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Rates of oxalate degradation by mixed bacterial populations in cecal contents from wild rats ranged from 2.5 to 20.6 μmol/g (dry weight) per h. The oxalate-degrading activity in cecal contents from three strains of laboratory rats (Long-Evans, Wistar, and Sprague-Dawley) from four commercial breeders was generally lower, ranging from 1.8 to 3.5 μmol/g (dry weight) of cecal contents per h. This activity did not increase when diets were supplemented with oxalate. When Sprague-Dawley rats from a fifth commercial breeder were fed an oxalate diet, oxalate-degradation rates increased from 2.0 to 33.3 μmol/g (dry weight) per h. Obligately anaerobic, oxalate-degrading bacteria, similar to ruminal strains of Oxalobacter formigenes, were isolated from the latter group of laboratory rats and from wild rats. Viable counts of these bacteria were as high as 10^9/g (dry weight) of cecal contents, which was less than 0.1% of the total viable population. This report presents the first evidence for the presence of anaerobic oxalate-degrading bacteria in the cecal contents of rats and represents the first direct measurement of the concentration of these bacteria in the large bowel of monogastric animals. We propose that methods used for the maintenance of most commercial rat colonies often preclude the intestinal colonization of laboratory rats with anaerobic oxalate-degrading bacteria.

Oxalate is degraded by microbial populations in the gastrointestinal tracts of humans (4, 9), ruminants (21, 28), and certain nonruminant herbivores (7). Oxalate degradation rates by microbial populations from the rumen and the bowel of nonruminants increase dramatically as a result of the selection of obligately anaerobic, first late-degrading bacteria (3). Dawson et al. (14) reported the presence of anaerobic oxalate-degrading bacteria (Barium and Chemicals, Inc., Steubenville, Ohio) added. The animals were provided diets and water ad libitum for at least 15 days before being sacrificed. Feed consumption was the same for both diets. Wild rats were captured from the area surrounding Ames, Iowa, and transported to the laboratory within 24 h. Rats from a single collection, two to four animals, were sacrificed and analyzed as a group.

Rats were sacrificed by CO₂ narcosis. Cecal contents from a pair of laboratory rats or from a group of wild rats were pooled in a weighing dish. A 2-g sample was transferred to a Waring blender that contained 18 ml of anaerobic dilution solution (less the CaCl₂ (11]) and homogenized at high speed for 15 s under CO₂. In certain experiments, the contents of the small and large intestines from laboratory rats were also processed in the same manner.

Cultural method. Decimal dilutions of cecal homogenates were made in anaerobic dilution solution, and 0.2-ml portions of each dilution (10⁻⁴ to 10⁻⁹) were inoculated into duplicate roll tubes of enumeration medium. All procedures were performed under strictly anaerobic conditions (10, 19). A 20 mM oxalate medium employed for the enumeration and isolation of viable anaerobic oxalate-degrading bacteria was designated D agar. D agar contained (per liter): KH₂PO₄, 0.25 g; K₂HPO₄, 0.25 g; (NH₄)₂SO₄, 0.5 g; MgSO₄·7H₂O, 0.025 g; trace metals solution (22), 20 ml; sodium acetate, 0.82 g; sodium oxalate (Sigma Chemical Co., St. Louis, Mo.), 2.7 g; CaCl₂·2H₂O, 1.0 g; yeast extract, 1.0 g; resazurin, 0.001 g; agar, 15 g; Na₂CO₃, 4.0 g; and cysteine hydrochloride·H₂O, 0.5 g. Ingredients other than the last

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two were mixed, and the pH was adjusted to 6.8. After boiling, the mixture was maintained under CO₂ while it was cooled, while sodium carbonate and cysteine were added, and while 5-ml volumes were dispensed into culture tubes (18 by 150 mm). This medium was opaque because of the presence of calcium oxalate. Clear zones developed around colonies of oxalate-degrading bacteria. D broth was identical to D agar except that calcium and agar were omitted and the culture tubes contained 10 ml of medium.

Medium 10, used for enumeration of "total" viable bacteria in cecal homogenates (12), has been used for the enumeration of bacteria in human feces (4, 16). In preliminary experiments, we found that colony counts of bacteria from homogenates were greater in medium 10 than in CCA medium (8) or modified Balch medium (20).

With the aid of a stereoscopic microscope, colonies were counted after 7 to 10 days of incubation at 37°C. Few additional colonies appeared after 10 days. Colonies in D agar that were surrounded by clear zones were picked and streaked on roll tubes of D agar. After 5 to 14 days of incubation, colonies with clear zones were restreaked. Subsequent colonies with clear zones were transferred to D broth. Growth in broth was measured as absorbance at 600 nm against a blank of un inoculated medium by using a Spectronic 70 colorimeter (Bausch and Lomb, Rochester, N.Y.). The calcium precipitation test was used to detect the presence of oxalate (15).

For electron microscopy, cultures were grown in D broth that contained 100 mM sodium oxalate. After incubation for 18 h, the cells were collected by centrifugation and prepared for examination by the procedures of Ritchie and Fernellus (23).

**Analytical methods.** Oxalate degradation rates were estimated from measurements of [14C]CO₂ production. Duplicate 1.8-ml portions of a sample plus 0.2 μl of sodium [14C]oxalate (0.1 M; 0.02 μCi/μl; New England Nuclear Corp., Boston, Mass.) were incubated in rubber-stoppered test tubes (13 by 100 mm) under CO₂ at 38°C for 1 or 2 h. The reactions, including 0-min controls, were stopped by injecting 1 ml of 3 N NaOH through the stopper. [14C]CO₂ was measured after diffusion of [14C]CO₂ from an acidified reaction mixture (13) into phenethylamine (6). Radioactivity trapped in phenethylamine was counted in a model LS-9000 liquid scintillation counter (Beckman Instruments, Inc., Palo Alto, Calif.) with 10 ml of Bioflour (New England Nuclear). Counting efficiency (90%) was monitored by external standardization (H-number) and determined by the addition of [14C]toluene (Amersham Corp., Arlington Heights, Ill.). Oxalate was decarboxylated to CO₂ and formate by O. formigenes (5). However, when sodium [14C]formate (10 mM) was incubated with samples of cecal contents (2) and feces (this study) from laboratory rats, rates of [14C]CO₂ production were at least four times greater than oxalate degradation rates. Therefore, the production of 2 mol of CO₂ per mol of oxalate degraded was assumed for the calculation of oxalate degradation rates. The specific activity of [14C]oxalate in the reaction tubes was corrected to account for soluble oxalate present in the samples.

Intestinal homogenates were clarified by centrifugation at 12,000 × g for 10 min, and soluble oxalate in the supernatants was measured by gas chromatography of the dibutyl ester (6, 24). Reported concentrations are means of measurements for duplicate samples.

For wild rats, duplicate 2- or 3-ml portions of the cecal homogenates were weighed, lyophilized, and weighed again to determine water content. This dried material was subsequently analyzed for total oxalate by the gas chromatographic procedures just described. For laboratory rats, duplicate 4-ml volumes of the homogenates were oven-dried at 55°C until a constant weight was achieved. Results are reported per unit of dry weight unless indicated otherwise.

Statistical evaluations of oxalate degradation rates were performed with Student's t test (27).

**RESULTS**

Oxalate degradation by contents from the intestinal tracts of laboratory and wild rats. In a preliminary series of experiments, contents of the small intestines, ceca, and large intestines from Sprague-Dawley rats (Haran Sprague-Dawley, Inc.) were examined to determine whether oxalate-degrading activity could be increased by the addition of oxalate to the diet. Mean values for oxalate degradation rates (means ± standard error, six pairs of rats per diet) were 2.1 ± 0.2 and 1.4 ± 0.1 μmol/g per h for samples from the cecum and large intestines, respectively, of rats fed the oxalate diet. The values were 2.7 ± 0.3 and 1.5 ± 0.2 μmol/g per h for cecum and large intestine samples, respectively, for rats fed the control diet. These low rates of [14C]CO₂ production from [14C]oxalate were thus not affected by adding oxalate to the diet, and no trends related to length of time on the diets (15, 30, and 60 days) were detected. Oxalate-degrading activity was not observed in any sample of small intestinal contents. Tests to determine factors responsible for the low levels of [14C]CO₂ production were performed. Oxalate-degrading activity was not observed when samples of anaerobic dilution solution, control diet, or rat cecal tissue were incubated with [14C]oxalate. After centrifugation of diluted cecal contents at high speed, all the oxalate-degrading activity was recovered in the pellet. Attempts to isolate oxalate-degrading microbes from the cecal contents of these rats, either by enrichment culture in D broth or by direct isolation on D agar, were unsuccessful. These and other results suggest that the low levels of oxalate-degrading activity were not due to microbes such as O. formigenes, which require oxalate as a source of carbon and energy and are selected for by diets high in oxalate.

In a second series of experiments, laboratory rats from different commercial breeders were surveyed for the presence and the selection of oxalate-degrading intestinal microbes. The oxalate-degrading activity was low (1.8 to 3.0 μmol/g per h) in samples of cecal contents from Sprague-Dawley rats from four breeders, Wistar rats from two breeders, and Long-Evans rats from a single breeder, and did not increase when animals were fed the oxalate diet (Table 1). When Sprague-Dawley rats from a fifth breeder (Charles River Breeding Laboratories, Inc.) were fed the oxalate diet, oxalate degradation rates in samples of cecal contents increased from 2.0 to 23.1 μmol/g per h. The latter value is of the same magnitude as the rates measured in samples of cecal contents from other laboratory animals (guinea pigs, rabbits) adapted to diets high in oxalate (2). Tests for the presence of oxalate degraders were also made by inoculating D broth with 10⁻² to 10⁻³ g (wt weight) of cecal contents from rats fed the oxalate diet (Table 1). Oxalate was degraded within 7 days to a level which could not be detected in D broth that had been inoculated with cecal contents from Sprague-Dawley rats from breeder 5. After 21 days of incubation, however, no loss of oxalate was detected in D broth cultures that had been inoculated with cecal contents from any of the other rats (data not shown).

The mean rate of oxalate degradation from samples of cecal contents from five groups of wild rats (16 animals) was
Three pairs of rats (one pair of rats per cage) were sacrificed per strain per cecal contents from Sprague-Dawley rats (Charles River diet. Cecal contents from a pair of rats were pooled before analysis. 

Harlan Sprague-Dawley, Inc. so

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In wild rats, total oxalate concentrations varied from 2.1 to 12.6 μmol/g of cecal contents. Of the total oxalate present, soluble oxalate represented anywhere from 0 to 45%. Soluble oxalate concentrations in samples of intestinal contents from laboratory rats fed the control diets were negligible; oxalate was not detected in the supernatant fluid of any of these samples. In samples of intestinal contents from oxalate-fed laboratory rats, soluble oxalate concentrations ranged from 0 to 4 μmol/g of contents (data not shown).

**Enumeration, isolation, and characterization of oxalate-degrading anaerobes.** In initial cultural studies with cecal samples from wild rats, colony formation was inhibited when the medium contained 40 mM sodium oxalate (data not shown). When the oxalate content of the medium was 20 mM (D agar), colony counts of anaerobic oxalate-degrading bacteria from wild rats and from Sprague-Dawley rats (Charles River Breeding Laboratories, Inc.) adapted to a high-oxalate diet ranged from 7.24 to 8.09 log_{10} per g of cecal contents (Table 2). When the level of yeast extract was increased from 0.1 to 0.3% in D agar, the colony count of oxalate-degrading bacteria from the cecal contents of these laboratory rats increased nearly twofold (Table 3). An additional threefold increase in the colony count was observed when the CaCl_2 concentration was increased from 7 to 14 mM (D3 agar). However, with nearly a sevenfold increase over D agar in the colony count of oxalate-degrading bacteria, D5 agar, which contained 10 rather than 20 mM oxalate, 7 mM CaCl_2, and 0.3% yeast extract, was the optimum medium in this study.

Nine oxalate-degrading isolates (OxWR1, OxWR2, and OxWR4 through OxWR10) were obtained from wild rats. Six isolates (OxCR1 to OxCR6) were obtained from Sprague-Dawley rats (Charles River Breeding Laboratories, Inc.). Isolates were gram-negative, nonmotile, nonsporeforming, slightly curved rod-shaped cells, occurring singly and in pairs (Fig. 1). Typical cell dimensions were 1.1 to 1.8 μm by 3.1 to 9.4 μm. No significant relationship was observed between cell morphology and culture conditions. All isolates degraded oxalate to CO_2 and formate.

Dawson et al. (14) reported that OxB, an oxalate-degrading bacterium isolated from ruminal contents by using enrichment medium that contained 45 mM oxalate, was capable of growth in medium containing oxalate concentrations as high as 111 mM. In the present study, all strains of oxalate-degrading bacteria grew well in D broth (20 mM oxalate). Maximum absorbance typically occurred after about 24 h of incubation. After several passages, these strains were used to inoculate D broth that contained either 40 or 100 mM oxalate. Only one strain (OxWR1) grew in medium containing 100 mM oxalate, but all strains grew within 7 days in the 40 mM oxalate medium.

None of the oxalate-degrading isolates grew in either medium 10 broth or PYG medium (18) without oxalate. Medium 10 broth was often inoculated as a test for contamination of oxalate-degrading cultures.

Enumerated bacteria were obligate anaerobes and did not grow in D broth in which resazurin had turned pink (oxidized) or D broth (minus cysteine and sodium carbonate) prepared under aerobic conditions.

Dawson et al. (14) tested a limited number of substrates for their ability to support growth of OxB and found that none could replace oxalate as a growth substrate. In addition, Allison et al. (5) reported that none of a wide variety of substrates, when present with oxalate, would enhance the growth of OxB. In the present study, a large number of substances were tested as possible growth substrates with strain OxWR1 in D broth, in both the presence and absence of 20 mM oxalate. Growth of OxWR1 was not enhanced or supported by the addition of any of the following filter-sterilized substances at a concentration of 20 mM: acetate, acrylate, adipate, alanine, aspartate, benzoate, butyrate, caproate, citrate, lactate, malate, pyruvate, succinate, and threonate. OxB did not grow when the concentration of oxalate was increased to 140 mM (D5 broth). Enforced growth of OxB occurred in D5 broth when the concentration of yeast extract was increased to 0.3% (Table 3). No growth occurred in D5 broth containing 0.3% yeast extract and 140 mM oxalate.

**TABLE 1. Oxalate degradation by the cecal contents from laboratory rats obtained from various commercial breeders**

<table>
<thead>
<tr>
<th>Breeder</th>
<th>Strain</th>
<th>Mean oxalate degradation rate (μmol/g per h) ± SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holtzman Co.</td>
<td>SD</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>Harlan Sprague-Dawley, Inc.</td>
<td>SD</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>King Animal Laboratories, Inc.</td>
<td>SD</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>BioCore Corp.</td>
<td>SD</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Charles River Breeding Laboratories, Inc.</td>
<td>SD</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>WI</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>LE</td>
<td>1.8 ± 0.1</td>
</tr>
</tbody>
</table>

* Diets were fed for a minimum of 15 days before animals were sacrificed. Three pairs of rats (one pair of rats per cage) were sacrificed per strain per diet. Cecal contents from a pair of rats were pooled before analysis.

**TABLE 2. Colony counts of anaerobic oxalate-degrading bacteria from the cecal contents of wild and laboratory rats**

<table>
<thead>
<tr>
<th>Breeder</th>
<th>Mean oxalate degradation rate (μmol/g per h) ± SE</th>
<th>Oxalate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild</td>
<td>4.1 ± 0.8</td>
<td>7.78 ± 0.16</td>
<td>11.12 ± 0.08</td>
</tr>
<tr>
<td>Laboratory</td>
<td>17.4</td>
<td>7.24</td>
<td>10.71</td>
</tr>
</tbody>
</table>

* Three pairs of wild rats were tested. Laboratory rats were Sprague-Dawley animals (Charles River Breeding Laboratories, Inc.) that had been fed the oxalate diet for 24 days. Cecal contents from each pair of rats were pooled before analysis.

**TABLE 3. Comparison of medium modifications: colony counts of anaerobic oxalate-degrading bacteria from the cecal contents of laboratory rats**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Sodium oxalate (mM)</th>
<th>Yeast extract (%)</th>
<th>CaCl_2 (mM)</th>
<th>Colony count</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>20</td>
<td>0.1</td>
<td>7</td>
<td>7.24</td>
</tr>
<tr>
<td>D2</td>
<td>20</td>
<td>0.3</td>
<td>7</td>
<td>7.46</td>
</tr>
<tr>
<td>D3</td>
<td>20</td>
<td>0.3</td>
<td>14</td>
<td>7.73</td>
</tr>
<tr>
<td>D4</td>
<td>10</td>
<td>0.1</td>
<td>7</td>
<td>7.88</td>
</tr>
<tr>
<td>D5</td>
<td>10</td>
<td>0.3</td>
<td>7</td>
<td>8.11</td>
</tr>
</tbody>
</table>

* Cecal contents were from the same pair of oxalate-fed Sprague-Dawley rats described in Table 2.

* Log_10 colonies per gram (dry weight) of cecal contents. Counts were from colonies producing clear zones after 7 to 10 days of incubation. Each value is the mean ± standard error of duplicate tubes.

* Colonies producing clear zones in D agar.

* Total viable count in medium 10 (12).
ate, citrate, ethanol, ethylene glycol, formamide, formate, fumarate, glutarate, glyceraldehyde, glycerol, glycine, glycolate, glyoxal, glyoxylate, isocitrate, itaconate, ketoglutarate, lactate, malate, maleate, malonate, methanol, oxaloacetate, oxamate, parabanate, phenylpyruvate, phthalate, propionate, pyruvate, serine, succinate, tartarate, tartonate, or urea. Although both parabanate and dimethyl oxalate supported the growth of strain OxB of *O. formigenes* (5), only dimethyl oxalate supported growth of strain OxWR1.

Antibiotics were tested for their effects on the growth of strains OxCR6 and OxWR1 in D broth that contained 20 or 100 mM oxalate, respectively. Growth of both strains was less than growth in control tubes in the presence of chloramphenicol (12 μg/ml), colistin (2 μg/ml), or tetracycline (6 μg/ml). Strain OxWR1 was resistant to kanamycin (6 μg/ml), erythromycin (3 μg/ml), vancomycin (6 μg/ml), rifampin (1 μg/ml), streptomycin (2 μg/ml), penicillin (2 U/ml), carbenicillin (20 μg/ml), and ampicillin (4 μg/ml). Both strains were resistant to cephalothin (6 μg/ml) and neomycin (6 μg/ml); however, only OxWR1 was resistant to clindamycin (1 μg/ml).

**DISCUSSION**

The results of several studies indicate that oxalate-degrading microbes are few or absent in laboratory rats (2, 17, 25). Data presented here provide the first evidence that anaerobic oxalate-degrading bacteria are present in certain laboratory rats and in wild rats and the first direct measurements of the concentrations of these bacteria in cultures of the cecal contents from monogastric animals. Of the three strains of laboratory rats from five breeders, only Sprague-Dawley rats from one breeder harbored significant cecal populations of anaerobic oxalate-degrading bacteria (Table 1). A different colony of Sprague-Dawley rats from the same breeder was also tested, and oxalate-degrading bacteria were not detected in these rats. Although the lack of a certain bacterial species among the normal flora inhabiting a specific group of mammals is not a new phenomenon, this is the first report involving oxalate-degrading bacteria. So far, each human, laboratory animal (other than rats), and farm animal that has been tested harbored gastrointestinal oxalate-degrading bacteria (1). Although these bacteria were present in wild rats at numbers as high as 10⁸/g (dry weight) of cecal contents (Table 2), they represented less than 0.1% of the total viable count of bacteria that were able to grow in medium 10. A similar ratio was noted between concentrations of anaerobic oxalate-degrading and the total viable count from human feces (4).

All strains of anaerobic oxalate-degrading bacteria isolated from wild and laboratory rats were similar in morphology and nutrition to the type strain of *O. formigenes*, strain OxB; to strains isolated from humans and a pig; and to rod-shaped bacteria isolated from lake sediments (5, 26). Of the two rat strains tested for antibiotic sensitivity, both were sensitive to essentially the same antibiotics reported as being effective against strain OxB (K. A. Dawson, Ph.D. dissertation, Iowa State University, Ames, 1979). The only difference was that strain OxWR1 was resistant to clindamycin. Other differences, based on tolerance to oxalate, were noted between strains. Unlike OxB, the growth of most rat strains was inhibited by high oxalate concentrations (100 mM) in the culture medium. Inhibition by high levels of oxalate was also observed with strains isolated from lake sediments (26).

The production of small amounts of ¹⁴CO₂ when [¹⁴C]oxalate was incubated with contents from the ceca and large intestines of laboratory rats that apparently did not harbor anaerobic oxalate-degrading bacteria is not yet explained (Table 1). However, results of this and other studies do indicate that this oxalate-degrading activity (i) is limited to
the particulate fraction of gut contents; (ii) is not associated with oxalate degradation in oxalate enrichment cultures or in roll tubes of D agar; (iii) is low in comparison with oxalate degradation rates found in populations where O. formigenes is present and does not increase when diets high in oxalate are given (Table 1); (iv) is not affected by antibiotics (cephalothin, chloramphenicol, tetracycline), gas phase (H2, O2, room air), or temperature (4 or 65°C); only autoclaving (121°C for 15 min) completely destroys this oxalate-degrading activity (S. L. Daniel, Ph.D. dissertation, Iowa State University, Ames, 1987); and (v) is neither proportional to the amount of gut contents nor linear with time (Daniel, Ph.D. dissertation). The above evidence suggests that this oxalate-degrading activity is the result of a nonspecific chemical reaction(s), although the process by which these nonspecific reactions occur remains to be resolved.

The reasons that some but not all laboratory rats harbor oxalate-degrading bacteria are unknown. Shirley and Schmidt-Nielsen (25) postulated that laboratory rats maintained for generations on diets low in oxalate have simply lost the capacity (microbes) for intestinal oxalate degradation. The control diet used here contained only about 0.1% oxalic acid; however, this level of oxalate was sufficient to maintain a population of oxalate-degrading microbes in one group of laboratory rats. Allison and Cook (2) suggested that laboratory rats lack intestinal oxalate-degrading microbes because of their limited contact with other herbivores. In support of this are preliminary studies showing that laboratory rats inoculated with mixed populations of microbes from wild rats develop populations of cecal microbes that have an increased capacity for oxalate degradation (S. L. Daniel, M. J. Allison, and P. A. Hartman. Abstr. Ann. Meet. Am. Soc. Microbiol. 1983, 1118, p. 159). Also, Smith et al. (26) suggested that sediments and soils may also provide a source of oxalate-degrading organisms. Thus, we propose that procedures used for the establishment (e.g., cesarean-originated) and maintenance of some commercial rat colonies limit the introduction and establishment of anaerobic oxalate-degrading bacteria.

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LITERATURE CITED


