Oxalobacter formigenes Colonization and Oxalate Dynamics in a Mouse Model

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Animal and human studies have provided compelling evidence that colonization of the intestine with Oxalobacter formigenes reduces urinary oxalate excretion and lowers the risk of forming calcium oxalate kidney stones. The mechanism providing protection appears to be related to the unique ability of O. formigenes to rely on oxalate as a major source of carbon and energy for growth. However, much is not known about the factors that influence colonization and host-bacterium interactions. We have colonized mice with O. formigenes OxCC13 and systematically investigated the impacts of diets with different levels of calcium and oxalate on O. formigenes intestinal densities and urinary and intestinal oxalate levels. Measurement of intestinal oxalate levels in mice colonized or not colonized with O. formigenes demonstrated the highly efficient degradation of soluble oxalate by O. formigenes relative to other microbiota. The ratio of calcium to oxalate in diets was important in determining colonization densities and conditions where urinary oxalate and fecal oxalate excretion were modified, and the results were consistent with those from studies we have performed with colonized and noncolonized humans. The use of low-oxalate purified diets showed that 80% of animals retained O. formigenes colonization after a 1-week dietary oxalate deprivation. Animals not colonized with O. formigenes excreted two times more oxalate in feces than they had ingested. This nondietary source of oxalate may play an important role in the survival of O. formigenes during periods of dietary oxalate deprivation. These studies suggest that the mouse will be a useful model to further characterize interactions between O. formigenes and the host and factors that impact colonization.

Oxalobacter formigenes is a Gram-negative, obligate anaerobic bacterium that requires oxalate as a carbon source for energy and growth (1). O. formigenes is part of the microbiota in the large intestine of many humans and other mammalian species (1-6). A review of colonization frequencies conducted worldwide indicated that 38% to 77% of a normal population is colonized with O. formigenes (7). Recent evidence suggests that a lack of colonization with this oxalate-degrading specialist is a risk factor for idiopathic recurrent current calcium oxalate kidney stone disease (8, 9). Controlled diet studies in healthy human subjects have also indicated that O. formigenes-colonized individuals excrete significantly less oxalate in 24-hour urine collections when they consume diets containing moderate oxalate (250 mg) and low calcium (400 mg) levels (10).

Little is known about how and when individuals become colonized or how O. formigenes persists over time. Studies to date suggest it occurs early in childhood (11), and if animal experiments provide any insight, it is obtained from the environment, not directly from the mother (12). Several studies have indicated that the intake of antibiotics can result in the loss of colonization (7, 13–15), and this is supported by decreased prevalence rates of O. formigenes in both cystic fibrosis patients (16) and calcium oxalate stone formers who are frequently prescribed antibiotics (15, 17).

Oxalate-degrading microbes are present in wild rodents (6, 18, 19) but are few or absent in laboratory rats (18, 20) and laboratory mice (21). It is postulated that procedures used for establishment and maintenance of commercial rodent colonies limit the introduction and establishment of anaerobic specialist oxalate-degrading organisms. It has been further suggested that maintenance of laboratory rodents for generations on diets high in calcium and low in oxalate has simply led to a loss of specialist oxalate-degrading organisms (22); however, some studies have shown that rodents do not lose colonization on such diets (18, 19), indicating that factors other than dietary oxalate are important for colonization.

Laboratory C57BL/6 mice have been shown to retain O. formigenes colonization on regular chow feed (21), whereas laboratory rats (23, 24) and a laboratory mouse strain deficient in the liver enzyme alanineglucosylaminotransferase (21) only retain colonization when fed high-oxalate diets, suggesting host or microbial factors may also be important in colonization.

Rodent studies have demonstrated that colonization with O. formigenes results in a reduction in urinary oxalate excretion (21, 23–25). However, much remains to be learned about the factors that influence the dynamics and stability of colonization. To better understand these parameters, a mouse model was used to systematically study the response of O. formigenes to diets with different levels of oxalate and calcium, including a purified diet containing negligible oxalate. Diets were supplemented with [13C2]oxalate to more accurately determine the contribution of dietary oxalate and the impact of O. formigenes colonization to urinary oxalate excretion. Cecal and fecal oxalate analyses were used to determine the role that a gut microbiome lacking O. formigenes plays in oxalate degradation.
TABLE 1 Custom purified diets with different calcium:oxalate mole ratios

<table>
<thead>
<tr>
<th>Diet acronym</th>
<th>Calcium:oxalate mole ratio</th>
<th>Sodium oxalate added (mg/g of diet)</th>
<th>Calcium chloride added (mg/g of diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LO</td>
<td>872</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>N</td>
<td>11.25</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>HO</td>
<td>0.75</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>LC</td>
<td>0.23</td>
<td>2.5</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*The acronyms for the diets are used throughout the text. The LO diet contained a very low background oxalate content (12.9 ± 1.1 µg oxalate/g of diet).

**Materials and Methods**

**Chemicals.** Reagent-grade chemicals were obtained from either Sigma-Aldrich Chemicals (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). Sodium oxalate and calcium chloride used in animal diets were purchased from Sigma-Aldrich, and [13C2]oxalic acid was obtained from Cambridge Isotopes (Andover, MA).

**Animals.** Male, 12- to 15-week-old inbred mice on a C57BL/6J background (The Jackson Laboratory), mean body weight (± standard deviation [SD]) of 28 ± 1 g (range, 26 to 30 g), were used for experiments. Mice were maintained in a barrier facility with a 12-hour light/dark cycle and an ambient temperature of 23 ± 1°C and had free access to food (NIH-31 open formula mouse/rat sterilizable diet, 7917) and water. Animals were euthanized with CO2, and intestinal contents from the entire small intestine and cecum were frozen at −80°C. All animal studies were approved by the Institutional Animal Use and Care Committee of the University of Alabama at Birmingham.

**Custom purified animal diets.** A custom calcium-deficient, high-sucrose basal diet (TD.130032) was designed by Harlan Laboratories (Madison, WI), to which calcium chloride and sodium oxalate were added at different levels (Table 1). The remaining balance was provided by additional sucrose when needed. Diets spanning a broad range of dietary calcium-to-oxalate mole ratios were tested, as it has been shown that this ratio can have a significant impact on urinary oxalate excretion and fecal *O. formigenes* numbers (10). Furthermore, the HO diet was tested in this study, as this level of dietary oxalate and calcium has been previously used in a mouse model of *O. formigenes* colonization (25); however, intestinal and fecal oxalate levels and intestinal *O. formigenes* cell densities were not measured. Each oxalate-supplemented diet contained 0.2 g/kg diet [13C2]oxalic acid. The mix contained, by weight, 19.6% protein (whey protein isolate), 57.7% carbohydrate (maltoextrin and sucrose), 6.6% fat (lard and corn oil), and 10.3% cellulose, and it provided 3.7 kcal/g.

**O. formigenes colonization.** Pure cultures of *O. formigenes*, strain OxCC13, were grown as previously described (26). *O. formigenes* colonization of mice received from the Jackson Laboratory were tested by both fecal culture (21) and by fecal DNA analyses using PCR (26), following ad libitum feeding with the LC diet for 3 to 5 days. For culture, approximately 50 mg fecal material was inoculated into 10 ml of anaerobic medium B (1) supplemented with 20 mM oxalate, and after incubation at 37°C for 6 to 7 days, loss of most of the oxalate in the medium was indicative of *O. formigenes* colonization. Culture and PCR analyses were used throughout the study to confirm *O. formigenes* colonization.

To colonize mice, animals were given ad libitum access to the LC diet for 3 to 5 days. After this feeding phase, 100 µl of a concentrated preparation of *O. formigenes* (≈106) was injected by gavage via a stainless steel curved feeding needle. This procedure was repeated once more 24 hours later. Colonization with *O. formigenes* was confirmed after 7 days free feeding with the LC diet. Animals remained on the LC diet for a further 7 days, the time required to obtain the culture result, and if they were colonized they were then immediately switched to the experimental diets with different levels of calcium and oxalate, as described below. Noncolonized animals were treated in the same way but were not administered *O. formigenes*.

**Dietary oxalate deprivation.** The maintenance of rodents on regular rodent chow has been suggested to result in the loss of *O. formigenes* colonization due to the food’s high calcium and low oxalate contents (22). In this study, *O. formigenes* colonization was determined after 1 month of ad libitum feeding with rodent chow NIH-31 7917, which contains 11 mg calcium and 0.78 ± 0.04 mg oxalate per gram diet. Colonized animals were either group housed (5 mice per cage, total of 6 cages) or singly housed (n = 10) in regular-sized mouse cages with an elevated metal grill, as such a setup may limit coprophagy.

The use of purified ingredients to prepare experimental diets also allowed the impact of a diet containing negligible oxalate to be tested. Colonized animals (n = 11) were singly housed in regular-sized mouse cages with an elevated metal grill and given free access to the LO diet, which contained a very low oxalate content (12.9 ± 1.1 µg oxalate per gram of diet). *O. formigenes* colonization was determined after 7 days on the LO diet. To confirm *O. formigenes* colonization status at the end of the 7-day oxalate deprivation phase, animals were switched to the LC diet for 3 days, and fresh fecal material was used to test colonization.

**Impact of diets on intestinal *O. formigenes* colonization and oxalate dynamics.** Intestinal *O. formigenes* cell densities andecal oxalate levels in *O. formigenes*-colonized and noncolonized animals were determined following 7 days of free access to the diets (n = 5 to 8 animals for each diet). Animals were singly housed in regular-sized mouse cages with an elevated grill.

**Twenty-four-hour urine and fecal collections.** Mice were singly housed in Nalgene metabolic cages to collect fecal material and urine and to allow accurate determination of dietary oxalate intake. Mice were acclimated for 7 days prior to collections (n = 4 animals for each diet) and had free access to food and water in the metabolic cages. Twenty-four-hour urine samples were collected on 1 ml mineral oil to prevent evaporation and 50 µl 2% sodium azide to prevent bacterial growth. Three to four 24-hour urine and fecal collections were performed for each mouse. Colonization of all mice was confirmed at the end of the urine collections following free feeding with the LC diet and collection of fecal material. The percent dietary oxalate absorption was determined by dividing the amount of urinary [13C2]oxalate excreted by the amount of dietary [13C2]oxalate ingested in 24 hours and multiplying by 100.

**Microbial degradation of dietary oxalate.** To determine the degradation of dietary oxalate by intestinal microbiota, dietary oxalate intake, fecal oxalate, and urinary excretion of dietary oxalate (determined from the percent absorption of dietary [13C2]oxalate) were measured for the N, HO, and LC diets (n = 4). The following equation was then used to determine the percent dietary oxalate degraded by the microbiota: 

\[
\text{[(oxalate intake – (urinary oxalate derived from diet + fecal oxalate)) × 100]}
\]

This calculation assumes that the contribution of host enteric oxalate secretion and potential production of oxalate from microbial metabolism to fecal oxalate excretion is insignificant compared to the contribution of dietary oxalate to fecal oxalate excretion.

**Microbial synthesis of oxalate in vitro.** To examine if microbial metabolism can produce oxalate, 50 mg fresh fecal material from noncolonized mice acclimated to the LO diet was cultured at 37°C in 10 ml Schaedler broth (n = 5). After incubation for 48 h, the oxalate content was measured, after subtracting the background oxalate in Schaedler broth (4.6 µg/ml) and the greatest amount of oxalate measured in 50 mg of feces (10.0 µg).

**Urine, fecal, and intestinal preparations.** For oxalate measurements, part of the 24-hour urine sample was acidified with 100 mM HCl to prevent oxalate crystallization with cold storage and the degradation of ascorbic acid to oxalate. The 24-hour fecal collections were ground to powder in liquid nitrogen prior to storage. Urine and fecal powder were stored at −80°C. Cecal and fecal oxalate levels were determined following homogenization with 2 M HCl (1:19, wt/vol). Acid homogenization ensures complete dissolution of all crystalline oxalate. The concentration of soluble oxalate in the cecum was determined by centrifuging fresh cecal
contents and recovering the cecal water for oxalate analysis. The dry weight of the cecum was determined in order to calculate the amount of cecal oxalate that was soluble.

**Enumeration of intestinal O. formigenes by qPCR.** Samples from animals that tested positive for *O. formigenes* by both culture and PCR analyses of fresh fecal material and cecal contents were used to determine *O. formigenes* density by quantitative PCR (qPCR). DNA was extracted from cecal contents by using the Maxwell 16 tissue DNA purification kit and Maxwell 16 system (Promega, WI, USA), according to the manufacturer’s instructions. qPCR was performed using GoTaq qPCR master mix (Promega) in a 10-μl reaction mixture containing 40 ng of template DNA and 0.25 μM each primer (forward primer oxc1, 5′-CAGTGACAAAACA CTCGATATG-3′; reverse primer oxc2, 5′-AGCGCATATCCATACCTC ATAAGC-3′). These primers amplify a 93-bp fragment of the oxalate dehydrogenase gene, *oxc*, from *O. formigenes* strain OxCC13. A standard curve was generated over 6 orders of magnitude using genomic DNA isolated from *O. formigenes*. Each assay was performed in triplicate. The assay was performed in a 384-well reaction plate from BrandTech Scientific (catalog number 781358S) with an optical adhesive film from Thermo (catalog number AB-0558). The reaction was performed with an initial incubation at 95°C for 15 min followed by 45 cycles of 94°C for 15 s, 58°C for 30 s, and 72°C for 35 s. A Roche LightCycler 480 real-time PCR machine was used. DNA samples isolated from noncolonized animals acclimated to the HO diet were always run in triplicate as a negative control. Enumeration of CFU of mid-log-phase cultures of *O. formigenes* indicated that 1 ng DNA corresponded to 5.5 × 10^{10} CFU (26).

**Analytical assays.** Creatinine levels were measured in nonacidified urine samples by using a Medica EasyRA clinical chemistry analyzer. Urinary creatinine values were used to ensure complete urine collections. Three to four urine collections were performed for each mouse, and any urine with a creatinine value ±20% of the mean was discarded. Oxalate was determined in acidified urine samples by ion chromatography (IC), as previously described (27). [13C2]oxalate was measured by ion chromatography/mass spectroscopy (Thermo Fisher Scientific Inc., Waltham, MA). The IC portion consisted of a Dionex ICS-5000 system with an AS11-HC 4-μm, 2- by 150-mm, anion exchange column at a controlled temperature of 30°C and a Dionex ERS 500 anion electrothermally regenerated suppressor. A gradient of KOH from 0.5 to 80 mM over 60 min at a flow rate of 0.38 ml min \(^{-1}\) was used to separate anions in samples. The MSQ-Plus mass detector was operated in electrospray ionization negative mode, needle voltage of 1.5 V, cone voltage of 40 V, and a temperature of 500°C. The column eluent was mixed with 50% acetonitrile at 0.38 ml min \(^{-1}\) using a zero-dead volume mixing tee prior to entry into the mass spectrometer. Selected ion monitoring (SIM) at mass/charge ratios of [13C2]oxalate (SIM 89) and [13C2]oxalate (SIM 91) was used to determine the relative abundance of [13C2]oxalate. The percent mole enrichment calibration curves were prepared using known amounts of [13C2]oxalate in the range of 0% to 20% enrichment, as previously described (27). Dietary oxalate absorption was determined by dividing the amount of [13C2]oxalate excreted in urine by the level of ingested dietary [13C2]oxalate and multiplying by 100.

**Statistical analyses.** The means of at least two 24-hour urine and fecal oxalate determinations were used to characterize excretions for each mouse. Effects of dietary changes on all measurements were analyzed by one-way analysis of variance (ANOVA). Student’s *t* test was used to compare differences between *O. formigenes*-colonized and noncolonized mice on each diet. Data are expressed as means ± SD. The criterion for statistical significance was a *P* value of <0.05.

**RESULTS**

*O. formigenes* colonization. None of the C57BL/6J mice received from The Jackson Laboratory were found to harbor *O. formigenes* prior to study initiation. The colonization procedure resulted in the colonization of all animals. None of the *O. formigenes*-colonized animals lost colonization after 1 week of feeding with the N, HO, or LC diet.

*O. formigenes* survival with dietary oxalate deprivation. Mice maintained on rodent chow NIH-31 7917 remained colonized with *O. formigenes* for 1 month when either group housed or singly housed in regular-sized mouse cages with an elevated metal grill to limit coprophagy. The ability to maintain *O. formigenes* colonization on a very low oxalate diet was tested with a purified custom diet. After 7 days on the LO diet, only 4 of 11 animals excreted *O. formigenes*. After switching animals to the LC diet for 3 days, fecal culture and PCR analyses indicated 9 of 11 animals were colonized, indicating 71% of the mice that were not excreting detectable levels of *O. formigenes* while ingesting the LO diet were still colonized.

**Dietary oxalate is not the only source of oxalate in the intestines.** Gut secretions and possibly microbial metabolism may be an additional source of oxalate for *O. formigenes*. The use of the LO diet, which contains negligible oxalate, and ion chromatography to measure low levels of oxalate allowed the measurement of nondietary sources of oxalate in fecal material. Noncolonized animals, following 7 days of free access to the LO diet, excreted approximately 2-fold more oxalate (99.7 ± 31.9 μg) than they ingested (49.9 ± 14.5 μg) (*P* < 0.05) in 24 hours. To examine if microbial metabolism could account for some of the oxalate generated, fresh pellets from noncolonized mice fed the LO diet were cultured in nutrient-rich broth. After incubation for 48 hours, the oxalate content had increased by 27 ± 7 μg per 50 mg of fecal material.

**Intestinal O. formigenes colonization and oxalate dynamics.** Diets with different calcium and oxalate contents (Table 1) were tested in colonized and noncolonized animals to examine the relationship between intestinal oxalate and *O. formigenes* colonization. *O. formigenes* was not detected in the small intestine following ingestion of any of the diets. Dietary changes resulted in significant effects on cecal *O. formigenes* density (*P* < 0.05) (Fig. 1). Tukey’s multiple-comparison analysis showed both the LC and LC diet resulted in higher *O. formigenes* cell densities than the LO and N diets; however, there were no significant differences in *O. formigenes* cecal densities between animals fed the LO versus N diets or between those fed the LC and HO diets. We hypothesized that *O. formigenes* colonization decreases the level of oxalate in the gut. Total cecal oxalate (Fig. 2A) and soluble levels of cecal oxalate (Fig. 2B) were significantly lower in colonized animals fed the LC
diet (P < 0.05), which was the diet where a significant decrease in 24-hour urinary oxalate excretion was observed (see Fig. 5, below). O. formigenes-colonized animals on the HO and LC diets had approximately 100-fold less soluble cecal oxalate than non-colonized mice. These results illustrate the efficient in vivo degradation of soluble oxalate by O. formigenes.

Microbial degradation of dietary oxalate. To determine the degradation of dietary oxalate by intestinal microbiota, the dietary oxalate intake (Table 2), fecal oxalate levels (Fig. 3), and amount of dietary oxalate excreted in urine (see Fig. 5) were measured. There were no significant differences in dietary oxalate intake between the colonized and noncolonized groups. Fecal oxalate levels were only significantly lower in colonized animals fed the LC diet (P < 0.05), keeping with the significantly lower cecal oxalate observed in colonized animals. Oxalate balance measurements (equation 1) indicated that microbiota in animals not colonized with O. formigenes degraded approximately 5%, 40%, and 20% of the dietary oxalate with the N, HO, and LC diet, respectively (Fig. 4). Colonization of animals with O. formigenes resulted in significantly greater degradation of dietary oxalate (Fig. 4) with the LC diet (P < 0.001).

O. formigenes colonization reduces urinary oxalate excretion. The 24-hour urinary oxalate level was measured to determine whether dietary changes and colonization with O. formigenes influenced urinary oxalate excretion. Dietary changes did not significantly impact urinary creatinine excretion, suggesting that the dietary levels of oxalate tested did not impact kidney function.

Table 2 The 24-hour dietary oxalate intake in noncolonized or O. formigenes-colonized animals

<table>
<thead>
<tr>
<th>Diet acronym</th>
<th>Daily dietary oxalate intake (mg)</th>
<th>O. formigenes-colonized</th>
<th>Noncolonized</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>3.7 ± 0.3</td>
<td>3.8 ± 0.4</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>LC</td>
<td>9.4 ± 0.7</td>
<td>9.2 ± 0.4</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>HO</td>
<td>45.1 ± 4.5</td>
<td>44.6 ± 4.5</td>
<td>0.87</td>
<td></td>
</tr>
</tbody>
</table>

Mean 24-hour urinary creatinine levels in colonized and noncolonized mice were 0.560 ± 0.104 and 0.499 ± 0.06 mg, respectively. The 24-hour urinary oxalate excretion (Fig. 5A) and the percentage of dietary oxalate absorbed (Fig. 5B) were significantly altered by the dietary changes in O. formigenes-colonized versus noncolonized animals (P < 0.01). Colonized animals excreted significantly lower 24-hour urinary oxalate amounts than noncolonized animals but only for those on the LC diet (P < 0.01). Absorption of dietary [13C2]oxalate was significantly lower in colonized mice than in noncolonized mice, but again only for those on the LC diet (P < 0.01), in concordance with 24-hour urinary oxalate excretion data, confirming that the effect was due to decreased intestinal absorption and not increased endogenous synthesis. The calcium-to-oxalate mole ratio of the diet significantly impacted dietary oxalate absorption; less than 1% of the dietary oxalate was absorbed with the N diet, and greater than 17% was absorbed with the LC diet (Fig. 5B).
and lower calcium levels than those utilized in this study were not possible since such diets are toxic to mice. Dietary oxalate deprivation showed that viable O. formigenes was not always excreted in feces. However, when mice were switched to a diet rich in soluble oxalate, O. formigenes was detected in the feces of 71% of the animals not excreting O. formigenes, suggesting that it may be prudent to provide an oxalate load to human subjects prior to determining their O. formigenes colonization status. Noncolonized mice fed a purified diet lacking oxalate (the LO diet) excreted more oxalate in fecal material than they ingested, indicating that the intestine contains nondietary sources of oxalate. These nondietary sources of oxalate may be important to the survival of O. formigenes during periods of dietary oxalate deprivation, as might occur with certain diets, such as high-protein–high-fat diets, fasting, and enteral and parenteral feeding. Gut secretions and microbial metabolism are possible nondietary sources of oxalate. Oxalate may be synthesized as part of the tricarboxylic acid cycle with the glyoxylate bypass in enteric bacteria (28), as glyoxylate is the major precursor of oxalate (29). The culturing of fresh fecal pellets in nutrient-rich broth resulted in the generation of oxalate; however, whether microbial metabolism in the intestines results in production of oxalate is not known and warrants further investigation. Enteric oxalate secretion will contribute to the intestinal oxalate pool (30), which may increase when the oxalate content in the intestinal lumen is low. The use of the Skl26a6 null mouse model, which has reduced enteric oxalate secretion (31, 32), may allow the role of enteric oxalate secretion in O. formigenes survival to be more carefully examined. Studies with rodents have suggested that O. formigenes can enhance the oxalate secretory properties of intestinal epithelia (21, 23–25); however, the mechanisms by which this occurs are not known. A study with the probiotic Lactobacillus acidophilus and intestinal cells suggested that this organism secretes a soluble effector molecule(s) that stimulates apical chloride/hydroxyl exchange activity, which may explain the anti diarrheal activity of L. acidophilus (33). It is possible that O. formigenes secretes soluble factors that induce enteric oxalate secretion. This warrants further investigation. Work with other symbiotic bacteria has shown that species-specific physical interactions with the host promote stable and resilient gut colonization. With the model symbiont, Bacteroides fragilis, a small per-
centration of the bacteria penetrates the colonic mucus and resides deep within crypt channels (34). This population is hypothesized to serve as a reservoir to repopulate the gastrointestinal tract after environmental stress, such as may occur during nutrient starvation. Nothing is known about whether O. formigenes can tightly associate with the intestinal mucosal layer. Motility and adhesion are two important mechanisms by which bacteria enhance residence time in the harsh environment of the gastrointestinal tract. However, O. formigenes is nonmotile, although genomic sequence data have revealed the presence of various outer surface proteins known to be involved in adhesion mechanisms. Outer membrane structures on Gram-negative bacteria that are known to promote adhesion include lipopolysaccharide chains and fimbriae or pili. O. formigenes strain OxCC13 expresses four proteins homologous to the type 1 pilus proteins that appear to be in an operon (35). If O. formigenes can closely associate or adhere to intestinal epithelial cells, this may allow the bacterium to utilize host-secreted oxalate more efficiently and persist during periods of dietary oxalate deprivation. Work by Sonnenburg et al. (36) suggested that bacterial consortia assemble on nutrient scaffolds composed of partially digested plant glycans, shed mucus, or exfoliated cells. These scaffolds interact with one another and with the intact mucus layer, serving to oppose bacterial washout from the gut bioreactor and also enhance nutrient harvest and exchange with other members of the microbiota. Such interactions may also play a vital role in the persistence of O. formigenes during periods of nutrient starvation and environmental stress. The development of a gnotobiotic mouse model and the use of the sequenced O. formigenes strain OxCC13 would permit a more detailed analysis of specific O. formigenes-host interactions and may identify the proteins and pathways important in colonization and survival in the intestine.

Microbial degradation of dietary oxalate. Many species of bacteria have the capacity to degrade oxalate (37). Oxalate balance measurements in this study indicated microorganisms in the intestines of mice not colonized by O. formigenes have the ability to degrade dietary oxalate, and the capacity of these organisms to degrade oxalate increases with acclimation to higher oxalate intake. This finding is in keeping with oxalate degradation studies in ruminants (38). The wild woodrat tolerates much higher levels of dietary oxalate intake than laboratory rodents, primarily due to the very high oxalate degradation that occurs in the foregut of this animal (6). Utilization of the wild woodrat model to examine O. formigenes colonization, the diversity and number of other “generalist” oxalate-degrading bacteria, and the factors that influence oxalate degradation are of interest.

Urinary oxalate and O. formigenes colonization. Oxalate-degrading bacteria and divalent cations are major factors that influence the level of soluble oxalate in the intestine (39). Increasing dietary calcium consumption has been demonstrated to reduce oxalate excretion (40, 41). This response has been attributed to calcium binding to oxalate in the intestinal tract, reducing the amount of oxalate available for absorption and subsequent urinary excretion. The use of purified diets in this study demonstrated that as the molar excess of calcium over oxalate increased, less dietary oxalate was absorbed. Previous mouse studies utilizing a diet with the same oxalate and calcium contents of the HO diet have shown that O. formigenes colonization of animals with either the human strain HC-1 or rat strain OxWR results in a significant reduction in urinary oxalate excretion (21, 25). However, no significant impact of colonization on urinary oxalate excretion was observed in this study with the HO diet. The reasons for this are unclear. Differences in ingredients used to prepare diets, differences in dietary oxalate absorption throughout the gastrointestinal tract, the use of a different O. formigenes strain, differences in O. formigenes intestinal number, and differences in the diversity and number of other oxalate-degrading bacteria in the intestines may have played a role. O. formigenes colonization resulted in an ~30% reduction in 24-hour urinary oxalate excretion with the LC diet, which had the lowest calcium-to-oxalate mole ratio of all the tested diets (0.23:1). The low soluble oxalate concentrations in colonized mice may have also induced gut secretion of oxalate and further lowered urinary oxalate excretion. As a diet low in calcium can increase the risk of calcium oxalate stone formation (42), these data suggest that colonization with O. formigenes may significantly reduce dietary oxalate absorption during periods of low calcium intake. This is consistent with a study we conducted which showed that colonization had a significant impact on urinary oxalate excretion when healthy control participants ingested a diet low in calcium (400 mg) and moderate in oxalate (250 mg) (10). Total bacterial density in the ceca of mice has been reported to be ~10^{11} cells per gram (43). In contrast, O. formigenes cell densities in the cecum did not exceed 3.1 × 10^8 CFU/g, indicating O. formigenes represented <0.3% of the total microbiome. Considering the impact this organism can have on urinary oxalate excretion, these data highlight how low-abundance bacteria can have a significant impact on host physiology. There are limitations in using a mouse to model human intestinal activity, namely, the differences in gastrointestinal anatomy and physiology between the two species. However, systematic examination of the relationship between intestinal numbers and intestinal oxalate dynamics is not possible in human studies, where fecal collections can only be used as a surrogate of intestinal activity. The use of purified ingredients, including supplemental sodium oxalate and calcium chloride, may not reflect how foodborne calcium and oxalate are handled in the intestine; however, purified ingredients allowed diets containing negligible oxalate to be tested and the calcium-to-oxalate mole ratio of diets to be carefully controlled. While it is probably true that correlating changes in urinary oxalate with colonization density and intestinal oxalate in a mouse model may not translate to human health, it is interesting that both this mouse study and our human study indicated that colonization reduces host urinary oxalate excretion only during a period of low calcium intake and moderate oxalate intake.

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O. formigenes and Oxalate Dynamics in Mice


