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Intestinal Colonization of Laboratory Rats with *Oxalobacter formigenes*

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Six strains of *Oxalobacter formigenes* (anaerobic oxalate-degrading bacteria) were examined for their ability to colonize the gastrointestinal tracts of adult laboratory rats. These rats did not harbor *O. formigenes*. Strain OxCR6, isolated from the cecal contents of a laboratory rat that was naturally colonized by oxalate-degrading bacteria, colonized the ceca and colons of adult rats fed a diet that contained 4.5% sodium oxalate. Five days after rats were inoculated intragastrically with 10^9 viable cells of strain OxCR6, oxalate degradation rates in cecal and colonic contents increased by 19 and 40 times, respectively. Viable counts of strain OxCR6 from these rats averaged 10^8/g (dry weight) of cecal contents. Strain OxCR6 was not detected in the cecal contents of inoculated rats fed diets that contained less than 3.0% sodium oxalate. Strains of *O. formigenes* isolated from the cecal contents of swine, guinea pigs, and wild rats and from human feces also colonized the ceca of laboratory rats; a ruminal strain failed to colonize the rat cecum.

Bacterial degradation of oxalate occurs in the intestines of humans and animals (1). In the rumen, a selection for increased numbers of oxalate-degrading bacteria occurs with the addition of oxalate to the diet. This selection is based on oxalate availability, and the elevation of numbers of oxalate-degrading bacteria serves to limit the absorption and, thus, toxicity of high levels of dietary oxalate (5).

Laboratory rats are widely used as models for the absorption and excretion of oxalate in humans (11); however, there is now evidence that laboratory rats are unique in that most are not colonized by oxalate-degrading bacteria (2, 10, 13). How this fact affects conclusions from data obtained with rats is not known because the role of oxalate-degrading bacteria in the colon has yet to be defined. In a survey of commercially available rats, Daniel et al. (8) discovered that rats from only one of the five commercial breeders tested harbored colonic oxalate-degrading bacteria. Oxalate-degrading isolates from these rats were similar to isolates from the rumen (9), from the bowel of humans and certain nonruminant herbivores (4), and from aquatic sediments (14). A new genus and species, *Oxalobacter formigenes*, has been proposed for this unique group of anaerobic bacteria that use oxalate as a major source of carbon and energy (4).

Reasons for the absence of *O. formigenes* from the intestinal tracts of most laboratory rats are unknown. In this study, we used rats which did not harbor *O. formigenes* to determine whether different strains of *O. formigenes* could colonize the intestinal tracts of adult laboratory rats. We also studied some of the conditions (e.g., time, dietary oxalate levels, and strain specificity) required for colonization.

MATERIALS AND METHODS

Animals and diets. Outbred male Sprague-Dawley rats (250 to 350 g; designated pathogen-free) from a single commercial colony (building 207; Harlan Sprague-Dawley, Inc., Madison, Wis.) were used. Previous studies (8) indicated that *O. formigenes* was not present in the gastrointestinal tracts of these rats. Pairs of animals were housed in plastic shoebox-type cages and maintained as described previously (8). The control diet (Teklad 4% Fat Mouse/Rat Diet; Teklad, Winfield, Iowa) contained less than 0.1% oxalate. Oxalate diets consisted of the control diet plus sodium oxalate at a dry weight concentration of 1.5, 3.0, or 4.5%. All diets were mixed and then pelleted. The animals were provided diets and water ad libitum.

Preparation of *O. formigenes* inoculum. *O. formigenes* strains used were OxB (sheep rumen [9]); POxC (pig cecum [4]); OxK (human feces [3]); OxGP (guinea pig cecum [C. Fisher, unpublished data]); OxWR1 (wild rat cecum [8]); and OxCR6 (laboratory rat cecum [8]).

Strains of *O. formigenes* were grown under anaerobic conditions at 37°C without agitation in 600-ml sidearm (diameter, 18 mm) flasks that contained 500 ml of prereduced D broth (8). The inoculum (1 to 1.5%) was grown in culture tubes (18 by 150 mm) of D broth, and cell densities were measured at 600 nm with a Spectronic 70 colorimeter (Bausch & Lomb, Inc., Rochester, N.Y.). Cells at the mid- to late exponential phase of growth (A_{600} = 0.10 to 0.14; concentration = 4×10^7 to 6×10^7 viable cells per ml) were harvested by centrifugation (14,600 × *g* for 10 min at 4°C), washed once in anaerobic dilution solution (less the $CaCl_2$ [6]), and resuspended in anaerobic dilution solution to a final concentration of 0.5×10^{10} to 1×10^{10} viable cells per ml in CO_2-flushed 10- or 25-ml serum bottles.

Inoculation of rats with *O. formigenes*. In all colonization experiments, rats were switched from the control diet to the 4.5% sodium oxalate diet at least 4 days before inoculation. Both rats in a pair then received 0.5 or 1.0 ml of a cell suspension of a strain of *O. formigenes* administered intragastrically with a slightly curved 19-gauge feeding needle. Uninoculated rats that had been fed the 4.5% sodium oxalate diet or the control diet were included as controls.

Cultural and analytical methods. At the conclusion of a colonization experiment (time intervals after inoculations are given in Results), rats were sacrificed by CO_2 narcosis and the cecal contents from a pair of rats were pooled before analysis. In some experiments, contents from the small

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intestines and colons were each pooled also. Techniques used for the measurement of rates of oxalate degradation and for the cultural detection and enumeration of *O. formigenes* have been described previously (8). The rate of oxalate degradation and the concentration of *O. formigenes* in homogenized pooled contents from a pair of rats were determined from measurements in duplicate and triplicate tubes, respectively.

Intestinal tracts were considered colonized when rates of oxalate degradation in intestinal contents were greater than rates measured in intestinal contents from uninoculated animals or when viable cells of the *O. formigenes* strain were recovered from the intestinal contents.

RESULTS

Rates of oxalate degradation in cecal and colonic contents from rats fed a 4.5% oxalate diet and inoculated with 5×10^9 viable cells of strain OxCR6 increased by 5- to 10-fold at 3 days after inoculation (Table 1). Rates of oxalate degradation from colonized rats were always greater in colonic contents than in cecal contents. Oxalate-degrading activity was not detected in small intestinal contents. Five days after inoculation, rates of oxalate degradation in cecal and colonic contents were 19 and 40 times greater than rates measured in cecal and colonic contents from uninoculated rats, respectively. Based on these results, tests for *O. formigenes* colonization were subsequently conducted with cecal contents at 5 to 10 days after inoculation. In other studies with inoculated rats, cecal and colonic rates of oxalate degradation remained high for 68 days after inoculation (S. L. Daniel, P. A. Hartman, and M. J. Allison, manuscript in preparation).

Although some of the carbon-14 from [^{14}C]oxalate was recovered as $^{14}CO_2$ after incubation with cecal contents from rats fed the control diet (Table 2), this activity did not increase when rats that had not been inoculated with *O. formigenes* were given the 4.5% sodium oxalate diet. In addition, clear zones of *O. formigenes* were not observed in tubes of D5 agar inoculated with dilutions that contained 10^{-1} to 10^{-6} ml of cecal contents from these rats. These results support previous conclusions that *O. formigenes* is absent in the ceca of conventional adult Sprague-Dawley rats from this supplier and that the low level of oxalate-degrading activity observed in cecal contents from these rats (before inoculation with *O. formigenes*) was the result of nonspecific chemical or microbial degradation (8). Ten days after rats fed the 4.5% sodium oxalate diet were inoculated with 3×10^9 viable cells of strain OxCR6, rates of oxalate degradation averaged 19.1 μmol/g (dry weight) per h and population densities of strain OxCR6 averaged 8.29 $\log_{10}$/g (dry weight) in the cecal contents of colonized rats (Table 2).

Experiments were conducted to determine what effects different levels of dietary oxalate would have on numbers of *O. formigenes* in the cecal contents of colonized rats. While on the 4.5% sodium oxalate diet, 16 rats (eight pairs) were each inoculated with 3×10^9 viable cells of strain OxCR6. Six days after inoculation, two pairs of rats were sacrificed and two pairs each were switched to 3.0, 1.5, and 0% (control) sodium oxalate diets. These diets were fed for 6 days before the rats were sacrificed. Strain OxCR6 was present at a concentration of 10^8 viable cells per g (dry weight) of cecal contents in the ceca of both pairs of rats fed the 4.5% sodium oxalate diet (Table 3). Only one of the two pairs of rats fed the 3% sodium oxalate diet and none of the rats switched either to the 1.5% sodium oxalate diet or to the control diet harbored detectable levels of strain OxCR6.

Strains of *O. formigenes* isolated from the gastrointestinal tracts of a variety of mammals were also tested for their

TABLE 1. Time course and sites of colonization by *O. formigenes* in the intestinal tracts of laboratory rats fed a diet with 4.5% sodium oxalate

Day[a]	Oxalate degradation rate[b]	
	Cecum	Colon
0	2.4 ± 0.1	1.3 ± 0.5
3	12.7 ± 5.0	16.8 ± 6.1
5	45.4 ± 0.2	51.5 ± 5.7
7	17.4 ± 0.7	26.4 ± 1.7
10	28.4 ± 13	44.2 ± 7.1

[a] Days after inoculation (two pairs of rats per time period). Rats (one pair per cage) were each inoculated intragastrically with 5×10^9 viable cells of strain OxCR6. Contents of each intestinal segment from a pair of rats were pooled before analysis.

[b] Micromoles of oxalate degraded per gram (dry weight) of intestinal contents per hour. Each value is the mean ± standard error of two determinations. There was no oxalate-degrading activity in the small intestine.

TABLE 2. Oxalate degradation rates and colony counts of *O. formigenes* in the cecal contents of laboratory rats

% Sodium oxalate in diet[a]	Inoculated[b]	Oxalate degradation rate[c]	Colony count[d]	
			Oxalate[e]	Total[f]
0	−	2.4 ± 0.2	ND[g]	10.79 ± 0.06
4.5	−	2.0 ± 0.1	ND	10.84 ± 0.02
4.5	+	19.1 ± 3.3	8.29 ± 0.10	10.73 ± 0.05

[a] Three pairs of rats (one pair per cage) per treatment.

[b] Rats were each inoculated intragastrically with 3×10^9 viable cells of strain OxCR6 and were sacrificed 10 days after inoculation. Cecal contents from a pair of rats were pooled before analysis.

[c] Micromoles of oxalate degraded per gram (dry weight) of cecal contents per hour. Each value is the mean ± standard error of three determinations.

[d] $\log_{10}$ per gram (dry weight) of cecal contents. Each value is the mean ± standard error of three determinations.

[e] Colonies producing clear zones in D5 agar (8).

[f] Total colony count in medium 10 (7).

[g] ND, Not detected (≤200 CFU/g [dry weight] of cecal contents).

TABLE 3. Effect of dietary oxalate concentrations on the maintenance of *O. formigenes* in the cecal contents of colonized laboratory rats

% Sodium oxalate in diet	Pair no.[a]	Colony count[b]	
		Oxalate[c]	Total[d]
4.5	1	8.19	10.45
	2	8.22	10.27
3.0	1	6.72	10.56
	2	ND[e]	10.66
1.5	1	ND[f]	10.74
	2	ND[f]	10.88
0	1	ND[f]	10.63
	2	ND[f]	10.70

[a] Rats (one pair per cage) were each inoculated intragastrically with 2.5×10^9 viable cells of strain OxCR6. Rats were fed the diets for 6 days and then sacrificed. Cecal contents from a pair of rats were pooled before analysis.

[b] $\log_{10}$ per gram (dry weight) of cecal contents. Each value is the mean of triplicate tubes.

[c] Colonies producing clear zones in D5 agar (8).

[d] Total colony count in medium 10 (7).

[e] ND, Not detected (≤2,000 CFU/g [dry weight] of cecal contents).

[f] ND, Not detected (≤200 CFU/g [dry weight] of cecal contents).

TABLE 4. Rates of oxalate degradation in cecal contents from laboratory rats fed a diet with 4.5% sodium oxalate and inoculated with different strains of *O. formigenes*

Strain[a]	Source	Oxalate degradation rate[b]		
		Pair no. 1	Pair no. 2	Pair no. 3
Control		2.1	2.1	1.8
OxB	Sheep rumen	1.8	2.1	1.8
POxC	Pig cecum	2.1	1.8	8.0
OxK	Human feces	3.3	7.6	8.9
OxGP	Guinea pig cecum	19.5	34.1	25.8
OxWR1	Wild rat cecum	34.6	3.6	1.6
OxCR6[c]	White rat cecum	36.2	25.1	1.7

[a] Three pairs of rats per strain. Rats (one pair per cage) were each inoculated intragastrically with 0.7×10^{10} to 1×10^{10} viable cells of a strain of *O. formigenes* and were sacrificed 8 days after inoculation. Cecal contents from a pair of rats were pooled before analysis.

[b] Micromoles of oxalate degraded per gram (dry weight) of cecal contents per hour. Each value is the mean of duplicate tubes.

[c] Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.).

ability to colonize the ceca of laboratory rats. In this experiment, six rats (three pairs) on the 4.5% sodium oxalate diet were each inoculated with 0.7×10^{10} to 1×10^{10} viable cells of a single strain of *O. formigenes*. Based on measurements of rates of oxalate degradation, strains POxC, OxK, OxGP, OxWR1, and OxCR6 each colonized the ceca of at least one pair of rats (Table 4). Only strain OxB failed to colonize the ceca of any of three pairs of rats. However, even strain OxCR6, which was isolated from the cecal contents of laboratory rats, failed to colonize the ceca of one pair of rats.

DISCUSSION

O. formigenes colonized the ceca of adult laboratory rats fed a diet containing 4.5% sodium oxalate. Our evidence for colonization by specific strains of *O. formigenes* is indirect because we currently have no marker that would enable us to distinguish between strains. We did not, however, observe colonization of any rats in the laboratory that were not purposely inoculated. This includes observations of at least 35 pairs of rats that were not inoculated. Colonization by strain OxCR6 is, however, the most plausible explanation for our findings because large numbers of viable cells of an organism resembling strain OxCR6 were detected in the cecal contents of rats that had been inoculated, whereas none were detected in cecal contents from uninoculated rats. Furthermore, rates of oxalate degradation measured in cecal contents from rats inoculated with strain OxCR6 were markedly greater than rates measured in cecal contents from uninoculated rats (Table 2). These increased rates were similar to those in cecal contents from guinea pigs and rabbits and in naturally colonized laboratory rats that had been adapted to diets high in oxalate (2, 8).

Although strain OxCR6 attained levels of 10^8/g (dry weight) of cecal contents, it made up less than 1.0% of the total viable count of bacteria that grew in medium 10 (Table 2). These concentrations of strain OxCR6 were similar to the concentrations of *O. formigenes* in the cecal sample from which strain OxCR6 was isolated (8). When the sodium oxalate level in the diet was decreased to 3.0%, the concentration of strain OxCR6 in the cecal contents from one pair of colonized rats was about 3% of the concentration found in colonized rats on 4.5% sodium oxalate (Table 3). Strain OxCR6 was not detected by colony counts in the cecal contents from the other pair of rats fed 3% sodium oxalate nor was it detected in the cecal contents of inoculated rats fed 1.5% or less sodium oxalate. The concentration of oxalate in the diet, therefore, appeared to influence both colonization by strain OxCR6 and its concentration in cecal contents. Reductions in dietary oxalate also reduced concentrations of *O. formigenes* in ruminal contents of sheep (S. L. Daniel, H. M. Cook, and M. J. Allison, manuscript in preparation).

Host specificity has been observed in the colonization by lactobacilli of the gastrointestinal tracts of mice (15). In the present study, of the six strains of *O. formigenes* tested, five colonized the ceca of adult laboratory rats fed the 4.5% sodium oxalate diet (Table 4). All five of these strains were originally isolated from monogastric mammals. The ruminal strain, OxB, was the only strain from a pregastric intestinal tract site and also the only strain that did not colonize the rat cecum. In addition, rates of oxalate degradation in most cecal contents from rats colonized with rodent strains (OxGP, OxWR1, and OxCR6) were 2 to 12 times greater than the rates measured in cecal contents from rats colonized with swine and human strains (POxC and OxK), suggesting that population levels of rodent strains were greater than those of swine and human strains in colonized rats. Also in this study, of the five strains that were capable of colonizing the rat cecum, only two (strains OxK and OxGP) colonized the ceca of all pairs of rats tested (Table 4). The regulation of microbial communities in the gastrointestinal tract is complex and, as yet, poorly understood (12). While additional data would be needed for firm conclusions, our results suggest that host specificity and natural exclusion processes by indigenous flora operate to influence the colonization of the rat bowel by given strains of *O. formigenes*. By administering high concentrations of dietary oxalate, we attempted to overcome barriers to colonization by strains of *O. formigenes*. However, with some rats, this evidently was insufficient.

We currently do not know why *O. formigenes* is absent from the intestinal tracts of most laboratory rats. Procedures used by most commercial breeders for the establishment (e.g., caesarean originated) and maintenance (e.g., barrier sustained) of rat colonies may limit the contact of rats with exogenous microorganisms and thus with *O. formigenes* (8). However, even if conventional adult rats are exposed to *O. formigenes*, the results of the present study indicate that a laboratory diet that is low in oxalate greatly reduces the chances of *O. formigenes* becoming established in the intestinal tract. However, the isolation of *O. formigenes* from a colony of laboratory rats that had been maintained on a diet containing less than 0.1% oxalate suggests that the same may not be true of newborn rats (8). An alternate possibility is that other microorganisms present in the laboratory rats that were naturally colonized are important for the establishment of *O. formigenes* when dietary oxalate is limited, and these microorganisms were not present in rats inoculated here. Additional studies, possibly with newborn or germ-free rats, may provide insight into the dynamics of intestinal colonization by *O. formigenes*.

The ability to implant *O. formigenes* into the indigenous intestinal flora of adult laboratory rats should provide an animal model in which the influence of *O. formigenes* on the fate of dietary oxalate in mammals can be studied. Studies with these rats may be useful for gaining a better understanding of factors influencing the absorption of dietary oxalate and its relation to urinary stone formation in humans (11).

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