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Inactivation of Bacteriocins in the Intestinal Canal and Oral Cavity

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Colicinogenic *Escherichia coli* colonized gnotobiotic mice harboring susceptible or resistant strains comparably. Intestinal contents and dental plaque containing proteases inactivated several colicins and streptococcal bacteriocins.

Bacteriocin production has been suggested to be an important factor in the ecology of mixed microbiotas, but several studies have failed to convincingly support or refute this hypothesis (1-6, 12, 13). The purpose of the present study was to investigate the influence of bacteriocins on microbial ecology by using a system consisting of gnotobiotic mice, monoinfected with strains of *Escherichia coli*, and to relate the findings to the normal situation of the mixed microbiotas of the intestine and dental plaque.

The strains of *E. coli* used were colicin-sensitive strain W3110, its colicin-resistant mutant W3110/E2, and colicinogenic strain W3110 (E2), derived from strain W3110 by conjugation (8). All strains were F^-. Germ-free mice were obtained from Charles River Breeding Laboratories (Wilmington, Mass.) and fed diet L356 (Gen. Biochem.) for 1 week prior to and during the experiment. The animals were first monoinfected in groups of four with one of the three strains; 1 week later the groups were combined. Animals harboring the colicinogenic strain W3110 (E2) were caged with animals harboring the sensitive strain W3110 and, for comparative purposes, with animals harboring the resistant strain W3110/E2. Crossover of bacteria between the animals was followed by periodic cultural examination of feces. Freshly voided fecal pellets were homogenized and serially diluted in 0.1% Bacto-Peptone (Difco), and suitable dilutions were plated on Trypticase Soy Agar plates (BBL). The fraction of colicinogenic colonies was estimated by applying an overlayer of a sensitive indicator strain (2).

It was found that crossover of strains between the animals took place slowly, and no marked differences were noted between the experimental and control groups (Table 1). After 5 to 6 weeks, the colicinogenic strain W3110 (E2) appeared to dominate the intestinal flora of all animals, comprising about 90% of the flora. However, since the rate of exchange and final composition of the flora was similar in all groups, this crossover cannot be ascribed to colicin activity.

To determine whether active colicin was present in the intestine of the animals, cecal contents were mixed with an equal volume of 0.1% peptone water by use of a Vortex mixer, clarified by centrifugation, and sterilized with chloroform. When dropped on lawns of sensitive bacteria (2), they failed to exhibit colicin activity. This suggested that either colicins were not formed or they were inactivated in the intestine. To determine whether intestinal contents would inactivate colicin, the cecal extracts were incubated for 30 min with supernatant liquor of a Trypticase Soy Broth culture of strain W3110 (E2), which contained colicin E2. It was found that the cecal extract inactivated the colicin (Fig. 1). Similar experiments demonstrated that colicin A, B, D, E1, E2, I, K, and V (produced by strains listed in Table 2) were also inactivated by the cecal contents, by human feces, and by upper ileal and cecal contents of conventional mice (Charles River Strain, fed diet L356 for 3 weeks). The inactivation of colicin by intestinal contents is presumably enzymatic, for colicins are sensitive to proteolytic enzymes which are abundant in the intestine (10).

A similar situation appears to exist in the mixed microbiota of human dental plaque. It was found that bacteriocinogenic streptococci were common in dental plaque, and that sensitive strains of streptococci often coexisted in the same plaque samples. These observations suggest that the bacteriocins were not active in the plaque. The potential of dental plaque and filter-sterilized saliva to inactivate streptococcal bacteriocins was therefore tested by applying plaque or saliva adjacent to stab cultures of 10 bacteriocinogenic strains (7) in agar plates before application of

TABLE 1. *Invasion of the colicinogenic strain W3310 (E2)*

Original flora	Percentage of colicinogenic bacteria in feces			
	Day 10	Day 15	Day 28	Day 35–44
Sensitive strain W3110	4 (0–12)[a]	66 (47–86)	60 (33–89)	94 (89–98)
Resistant strain W3110/E2	10 (0–21)	58 (51–62)	65 (58–73)	90 (82–96)

[a] Figures in parentheses indicate ranges.

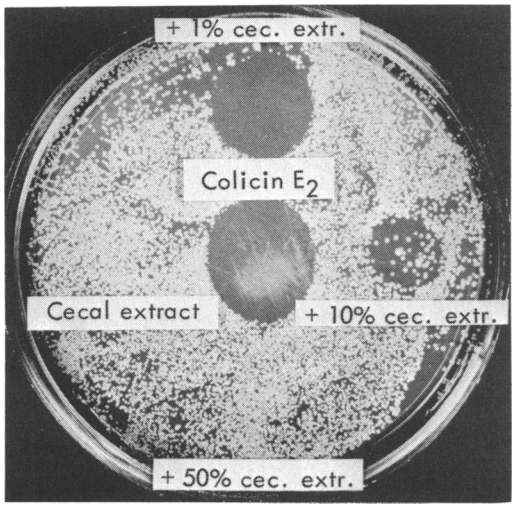

FIG. 1. *Inactivation of colicin E2 by incubation with various concentrations of cecal extracts, demonstrated by dropping on an agar plate, seeded with indicator strain W3110.*

the indicator overlayers. It was found that the bacteriocins were often inactivated, as indicated by partial or complete absence of inhibition zones in the overlayers. This seems to indicate that streptococcal bacteriocins may be inactivated in plaque and saliva, which are known to contain proteolytic enzymes (11).

These data indicate that protease-sensitive bacteriocins are inactivated in the mixed microbiotas of the intestine and dental plaque, presumably by proteolytic enzymes in the environment. This observation would seem to explain, why studies in the past have failed to demonstrate a significant role of bacteriocins in the microbial ecology of the intestinal canal.

Other possible advantages of bacteriocinogeny, such as phage resistance, increased genetic transfer, etc. (for review see reference 9), remain undetermined and were not considered in this investigation.

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TABLE 2. *List of strains*

Strain designation	Colicin produced
Escherichia freundii CA31[a]	A
E. coli CL139[b]	B
E. coli CA23[a]	D
Salmonella typhimurium SL635[c]	E1
S. typhimurium SL636[c]	E2
S. typhimurium SL486[c]	I
E. coli K235[b]	K
E. coli CA7[a]	V
E. coli W3110 (E2)[d]	E2
E. coli W3110/E2[d]	E2-resistant
E. coli W3110[d]	Sensitive
E. coli K-12[c]	Sensitive

[a] Obtained from H. Richardson.
[b] Obtained from S. E. Luria.
[c] Obtained from K. Vosti.
[d] Obtained from M. Nomura.

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