Relation Between Bacteriocin Production and Virulence of *Streptococcus faecalis* var. *liquefaciens*[1] (37700)

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Infection by bacteriophage may influence the virulence of the infected bacteria. Zabriskie (1) reviewed the evidence of group A streptococcal bacteriophage-host interaction. We have examined the role of bacteriocin [which some have considered may be defective phage (2)] in the virulence of a group D streptococcus (*Streptococcus faecalis*). The present studies were designed to evaluate the relationship between bacteriocin production in *Streptococcus faecalis* and the ability of the organism to produce experimental hematogenous pyelonephritis in the rat. Bacteriocin production was determined on the basis of effect on five bacterial L-forms. We had previously shown that all L-forms tested were susceptible to bacteriocin(s) produced by enterococci even when parent bacterial forms were resistant (3). It was felt, therefore, that L-forms were sensitive indicators of bacteriocin production.

Materials and Methods. L-forms. The L-forms used as bacteriocin indicators were (a) *Streptococcus faecalis* strain T53, originally produced with penicillin, and required 0.5 *M* sucrose as osmotic stabilizer and (b) its derivative, T531, which has been cultured without osmotic stabilizer; (c) *S. faecalis* var. *zymogenes*, originally produced with penicillin, and required 0.5 *M* sucrose as osmotic stabilizer; (d) *S. faecium* strain F24, obtained from Dr. Harry Gooder, originally prepared by lysozyme treatment of *S. faecium* strain F24, and which required osmotic stabilization with 0.5 *M* sucrose; and (e) *Escherichia coli* strain Yale, originally produced with penicillin, and required 0.5 *M* sucrose as osmotic stabilizer. All five L-forms were stable, *i.e.*, had not reverted to the parent bacterial form after many transfers without the original inducing agent.

Bacteria. Eights strains of *S. faecalis* var. *liquefaciens* which were clinical isolates were tested for bacteriocin production. Four bacteriocin-producing and four nonproducing strains were obtained.

Bacteriocin production. The method of detecting bacteriocin production was a slight modification of that of Brock *et al.* (4), as has been previously described (3). A macrocolony of the organism being tested as bacteriocin producer was created on agar and incubated overnight to allow bacteriocin production. The following day, the microorganism used as bacteriocin indicator was gently overlaid in soft agar on the plate containing the macrocolony, and the plates reincubated for 48 hr. Bacteriocin activity was indicated by a clear zone around the macrocolony of the bacteriocin producer organism.

Production of pyelonephritis. The method of maintaining cultures, producing hemato-

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TABLE I. Comparison of Pyelonephritis Induced by 4 Bacteriocin-Producing and 4 Nonproducing Strains of *Streptococcus faecalis* var. *liquefaciens*.

Sacrifice time (weeks)	Parameter	Bacteriocin-producing strains[a]	Nonproducing strains
2	Log bacteria/g[b]	5.12 ± 0.18[c]	4.56 ± 0.25
	Proportion infected kidneys	76/80	67/80
4	Log bacteria/g	4.83 ± 0.21	4.42 ± 0.23
	Proportion infected kidneys	71/80	71/80
6	Log bacteria/g	4.73 ± 0.21	4.30 ± 0.24
	Proportion infected kidneys	71/80	67/80
Overall	Log bacteria/g	4.89 ± 0.12	4.42 ± 0.14
	Proportion infected kidneys	218/240	205/240

[a] Mean inoculum bacteriocin producing strains, 1.13×10^9 bacteria; nonproducing strains, 0.94×10^9 bacteria.

[b] Infected kidneys only.

[c] Mean ± standard deviation of mean.

genous pyelonephritis in outbred Wistar rats, and bacteriologic evaluation of the kidneys has been described (5). In order to obtain comparable data, all bacterial strains were handled in a standardized manner. The animal inocula were adjusted to 1×10^9 bacteria in 1 ml, which was injected into a tail vein. Groups of 8–10 animals were sacrificed at 2, 4, and 6 weeks, and the microbial population determined for each kidney. Several colonies from each plate were confirmed as group D streptococci by growth in S. F. medium, growth in 6% NaCl, and failure to produce catalase. Kidneys containing any streptococci were considered to be infected.

Results. The results are shown in Table I. Pyelonephritis was produced and persisted for at least 6 weeks with high numbers of *S. faecalis* in the kidneys. The renal microbial population was similar to that seen with hematogenous pyelonephritis previously reported with another strain of *S. faecalis* (5) that produced chronic nonobstructed pyelonephritis in the rat. The renal microbial population (log 4.89 ± 0.12/g kidney tissue) in animals inoculated with bacteriocin-producing strains was higher than in those receiving nonbacteriocin-producing strains (log 4.42 ± 0.14/g kidney tissue, $p < 0.02$). Proportion of infected kidneys in animals receiving bacteriocin-producing strains, 218/240, was higher than among those receiving nonproducing strains, 205/240, although the difference was not statistically significant, $p < 0.10$.

Discussion. These studies were interpreted as demonstrating a possible association between bacteriocin production by *S. faecalis* var. *liquefaciens* and ability to produce hematogenous pyelonephritis in the rat.

Bacteriocins are antibiotic-like substances produced by many bacteria which differ from usual antibiotics in that they act only on other strains of the same or closely related species. While the role of bacteriocins in ecology has been studied, little is known of any relation between bacteriocin production and virulence. The production of bacteriocins by group A streptococci with particular regard to differentiation of nephritogenic and nonnephritogenic types was studied by Kuttner (6). Strains of group A streptococci of the nephritogenic types 12, 4, and 49 produced bacteriocins most consistently. Strains of other types isolated from patients with acute rheumatic fever or from children with uncomplicated streptococcal pharyngitis produced bacteriocins infrequently. However, Overturf and Mortimer (7) were unable to confirm this observation. In their study, no significant difference was noted between strains isolated from cases of nephritis and those from a group of strains isolated from patients with other conditions. In addition, no correlation between serotype and bacteriocin production

was observed.

Evidence is available which indicates that infections by bacteriophage may influence production of extracellular products by the infected bacteria. This may be important, since at least some bacteriocins may be defective bacteriophage (2). Zabriskie (8) noted a relation between production of scarlatinal toxin and phage lysogeny in group A streptococci. However, Wannamaker *et al.* (9) could not recognize any distinctive phage or group of phages common to nephritogenic strains of group A streptococci. We know of no clinical data to support the observation that bacteriocins may relate to the virulence of *S. faecalis* producing pyelonephritis, and it would be of interest to examine the percentage of bacteriocin positive strains of *S. faecalis* causing clinical urinary tract infections.

The biological basis for a possible association between bacteriocin production and virulence of enterococci is not known. The bacteriocin may be toxic to kidney tissue, or, alternatively, may depress normal defense mechanisms. If bacteriocins of *S. faecalis* var. *liquefaciens* are defective bacteriophage it is also possible that bacteriocin serves as a marker for virulence factors carried by the bacteriophage.

Summary. Eight strains of *Streptococcus faecalis* var. *liquefaciens* were classified as bacteriocin producing or nonproducing on the basis of their effect on microbial L-forms. Four strains produced bacteriocin, and four did not. There was a significant association between bacteriocin production and ability to produce hematogenous pyelonephritis in the rat.

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