Oxalate Consumption by Probiotic Microorganisms

I-048

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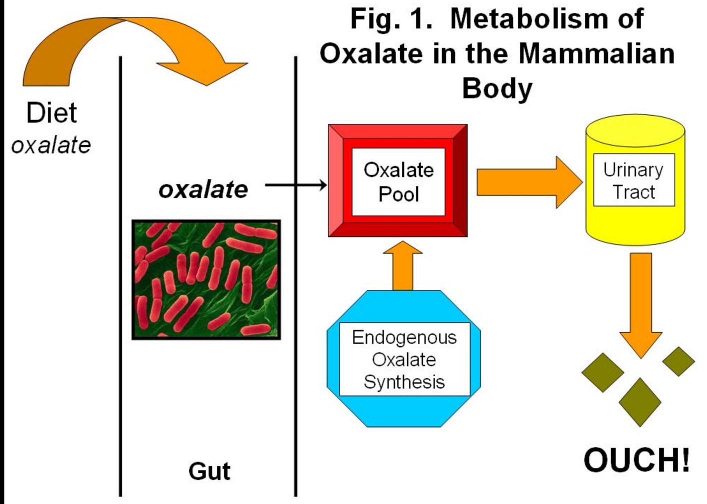
Introduction

Oxalate and Kidney Stones

- Oxalate is found in human and animal diets
- Oxalate is also produced endogenously as an end product of cellular metabolism
- Oxalate (absorbed from diet and endogenously synthesized) eliminated by urinary excretion
- High levels of oxalate in the urine (hyperoxaluria) can lead to kidney stones (**see Figure 1**)

Fig. 1. Metabolism of Oxalate in the Mammalian Body



Risk Factors for Kidney Stones

- Increased dietary oxalate intake
- Increased endogenous oxalate synthesis
- Increased absorption of oxalate due to the absence of oxalate-degrading bacteria in the gut
- **Oxalate-degrading gut bacteria impact oxalate metabolism in humans and animals**
- **Oxalate-degrading gut bacteria represent novel therapies for the prevention and treatment of kidney stones**

Oxalobacter formigenes

- Primary oxalate-degrading bacterium in the intestinal tracts of humans and animals (Allison et al., 1995)
- Anaerobe (a "specialist"): uses only oxalate as an energy source and converts it to formate and CO_2
- Colonization starts at 1 year of age in humans
- 70-80% of adult population are colonized
- Absence from the gut has been linked to increased urinary oxalate excretion and increased risk of kidney stone formation
- Currently being developed as a medical probiotic to prevent hyperoxaluria and stone formation

So, are there other oxalate-degrading bacteria in the gut?

Oxalate Degradation by Gut and Probiotic Bacteria

- *Eubacterium lentum* (Ito et al., 1996)
- *Enterococcus faecalis* (Hokama et al., 2000)
- Lactic Acid Bacteria (LAB)
  - *Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Bifidobacterium infantis* (Campieri et al., 2001)
  - *Leuconostoc lactis*, *Lactobacillus plantarum*, *Lactobacillus acidophilus* (Weese et al., 2004)
  - *Bifidobacterium lactis*, *Bifidobacterium breve*, *Bifidobacterium animalis*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium adolescentis* (Federici et al., 2004)
  - *Lactobacillus casei* (Kwak et al., 2006)
- All the above are "generalists" (substrates, other than oxalate, used for growth during oxalate degradation)

Impact of Probiotic Bacteria on Urinary Oxalate Excretion

- Pilot study by Campieri et al. (2001)
  - Freeze-dried mix of 5 Lactic Acid Bacteria (LAB) (10^{11}/g, *Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Bifidobacterium infantis*, *Lactobacillus plantarum*, *Lactobacillus breve)*
  - Urinary oxalate excretion reduced in all LAB-fed patients with stone disease and mild hyperoxaluria
  - LAB mixture developed into commercial probiotic called "Oxadrop" (sold by VSL Pharmaceuticals)
- 2nd study by Lieske et al. (2005)
  - Urinary oxalate excretion reduced in Oxadrop-fed patients with inflammatory bowel disease

Objectives

(1) To screen commercially available human and animal probiotics for their ability to consume oxalate *in vitro*

(2) To evaluate the impact of commercial probiotics on oxalate degradation by *Oxalobacter formigenes* under aerobic and anaerobic conditions

Methods

Probiotics Screened

Probiotic	Manufacturer	Probiotic Microbes Present
VSL#3	VSL Pharmaceuticals	*S. thermophilus, B. breve, B. longum, B. infantis, L. acidophilus, L. plantarum, L. casei, L. bulgaricus*
4 x 6 Acidophilus	NOW Foods	*L. acidophilus, B. bifidum, B. longum, S. thermophilus, L. bulgaricus, L. paracasei*
Saccharomyces boulardii	Jarrow Formulas	*S. boulardii*
Jarro-Dophilus	Jarrow Formulas	*L. rhamnosus, L. casei, L. plantarum, L. acidophilus, B. longum, B. breve*
Kyo-Dophilus	Wakunaga of America Co.	*L. acidophilus, B. bifidum, B. longum*
Super Potent Acidophilus	Rexall	*L. acidophilus*
Acidophilus Plus	Nature's Valley	*L. acidophilus, B. bifidum, B. longum*
Fastrack	Conklin	*E. faecium, L. acidophilus, S. cerevisiae*
Pet Inoculant	Wysong	*B. bifidum, L. lactis, E. faecium, L. acidophilus*
Oxalobacter formigenes	Dr. Milt Allison	*O. formigenes – active broth culture*

Processing Probiotics and Culture Conditions

- Commercial probiotic (recommended daily dose) added to 25 ml of culture medium in 60-ml serum bottles
  - Anaerobic medium: 10 mM oxalate, 50 mM glucose, 0.1% yeast extract, minerals, metals, cysteine, resazurin, and CO_2/HCO_3^- in crimp-sealed bottles
  - Aerobic medium: same as above except medium lacked cysteine, resazurin, and CO_2/HCO_3^- and was prepared aerobically in capped bottles
- Mixed by shaking for 1 hour to resuspend probiotic
- Probiotic cultures incubated at 37°C for 48 hours
- Oxalate measured at T_0 and at T_{48} by HPLC analysis to determine the amount of oxalate consumed

Results

- In the absence of glucose, Jarro-Dophilus, Acidophilus Plus, Super Potent Acidophilus, Kyo-Dophilus, 4x6 Acidophilus, and Fastrack consumed very little of the oxalate (0-1%) (**see Table 1**).
- *S. boulardii* and Pet Inoculant cultures consumed 5 and 8% of the oxalate, respectively. In contrast, VSL#3 cultures consumed all (100%) of the oxalate.
- In the presence of glucose, oxalate consumption increased slightly for several of the probiotics tested (4x6 Acidophilus, *S. boulardii*, Jarro-Dophilus, Kyo-Dophilus, and Super Potent Acidophilus).
- VSL#3 cultures consumed oxalate under both anaerobic and aerobic conditions. *S. boulardii*, Jarro-Dophilus, and *O. formigenes* were more active under anaerobic conditions (**see Table 2**).

Table 1. Anaerobic Oxalate Consumption by 10 Different Probiotics in the Absence and Presence of Glucose

Probiotic	Dose (inoculum)	% Oxalate Consumed[a] Anaerobic (- Glucose)	% Oxalate Consumed[a] Anaerobic (+ Glucose)
VSL#3	5 g	100	92
4 x 6 Acidophilus	1 g	1	16
Saccharomyces boulardii	1 g	5	7
Jarro-Dophilus	1 g	0	4
Kyo-Dophilus	1 g	0	2
Super-Potent Acidophilus	1 g	0	1
Acidophilus Plus	0.5 g	0	0
Pet Inoculant	1 g	8	6
Fastrack	2 ml	0	0
Oxalobacter formigenes	0.5 ml	100	100

[a] Initial oxalate concentration was 10 mM; values are the means of duplicate cultures after 48 hours of incubation.

Table 2. Oxalate Consumption by 4 Different Probiotics under Aerobic and Anaerobic Conditions

Probiotic	Dose (inoculum)	% Oxalate Consumed[a] Aerobic (+ glucose)	% Oxalate Consumed[a] Anaerobic (+ glucose)
VSL#3	5 g	90	94
Saccharomyces boulardii	1 g	0	7
Jarro-Dophilus	1 g	1	4
Oxalobacter formigenes	0.5 ml	0	100

[a] Initial oxalate concentration was 10 mM; values are the means of duplicate cultures after 48 hours of incubation.

- Oxalate consumption by *O. formigenes* under aerobic conditions was not observed (**see Figure 2; Table 2**).
- Oxalate consumption was **NOT** observed in the *O. formigenes* + *S*. boulardii mixture under aerobic conditions indicating that the probiotic yeast failed to protect *O. formigenes*.
- Aerobic oxalate consumption was observed in the *O. formigenes* + VSL#3 mixture, although it is unclear as to whether this was due to VSL#3, to *O. formigenes* or to both (**see Figure 3**).
- Under anaerobic conditions, *O. formigenes* + VSL#3 mixture consumed oxalate at a much slower rate than *O. formigenes* alone, suggesting VSL#3 slightly repressed oxalate consumption by *O. formigenes*.
- Nonetheless, *O. formigenes* appeared to be very compatible with the 2 probiotics tested.

Figure 2. Oxalate Consumption by *Oxalobacter formigenes* and *Saccharomyces boulardii* under Aerobic and Anaerobic Conditions

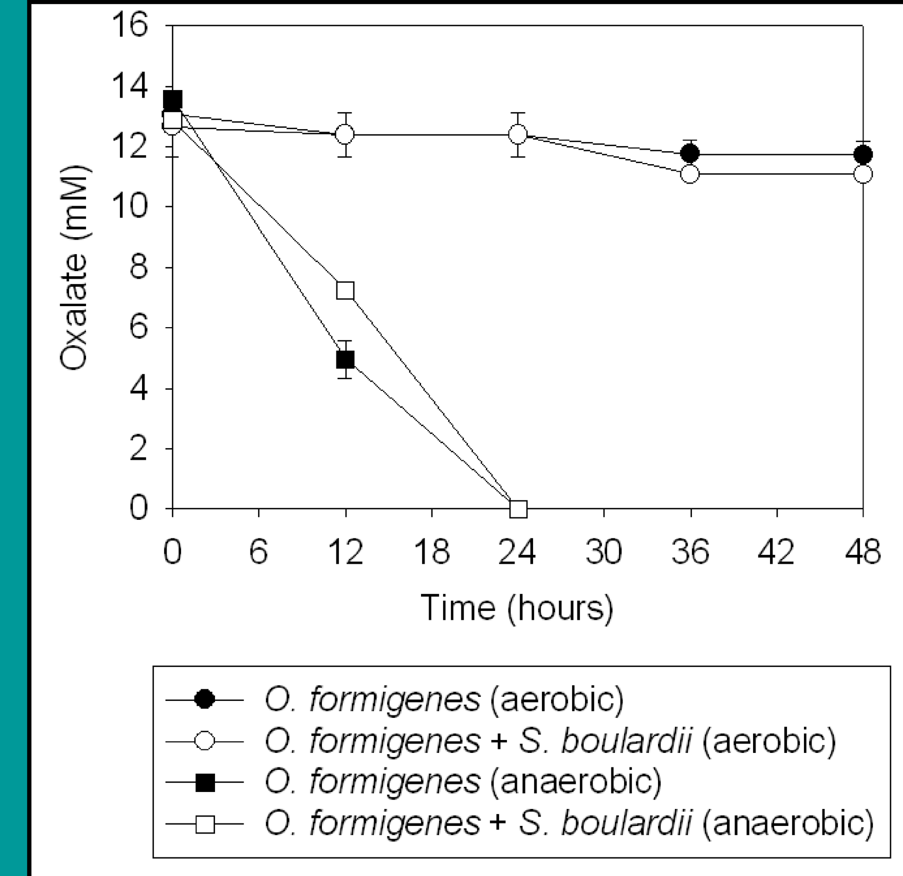
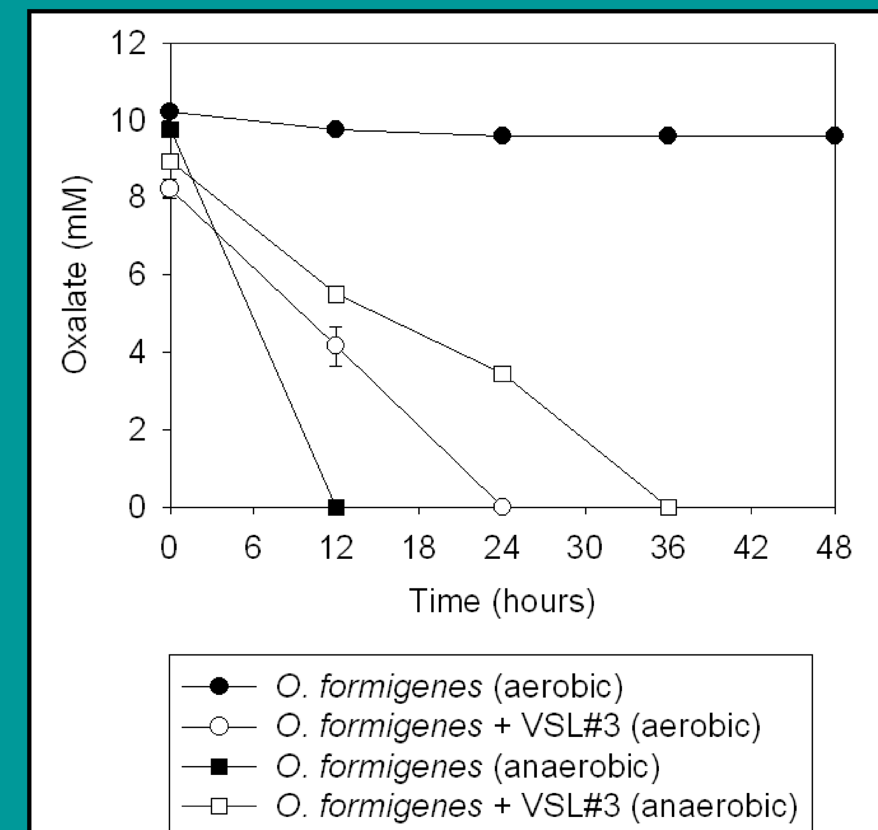


Figure 3. Oxalate Consumption by *Oxalobacter formigenes* and VSL#3 under Aerobic and Anaerobic Conditions



Summary

- Of the 9 different commercially available probiotics screened, only VSL#3 consumed significant amounts of oxalate
- Nature of the oxalate-consuming organisms or activities in VSL#3 is presently unknown
- Two probiotic (VSL#3 and *S. boulardii*) did not protect *O. formigenes* under aerobic conditions
- The combination of VSL#3 and *O. formigenes* in a single probiotic might provide a very potent and novel therapy for the prevention of calcium oxalate stones in humans and animals

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