolate on the basis of a McFarland 2.0 suspension from both the MRS and MRS-oxalate broth. A 100-µL aliquot of each diluent was inoculated and spread onto MRS plates and incubated anaerobically at 37°C for 24 hours. Overall growth was assessed by counting the number of colonies on plates containing 30 to 300 distinct colonies and multiplying by the dilution factor.

**Oxalate detection**

Samples were analyzed by use of an ion chromatography system with hydroxide-selective analytical column, anion self-regenerating suppressor, autosampler, and integrated dual-piston pump and conductivity detector. The eluent generator system produced a variable concentration of KOH eluent, which was regulated by commercial control software. The control program used a comprehensive anion elution method.

Sodium oxalate standard solutions of 16, 8, 4, 2, 1, and 0.25 mg/L were prepared from a sodium oxalate stock solution (16 mg/L). All assays were performed in triplicate.

**Table 1** — Oxalate degradation for *Lactobacillus* bacteria (*Lactobacillus acidophilus, Lactobacillus plantarum,* and *Lactobacillus casei* or *Lactobacillus zeae* [too closely related to differentiate]) cultured from 2 commercial veterinary probiotics, compared with results for a negative control sample, after incubation in an oxalate-containing broth for 72 hours.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Isolated bacteria</th>
<th>Mean ± SD oxalate concentration in supernatant (mg/L)</th>
<th>Oxalate degradation (mg/L)</th>
<th>Oxalate degradation (%)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control sample</td>
<td>None</td>
<td>393.6 ± 7.3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Positive control sample</td>
<td><em>L. acidophilus</em></td>
<td>308.3 ± 11.1</td>
<td>−85.3†</td>
<td>−21.7†</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Probiotic 1</td>
<td><em>L. acidophilus</em></td>
<td>231.7 ± 9.8</td>
<td>−161.9†</td>
<td>−41.2†</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td><em>L. plantarum</em></td>
<td>449.6 ± 11.8</td>
<td>56.1</td>
<td>14</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td><em>L. casei</em>†</td>
<td>406.4 ± 2.9</td>
<td>12.8</td>
<td>3</td>
<td>0.4</td>
</tr>
<tr>
<td>Probiotic 2</td>
<td><em>L. plantarum</em></td>
<td>429.7 ± 8.1</td>
<td>36.1</td>
<td>9</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Sterile broth containing a known quantity of sodium oxalate was used as the negative control sample, and an oxalate-degrading isolate of *L. acidophilus* (ATCC 53544) was used as the positive control sample. Assays were performed in triplicate for each isolate.

*Values were considered significant at P < 0.05. †Negative value denotes a decrease in oxalate concentration. ‡Isolates had 100% identity over 1,447 bases with the *L. casei* gene for 16S rDNA and 99% identity over 1,456 bases with the *L. zeae* gene for 16S rDNA. NA = Not applicable.
Lactobacillus (ATCC 53544) significantly (P < 0.001) decreased oxalate concentrations by 85.3 mg/L, whereas the L. acidophilus from probiotic 1 significantly (P < 0.001) decreased oxalate concentrations by 161.9 mg/L. Conversely, L. plantarum isolates from probiotics 1 and 2 significantly increased oxalate concentrations by 56.1 mg/L (P < 0.001) and 36.1 mg/L (P = 0.003), respectively. Lactobacillus casei (or L. zeae) isolated from probiotic 1 did not have a significant (P = 0.4) effect on oxalate concentrations (Table 1). Oxalate concentrations detected in the negative control sample and in all 6 standard stock solutions, including the undiluted 16 mg/L stock solution, were consistently higher (by a mean ± SD factor of 1.53 ± 0.04) than the oxalate concentrations that had been targeted.

Discussion

The objectives of the present study were to isolate Lactobacillus spp from veterinary probiotics and to assess the in vitro oxalate-degrading capability of each isolate. Of the probiotics that were evaluated, 3 Lactobacillus spp (L. acidophilus, L. plantarum, and L. casei (or L. zeae)) were isolated from probiotic 1 and 1 Lactobacillus sp (L. plantarum) was isolated from probiotic 2. Oxalate degradation appeared to be highly variable within Lactobacillus bacteria, with only the L. acidophilus isolates causing significant reductions in oxalate concentrations. Indeed, measurable oxalate concentrations within the broth media increased significantly with L. plantarum isolates, regardless of the probiotic from which the bacteria were isolated. Increases in oxalate concentrations possibly may have been attributable to de novo synthesis of oxalate by certain Lactobacillus spp, laboratory error, or the production of metabolites that falsely registered as oxalate during the ion-exchange process used to separate analytes for ion chromatography. For example, the techniques used to measure oxalate concentrations required processing of samples in a neutral or alkaline environment; samples that may contain ascorbic acid or ascorbate products were used in the undiluted 16 mg/L stock solutions, were consistently higher by a mean ± SD factor of 1.53 ± 0.04 than the oxalate concentrations that had been targeted.

Results of the present study corroborated data for other studies conducted to evaluate the oxalate-degrading activity of Lactobacillus spp. Investigators of 1 study, reported wide variability in the oxalate-degrading activity of multiple Lactobacillus spp and strains isolated from dairy and probiotic products. Specifically, all 32 of the L. acidophilus isolates were found to reduce in vitro oxalate concentrations, with degradation values ranging from 50% to 95%; 11 strains reduced oxalate concentrations by > 80%. Of the L. plantarum isolates evaluated, in vitro oxalate degradation ranged from 0% (4 strains) to 40% (1 strain). For L. casei isolates, reported oxalate degradation ranged from 0% (1 strain) to 48% (1 strain). Investigators of another study, also reported similar findings in that L. acidophilus isolates caused the most in vitro oxalate degradation, compared with that caused by other lactic acid bacteria species, whereas L. plantarum isolates degraded little or no oxalate, despite having robust growth in oxalate-enriched media. When supplemental lactic acid bacteria were provided to humans with idiopathic calcium oxalate urolithiasis and hyperoxaluria, urinary oxalate concentrations were significantly reduced after a 4-week period. However, the probiotic supplement contained a mixture of lactic acid bacteria, including L. acidophilus and L. plantarum, so conclusions as to the efficacy of individual species of Lactobacillus bacteria for reducing urinary oxalate concentrations in vivo could not be deduced.

Veterinary studies, have revealed that Lactobacillus spp isolated from the canine and feline gastrointestinal tract can degrade oxalate, both in vitro and in vivo in rodents, but results indicate that oxalate degradation appears to be highly species and strain dependent. In the study reported here, we specifically evaluated in vitro effects of commercial veterinary probiotics to reduce oxalate concentrations. Use of commercially available probiotics has the benefit of eliminating the need for isolating and purifying gastrointestinal or fecal strains of lactic acid bacteria prior to administration. Although most veterinary probiotics contain multiple species of lactic acid bacteria, analysis of results for the present study suggested that L. acidophilus may be the most efficient for reducing oxalate concentrations. However, this was a small study and additional evaluation with multiple replicates focusing specifically on L. acidophilus, rather than on mixed lactic acid bacteria, should be performed.

The study reported here had limitations. One of the major limitations was that the study was conducted in vitro and reported results may not correspond to in vivo outcomes. Specific points of consideration include viability and colonization following gastrointestinal tract transit as well as impact on oxalate degradation in the presence of other intestinal microflora.

Additionally, comparisons were made between each isolate and the negative control sample at the 72-hour time point, rather than against a baseline measurement obtained at time 0. Both the MRS and sodium oxalate solutions were single stock solutions, which were used for the negative control sample and all isolates. Thus, concentrations for the solutions containing each isolate should have been identical to the negative control sample at time 0, but this cannot be confirmed without a measurement at time 0. An unexpected finding was that the measured concentrations in the negative control sample and the 6 standard stock solutions were higher than the targeted or expected concentrations. However, the difference between expected and measured concentrations was highly consistent, with measured concentrations being 1.5 times as high as expected for all solutions. Because
ducted to evaluate only the labeled probiotic product. Future studies could focus on isolates based on the composition reported on the label of each probiotic product. The variability among individual bacterial species suggested that probiotics formulated with multiple strains of *Lactobacillus* bacteria may reduce oxalate degradation in vitro, whereas *L. plantarum* caused significantly higher oxalate concentrations. The variability among individual bacterial species suggested that probiotics formulated with multiple species of *Lactobacillus* bacteria may not be ideal for reducing oxalate concentrations. Future clinical studies should focus on preparations containing single or multiple strains of *L. acidophilus*.

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The authors declare that they have no conflicts of interest.

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**Footnotes**


b. Proviable-DC, Nutramax Laboratories Inc, Lancaster, SC.


e. PrepMan Ultra sample preparation reagent, Applied Biosystems, Grand Island, NY.

f. HotStarTag master mix kit, Qiagen, Valencia, Calif.

g. GeneAmp PCR system 9700 thermal cycler, Applied Biosystems, Grand Island, NY.

h. Lasergene software program Seqman NGen, DNASTAR, Madison, Wis.


k. MRS broth (*Lactobacillus* broth acc: to deMan, Rogosa, and Sharpe); Sigma-Aldrich, St Louis, Mo.

l. Sodium oxalate; Sigma-Aldrich, St Louis, Mo.

m. Millex GV filter unit, Millipore, Billerica, Mass.

n. ICS-2000 chromatography system, Dionex, Sunnyvale, Calif.

o. IonPac AS19 column, Dionex, Sunnyvale, Calif.

p. ASRS 300 suppressor, Dionex, Sunnyvale, Calif.

q. AS40 automated sampler, Dionex, Sunnyvale, Calif.

r. Chromleon control software, Dionex, Sunnyvale, Calif.

**References**


