Lactobacilli and Azoreductase Activity in the Murine Cecum

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Azoreductase activity in the ceca of mice lacking lactobacilli as members of the normal microflora (reconstituted-lactobacillus-free [RLF] mice) was compared with that of RLF mice whose gastrointestinal tracts were colonized by strains of Lactobacillus delbrueckii and Lactobacillus fermentum. Azoreductase activity was 31% lower in the ceca of mice colonized by lactobacilli.

Lactobacilli are members of the normal microflora of the digestive tract of several animal species, but despite a historical, putative association between intestinal lactobacilli and enhancement of health, little is known of the activities of lactobacilli inhabiting the gastrointestinal tract or of their specific influences on the animal host (6). A colony of mice that we have derived is proving useful in determining the influences of lactobacilli on host biochemistry (7, 8). The mice harbor a complex intestinal microflora which, on the basis of 26 microflora-associated characteristics, is functionally equivalent to that of conventional mice but from which lactobacilli are absent (7). The mice, referred to as reconstituted-lactobacillus-free (RLF) animals, are maintained in isolators by standard gnotobiotic technology. The biochemical and bacteriological characteristics of the intestinal tracts of these animals can be compared with those of RLF mice housed under identical conditions that have been colonized by lactobacillus strains present in conventional mice (8). Such comparisons permit the identification of lactobacillus influences in the intestinal ecosystem. Mice are suitable experimental subjects for elucidating the influences of lactobacilli because mice are colonized soon after birth by lactobacilli and large populations of these bacteria are present throughout the gastrointestinal tract during the remainder of the animal's life (6). Thus, if lactobacilli influence intestinal parameters, such influences are likely to be observed in mice.

We report here the influence of lactobacilli on azoreductase activity in the ceca of mice. Azoreductase enzymes present in the intestinal tract have received attention because they catalyze the reductive cleavage of azo bonds in dyes used in the food industry as coloring agents (5). A wide range of bacterial species that constitute the normal microflora of the large bowel synthesize azoreductases, and concern has been expressed that these enzymes can mediate the formation of potentially toxic aromatic amines in the intestinal ecosystem (5). Intestinal azoreductase activity is influenced by major dietary modification (for example, as in meat-versus grain-fed rats; 1). Supplementation of the diet of meat-fed rats with a culture of Lactobacillus acidophilus has been reported to reduce azoreductase activity, but it was not evident from that study whether lactobacilli were already present in the intestinal tract of the animals or whether the L. acidophilus strain colonized the intestinal tract (1). Similarly, ingestion of milk containing L. acidophilus by human subjects has been reported to lower fecal azoreductase activity, but evidence for colonization or survival of the lactobacilli in the intestinal tract was not provided (2).

We compared azoreductase activity in the ceca of 24 RLF mice with that of 21 RLF mice colonized with the three strains of lactobacilli (Lactobacillus delbrueckii 18 and 21 and Lactobacillus fermentum 20) that constitute the lactobacillus microflora of conventional animals in our facility (8). The RLF mice harboring lactobacilli were the progeny of mice colonized with these bacteria, hence the animals used in the experiments had harbored a lactobacillus microflora the same as that of conventional mice throughout life. The RLF mice were drawn from seven litters; the RLF animals harboring lactobacilli were from five litters. The animals were 6 weeks of age at examination and had been maintained in isolators as described previously (7). Azoreductase activity was measured by the method of Wise and colleagues (9), in which a cecal homogenate was prepared in an anaerobic glovebox (Forma Scientific, Marietta, Ohio) in prerred 0.01 M potassium phosphate buffer (pH 7.0) to give a 1:10 (wt/vol) dilution. Fifty microliters of 0.06 M amaranth (Aldrich Chemical Co., Inc., Milwaukee, Wis.) solution was added, and a 300-μl sample was removed immediately (zero time sample) to a tube containing 2.7 ml of 0.001 M sodium azide solution held on ice. The remainder of the homogenerate-amaranth mixture was placed at 37°C, and further 300-μl samples were removed and added to ice-cold sodium azide solution at timed intervals until the incubated mixture became colorless. The samples that had been held on ice were then centrifuged for 5 min at 5,000 × g, and the A520 of the supernatants was determined. The reaction rate was calculated from the following equation: (gradient of linear part of the curve formed by plotting A520 values against time/extinction coefficient [27.32] × 60. Azoreductase activity (in micromoles per hour per gram of cecum) equals the reaction rate times 100. Azoreductase activity in the cecum did not differ in male and female animals (Table 1). The results from males and females were therefore combined to compare, by Student's t test, the azoreductase activities of RLF mice and RLF mice harboring lactobacilli. Azoreductase activity was reduced by approximately 31% in the ceca of mice harboring lactobacilli compared with activity in lactobacillus-free animals (Table 1; P < 0.01).

We do not know the mechanism by which lactobacilli reduce azoreductase activity in the murine cecum. Colonization of the gastrointestinal tract by lactobacilli does not alter the population sizes of the numerically dominant members of the cecal microflora (7). Azoreductases are constitutively synthesized by those bacterial species that have been investigated in this respect (4). If lactobacilli do not alter population levels of azoreductase-producing bacteria, inac-
activation of the enzymes by lactobacilli may be a possibility, or their synthesis may be inhibited under lactobacillus-mediated conditions present in the ecosystem. Azoreductase activity in bacterial cultures is reduced by the addition of the unconjugated bile acid chenodeoxycholic acid, and cholic acid lowers the activity temporarily (3). It is noteworthy, therefore, that lactobacilli are responsible for approximately 86% of bile salt hydrolase activity in the small bowel of mice (74% in the cecum) and that this enzyme activity may increase the concentration of unconjugated bile acids in the intestinal ecosystem (8).

Although we have observed a statistically significant reduction in azoreductase activity associated with the colonization of the gastrointestinal tract by lactobacilli, we do not know if this is of significance biologically. In other words, we do not know if a 31% reduction in azoreductase activity is of any significance to the well-being of the mice. The RLF and RLF-lactobacillus mice should provide suitable systems, however, by which the generation of aromatic amines and their toxicities can be tested under microbiologically complex but controlled conditions. Our RLF mice should be suitable, too, for testing different lactobacillus strains for their potential to reduce azoreductase activity and for investigating the mechanisms by which such a reduction is mediated.

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REFERENCES

<table>
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<th>Murine host</th>
<th>Sex*</th>
<th>No. of mice</th>
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<td>4.9 (0.6)</td>
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<td>4.87 (0.37)</td>
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<tr>
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<td>3.34 (0.38)</td>
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* M, male; F, female.
* Combined data.