ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1976, p. 81-88

Vol. 9, No. 1
Printed in U.S.A.

Viridins, Bacteriocins of Alpha-Hemolytic Streptococci: Isolation, Characterization, and Partial Purification

ADNAN S. DAJANI,* MAY C. TOM, AND DAVID J. LAW

Division of Infectious Diseases, Children's Hospital of Michigan, and the Department of Pediatrics, Wayne State University School of Medicine, Detroit, Michigan 48201*

Received for publication 13 August 1975

Bacteriocin-like activities were detected in 78% of 120 alpha-hemolytic streptococcal isolates. Inhibitory substances from three such isolates (one *Streptococcus sanguis* strain and two *S. mitis* strains) were investigated further and termed viridins (A, B, and C). The viridins were unique among bacteriocins of gram-positive bacteria in that they inhibited many gram-negative bacteria in addition to inhibition of a variety of gram-positive organisms. Viridins were obtained in a cell-free state only after mechanical disruption of bacteriocinogenic cells but could not be isolated from supernatant fluids of cultures of such bacteria or from freeze-thaw liquor of agar on which the bacteria had been grown. Viridin B could be partially purified by ammonium sulfate precipitation and by gel filtration on a Sephadex G-100 column. This bacteriocin had some unusual properties including heat lability, a narrow pH range of activity, and lack of adsorptive capacity to susceptible bacteria. Although viridin B was bactericidal to a *Neisseria sicca* strain, it was only bacteriostatic against a coagulase-negative staphylococcus,

The phenomenon of bacterial interference has been recognized for over a century but has received a more pronounced emphasis in recent years. Several mechanisms have been postulated as the basis for this phenomenon, including pH changes, toxic metabolites, and depletion of nutrients. Production of bacteriocins by several organisms is one other mechanism of bacterial interference and has been a major interest in our laboratory for several years.

In 1968 Sprunt and Redman (16), using the deferred antagonism technique, demonstrated the inhibitory effect of alpha-hemolytic streptococci against a variety of gram-positive and gram-negative bacteria. This inhibition was shown not to be related to depletion of nutrients or to pH changes. Although in some instances production of hydrogen peroxide was thought to be responsible, the exact mechanism for this inhibition was not further elucidated. A year later Sanders (14), using the simultaneous antagonism method, reported a similar effect of bacterial interference by alpha-hemolytic streptococci against staphylococci, neisseria, and group A streptococci. He attributed this to a reduction in pH and to depletion of nutrients from the media.

This inhibitory effect of alpha-hemolytic streptococci is not only an in vitro phenomenon. Sprunt and co-workers (15) clearly demonstrated that the eradication of alpha-hemolytic streptococci from the oropharynx of individuals treated with parenteral antibiotics resulted in an overgrowth by gram-negative bacilli at this site. Moreover, selection for antibiotic-resistant strains of alpha-hemolytic streptococci in the throat prevented such overgrowth by enteric organisms when the patients were treated with the particular antibiotic. A report in 1973 by Crowe et al. (2) further defined the role of the throat normal flora in protecting children against colonization with group A streptococci. Such protection was shown to be directly proportional to the quantity of these bactericidal organisms in throat cultures.

In this report, data will be presented to show that the previously recognized bacterial interference of alpha-hemolytic streptococci is a function of a bacteriocin or bacteriocins produced by this group of organisms.

MATERIALS AND METHODS

Bacterial strains. All organisms were isolated from clinical specimens obtained at the Diagnostic Microbiology Laboratory, Children's Hospital of Michigan. Alpha-hemolytic streptococci were so identified by their colonial morphology, their hemolytic activity on 10% sheep blood agar plates, and their resistance to optochin. All enterococci and group D streptococci were excluded. Species identification was accomplished according to *Bergey's Manual* (1). Numbers appearing after bacterial

strains were assigned to the organisms in our diagnostic microbiology laboratory.

Media and chemicals. Dehydrated media were obtained from Difco Laboratories (Detroit, Mich.) and reconstituted according to the manufacturer's specifications. Sorensen phosphate buffer, 0.067 M, was used at pH 7.2 unless otherwise specified. Trypsin, 2× crystallized, was purchased from Grand Island Biological Co., Grand Island, N.Y. Its effect on bacteriocin activity was tested, as previously described (6), at pH 8.2. Papain and protease were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Sephadex G-100 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) was suspended in phosphate buffer and packed in a column (1.5 by 90 cm) at 4 C and stored at that temperature. The void volume of this column was determined as 140 ml.

Demonstration of bacteriocin activity. The simultaneous antagonism technique used tryptic soy agar plates. Producer strains were spotted on plates previously seeded with indicator strains, and after incubation at 37 C inhibition of the indicator strain was looked for around the growth of the producer strain. In the deferred antagonism method, producer strains were grown on tryptic soy agar plates for 24 to 48 h. The plates were then exposed to chloroform vapor for 30 min, aired, and overlayed with the indicator strains contained in 10 ml of melted tryptic soy agar. After 24 h of incubation at 37 C, plates were screened for inhibitory zones around the previous growth of producer strains.

Killing of bacteria by the partially purified, cell-free bacteriocin was determined by counting the number of colony-forming units per milliliter at appropriate intervals after mixing cells with the bacteriocin solution (5, 6).

Bacteriocin assays. The concentration of a bacteriocin solution was estimated by twofold dilutions of the preparation in phosphate buffer and then spotting 0.05 ml of each dilution onto sections of a tryptic soy agar plate previously seeded with the indicator strain (4). The reciprocal of the highest dilution showing a clear zone of growth inhibition was considered the titer of the assayed preparation.

A bacteriocin arbitrary unit (AU) was calculated as the amount of bacteriocin contained in 1.0 ml of the preparation (i.e., titer × 20).

RESULTS

Inhibitory capacity of alpha-hemolytic streptococci. Using the deferred antagonism technique, the susceptibilities of four indicator strains to the inhibitory capacities of 120 alpha-hemolytic streptococcal isolates were tested (Table 1). Bacteriocin-like inhibition was detected in 25 to 63% of the streptococcal strains against the single indicators. A higher frequency of inhibition (78%) can be noted, however, if the capacity to inhibit any one of the indicators is considered.

Further identification of the alpha-hemolytic streptococci was possible on 84 isolates. The frequency of inhibitory capacity and the sources of isolation of these identified species are shown in Table 2. Most isolates were from the upper respiratory tract; however, the site of isolation was not related to whether the isolate was inhibitory or not. The frequencies of production of inhibitory substances by *Streptococcus mitis* and *S. anginosus* were comparable. All five isolates of *S. sanguis* were producers, and the single *S. salivarius* strain did not produce any inhibitory substances.

Spectra of activities. Three strains of the viridans streptococci (*S. sanguis* strain 24658, *S. mitis* strain 42885, and *S. mitis* strain 42991) were selected for further testing of the spectra of their activities against various bacteria. The bacteriocin-like inhibitory substances from these three strains will be referred to as viridins A, B, and C, respectively.

The activities of the three viridins against a variety of gram-positive bacteria are shown in Table 3. In general, staphylococci, both coagulase negative and coagulase positive, were frequently susceptible. Streptococci of groups A and B were also susceptible but less commonly than staphylococci. Viridans streptococci, on the other hand, were usually resistant, and

TABLE 1. *Susceptibility of four indicators to 120 viridans streptococci*

Indicator	Inhibitory streptococcal strains	
	No.	%
Coagulase-negative staphylococcus 23709	75	63
Coagulase-negative staphylococcus 42658	30	25
Coagulase-negative staphylococcus 42687	61	51
Staphylococcus aureus 42774	51	43
Any one of the above	94	78

TABLE 2. *Sources of isolation and inhibitory capacity of four alpha-hemolytic streptococcal species*

Producer species	Sources	Inhibitory capacity	
		Frequency	%
Streptococcus mitis	Throat 54, nose 7, urethra 1	38/62	68
S. anginosus ..	Throat 14, nose 1, skin 1	9/16	69
S. sanguis	Throat 4, vagina 1	5/5	100
S. salivarius ..	Throat 1	0/1	0

none of four candida tested was susceptible. Another observation worthy of mention is the fact that within each group of indicator strains tested, a marked degree of variable susceptibilities was noted to the viridins, again suggesting heterogeneity among these bacteriocins at least in their spectra of activity.

Previously described bacteriocins from gram-positive bacteria, such as staphylococcins (4, 6, 8, 9, 11) and streptocins of group A (18, 19) and B (17) streptococci, have had no effect on gram-negative bacteria. When the three viridins were tested against such organisms, surprisingly large numbers were found susceptible (Table 4). Neisseria were frequently susceptible to all three bacteriocins. Although gram-negative bacilli were less commonly susceptible than *Neisseria* sp., various isolates of all six genera tested were inhibited. As with the gram-positive organisms, variable susceptibilities to the bacteriocins were also noted among all the gram-negative bacteria.

A *Neisseria sicca* strain (15362) found to be very susceptible to all three viridins was selected for use as the indicator in further studies reported here.

Isolation of inhibitory substances. To enable further characterization of the bacteriocin-like activities from these three streptococcal strains, attempts were made at recovery of such activities in a cell-free state in a variety of solid and liquid media. Bacteriocin-like activity could be easily and repeatedly demonstrated on tryptic soy agar plates by the deferred antagonism technique (Fig. 1). The inhibitory zone extended beyond the edge of the growth of the producer strain. Liquor obtained

TABLE 3. *Spectrum of activity of three viridins against gram-positive bacteria*

Indicator	No. of strains tested	Susceptibility (%) to:		
		Viridin A	Viridin B	Viridin C
Group A streptococci	17–19	53	29	12
Group B streptococci	30–36	56	47	0
Viridans streptococci	26	4	7	0
Coagulase-negative staphylococci	18–22	96	78	56
S. aureus	32	97	66	72
Candida	4	0	0	0

TABLE 4. *Spectrum of activity of three viridins against gram-negative bacteria*

Indicator	No. of strains tested	Susceptibility (%) to:		
		Viridin A	Viridin B	Viridin C
Neisseria gonorrhoeae	12–19	68	67	75
Neisseria sp.	15–17	80	76	82
Pseudomonas	21	29	19	14
Proteus	41–59	75	31	29
Escherichia coli	27	11	7	7
Klebsiella	9	0	11	11
Shigella	16–19	63	21	32
Salmonella	7	0	14	14

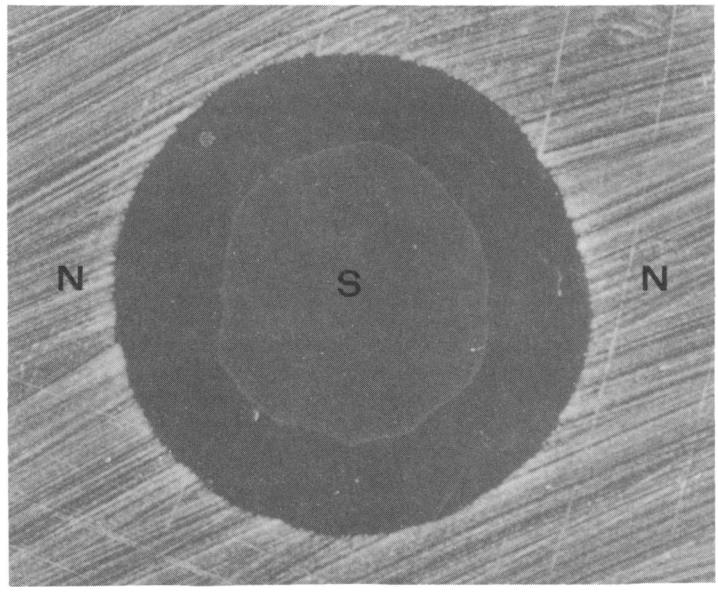

FIG. 1. *Inhibition of N. sicca (N) by S. mitis strain 42885 (S), using the deferred antagonism technique.*

after freeze-thaw treatment of agar on which producer strains had been previously grown and exposed to chloroform was tested for inhibitory activity. Although this procedure has been successful for obtaining bacteriocins from group A (19) and group B (17) streptococci, no activity could be recovered after similar treatment of the viridans streptococci. Elution of viridins from agar pieces adjacent to previous growth of producer strains also met with failure. The eluants used were 7 M urea, 1 M NaCl, 3 M KCl, 0.067 M phosphate buffer, and distilled water.

Attempts were also made to isolate the activity from supernatant fluid of various broth cultures incubated at 30 or 37 C under aerobic or anaerobic conditions. The broths used were brain heart infusion, Columbia, Trypticase soy, thioglycolate, and Todd-Hewitt. Although the strains grew well in these media under the conditions described, no inhibitory activity could be detected in the supernatant fluid. Concentration by ammonium sulfate precipitation, the addition of various amounts of sodium dodecyl sulfate (18), or induction by mitomycin C (4) was of no advantage.

Cells grown on tryptic soy agar or in tryptic soy broth always demonstrated activity whether tested by the deferred antagonism or the simultaneous antagonism techniques. Furthermore, cells previously killed by chloroform exposure or penicillin G treatment showed activities when placed on fresh tryptic soy agar plates and tested by both techniques. Elution or extraction of bacteriocins from live or killed cells using 3 M KCl, 7 M urea, various buffers, liquid chloroform, and ethanol all met with failure.

Disruption of streptococcal cells (*S. mitis* strain 42885), however, released substantial amounts of the bacteriocin (viridin B). The bacteria were grown in 1 liter of tryptic soy broth for 48 to 72 h at 37 C, and the cells were collected by centrifugation at 2,500 × *g* for 20 min. The cells were then washed in phosphate buffer and resuspended in 5 ml of the same buffer. Disruption of cells was accomplished by the use of a Potter-Elvehjem tissue homogenizer (Tri-R Instruments, Rockville Center, N.Y.). The tube was kept in an ice bath, and the homogenizer was used for 15-s intervals with 5-s pauses for a total of 15 min. The homogenized mixture was kept at 4 C overnight with constant stirring and then centrifuged at 13,000 × *g* for 90 min. The supernatant fluid, which contained most of the activity, was brought to 80% ammonium sulfate saturation. The resultant precipitate was subsequently dissolved in 5 ml of phosphate buffer, dialyzed for 18 h at 4 C against the same buffer, sterilized by filtration through a 0.45-μm membrane filter (Millipore Corp.), and used as the partially purified bacteriocin. Such preparations were stored at −20 C until used and usually contained 640 to 5,120 AU when tested against the *N. sicca* indicator.

Similar results were obtained with *S. sanguis* strain 24648 (viridin A) and with *S. mitis* strain 42991 (viridin C). Subsequent results reported hereafter deal with viridin B only, however.

Attempts to disrupt bacterial cells and release the bacteriocin by sonication instead of homogenization under similar experimental conditions were not successful for any of the three streptococcal strains.

Properties of viridin B. Using the partially purified viridin B, some of its physical and chemical properties were assessed. The bacteriocin did not pass through a dialysis membrane bag with a molecular weight cut-off of 12,000. It was filterable through a 0.2-μm membrane (Millipore Corp.).

The stability of viridin B to various temperatures is shown in Table 5. The initial bacteriocin concentration was 320 AU. It is evident that the bacteriocin is heat labile and that it is relatively unstable even at lower temperatures.

Figure 2 shows the activity of the bacteriocin at various pH values. A bacteriocin preparation, dissolved in phosphate buffer, pH 7.2, was divided into aliquots, and these were dialyzed against phosphate buffers of various pH values at 4 C for 18 h. The bacteriocin titer was determined in each aliquot. Stability was optimum at neutral pH, with total loss of the activity at low and at high pH values. At pH 4, a precipitate was noted that was totally inactive even after dissolving in buffer at neutral pH.

The effect of proteolytic enzymes on the activity of viridin B is shown in Table 6. Trypsin and protease totally abolished the bactericidal activity under the experimental conditions indicated. Papain had no such effect.

TABLE 5. *Stability of viridin B at various temperatures*

Temp (C)	Duration to inactivate bacteriocin totally
65	30 min
56	30 min
45	4 h
37	18–24 h
22	4 days
4	>15 days[a]
−20	>4 wk[a]

[a] Total inactivation was not detected, but marked reduction in titer occurred by indicated times.

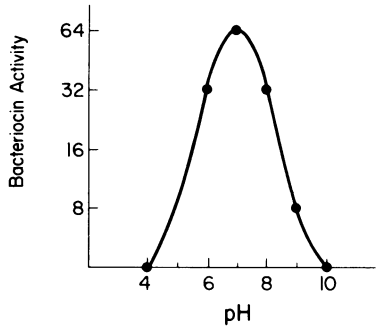

FIG. 2. *Activity (titer) of viridin B after dialysis at indicated pH values for 18 h at 4 C.*

TABLE 6. *Effect of proteolytic enzymes on viridin B*

Treatment[a]	Concn (mg/ml)	Titer	
		Initial	After treatment
Trypsin (2× crystallized)	0.5	16	0
Protease (Pronase B) ...	0.5	16	0
Papain	0.5	16	16
None (control)		16	16

[a] All incubations were done at 37 C for 30 min.

As pointed out earlier, although bacteriocin activity could be noted beyond the area of growth of the producer strain (Fig. 1), indicating that the bacteriocin diffuses in agar and that it may occur in a cell-free state, all attempts at recovery of activity from freeze-thaw liquor of agar met with failure. To assess whether binding of bacteriocin to agar occurs, a partially purified, cell-free preparation of viridin B was added to pieces of sterile tryptic soy agar and the mixture was incubated at 37 C. At intervals of 1, 2, 4, and 24 h after incubation, the titer of the bacteriocin was determined. A control of bacteriocin preparation without agar was assayed in a similar manner. Bacteriocin titers were comparable in the test and control specimens at 1, 2, and 4 h. Whereas the titer in the control specimen was reduced by eightfold at 24 h, probably due to heat inactivation of the bacteriocin, the titer in the test specimen remained unaltered. This finding suggests that the bacteriocin does not seem to bind to agar and that the agar stabilizes the bacteriocin under the experimental conditions described.

Inhibitory effect of viridin B. The action of viridin B was tested against *N. sicca* (strain 15362) and a coagulase-negative staphylococcus (strain 23709) to assess its bactericidal or bacteriostatic potential. Inhibition was tested by the rapid screening method devised by Hale and Hinsdill (10) and by the conventional kill-curve technique (5, 6), using different concentrations of the bacteriocin. By the rapid screening method, viridin B was noted to be bactericidal against the *N. sicca* but bacteriostatic against the coagulase-negative staphylococcus over a range of concentrations of the bacteriocin (320 to 1,280 AU).

The bactericidal effect of viridin B against *N. sicca* was observed also by the kill-curve method (Fig. 3). The presence of some nutrient medium (30% tryptic soy broth) was essential to demonstrate a bactericidal effect, and no killing was noted when the bacteriocin and organisms were mixed in phosphate buffer alone. Killing was minimal at 4 C, moderate at 37 C, and most pronounced at 22 C. The bactericidal effect of viridin B was comparable against logarithmic-phase and stationary-phase bacterial cells. By the same method, and using the same bacteriocin concentrations (320 to 1,280 AU), viridin B was only bacteriostatic against the coagulase-negative staphylococcus.

Adsorption of bacteriocin to cells. Four organisms were tested for their ability to adsorb viridin B, using a previously described technique (7). The organisms tested were: *N. sicca* (15362), which is killed by the bacteriocin; a coagulase-negative staphylococcus (23709) that

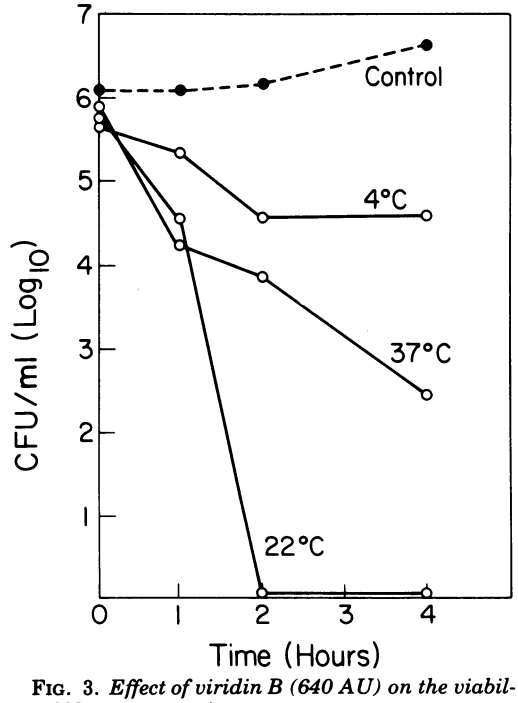

FIG. 3. *Effect of viridin B (640 AU) on the viability of N. sicca at various temperatures.*

is only inhibited by the bacteriocin; and two resistant bacteria, a *Klebsiella* sp. and a *Neisseria* sp. In each instance, 1 ml of the bacteriocin preparation was added to 5 g (wet weight) of the bacterial cells. The bacteriocin titer was not altered in any instance. Lack of adsorption was noted using a range of bacteriocin concentrations from 20 to 640 AU, using logarithmic- and stationary-phase organisms, in the presence or absence of additional tryptic soy broth, and after incubation at 22 and 37 C for intervals of 5 to 60 min.

Further purification of viridin B. The partially purified bacteriocin, after ammonium sulfate precipitation, was further purified by gel filtration on a Sephadex G-100 column (Fig. 4). The bactericidal activity was all contained in a single peak, and the bacteriocin could be separated from other substances lacking any bactericidal effect.

DISCUSSION

Although the inhibitory capacity of alpha-hemolytic streptococci has been recognized for some time (2, 14-16), the exact nature of such inhibition has not been elucidated previously. The present report strongly suggests that this inhibition is the function of a bacteriocin or bacteriocins produced by these organisms. The data presented indicate that the viridins produced by alpha-hemolytic streptococci are indeed bacteriocins, since these viridins are proteinaceous bactericidal substances, produced by one bacterial species, that inhibit the growth of other bacteria.

Several features of the viridins are unique when compared with previously described bacteriocins. Bacteriocins of gram-positive bacteria are now recognized to differ in many characteristics from bacteriocins of gram-negative bacteria. Such differences will be discussed in a forthcoming report (Tagg, Dajani, and Wannamaker, manuscript in preparation). In this paper, comparison of viridins to other bacteriocins of gram-positive bacteria will be the major concern.

The frequency of production of bacteriocins by alpha-hemolytic streptococci is considerably higher than the frequencies reported for other gram-positive cocci. Of 130 group A streptococci tested, 15 (11.5%) produced bacteriocins (19). In group B streptococci, only 2 of 105 isolates (1.9%) were found to be bacteriocinogenic (17). Among phage type 71 staphylococci of human origin, 25 of 65 strains (39%) produced a staphylococcin (3). Staphylococci of animal origin were found to be less commonly bacteriocinogenic (9). The rate of 78% bacteriocin production among the alpha-hemolytic streptococci reported here is, therefore, unusual.

Different patterns of inhibitory capacities were noted among the 120 viridans streptococci when tested against four indicator strains. Furthermore, when three viridins were tested against a large number of gram-positive and gram-negative bacteria, variable activity spectra were observed (Tables 3 and 4). These findings suggest that viridins may be a very heterogenous group of bacteriocins.

The susceptibility of many gram-negative

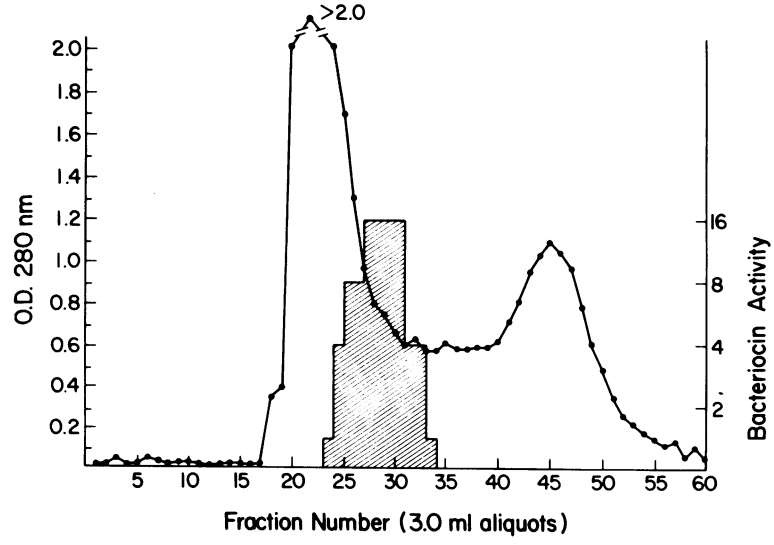

FIG. 4. *Gel filtration of partially purified viridin B on a Sephadex G-100 column. Each 3-ml fraction was assayed for protein content (●) and for bacteriocin titer (shaded area).*

bacteria to viridins is perhaps the most outstanding characteristic of these bacteriocins. In this study, gram-negative cocci were very commonly susceptible to all three viridins, and a variety of enteric gram-negative bacilli were also frequently inhibited. Although bacteriocins of gram-positive bacteria generally have a broader spectrum of activity than bacteriocins of gram-negative bacteria (13), no previously described bacteriocinogenic gram-positive bacterium has been shown to inhibit the growth of gram-negative bacteria (4, 6, 8, 9, 11, 13, 17-19).

The isolation of a bacteriocin from the producing bacteria is an essential prerequisite for purification and further characterization of the bacteriocin. Recovery of bacteriocins in a cell-free state has been achieved by different methods. Certain bacteriocins can be recovered in supernatant fluids of broth cultures of the bacteriocinogenic organism (5, 6, 18). Some other bacteriocins can be found in freeze-thaw liquor of agar on which the producing strain had been grown previously (17, 19). In some instances bacteriocins have been found to be closely associated with the bacterial cells, but they can be eluted off the cells after extraction with 7 M urea (9) or with 5% NaCl (12) or released from cells after mechanical cell breakage (8). In this regard, the viridins were recovered in a cell-free state only after homogenization of the bacterial cells, making them similar to staphylococcin 414 described by Gagliano and Hinsdill (8). Extensive efforts are needed, therefore, to achieve this purpose, and the optimum method has to be assessed individually. Difficulty in isolating bacteriocins in a cell-free state may explain, in part, the inability in the past to identify the inhibitory effect of alpha-hemolytic streptococci as a function of bacteriocins (2, 14, 16).

Other properties of the viridins deserve some discussion. The heat lability of viridin B is rather unusual for a bacteriocin of gram-positive bacteria. Such lability may explain the more pronounced killing effect at 22 C as compared with 37 C. Instability at lower temperatures can occur with certain bacteriocins, particularly after purification, and can be reduced upon addition of bovine serum albumin (17). Data presented in this report suggest that agar may also stabilize some bacteriocins.

The narrow pH range for optimal activity of viridin B is also unusual. Streptocin A has been shown to be active in an acid solution (pH 2 to 7) and loses activity only at pH levels above 11 (18). Similarly, streptocin B was quite stable at lower pH values (pH 2 to 6.5) and required boiling for 5 min at pH 13 to be completely inactivated (17). The bacteriocin from phage type 71 *S. aureus* had comparable activities in samples where the pH ranged from 4.0 to 8.5 (6).

Viridin B has been shown to be bactericidal against an *N. sicca* strain but had only a bacteriostatic effect on a coagulase-negative staphylococcus. This difference is probably not related to bacteriocin concentrations alone, since the amounts used ranged from 320 to 1,280 AU. A bacteriostatic rather than a bactericidal effect is most unusual for bacteriocins but has been reported for staphylococcin 462 by Hale and Hinsdill (10).

Viridin B is also similar to staphylococcin 462 (10) in that adsorption to susceptible bacteria could not be demonstrated for either bacteriocin. To demonstrate adsorption, it is important to use low bacteriocin concentrations and an excess of bacterial cells (7, 10), which was attempted in this study. Adsorption onto specific receptors is generally considered the initial stage in the killing effect of bacteriocins (13). Such adsorption may be specific (only to susceptible cells) (7, 13) or may be nonspecific (to one or more bacterial species whether individual strains are susceptible or not) (8, 11, 17). Apparently, in some instances, adsorption does not exist or at least cannot be demonstrated.

Partial purification of viridin B was achieved after ammonium sulfate precipitation and gel filtration of material obtained from mechanically disrupted cells. Additional purification is warranted before further chemical identification of the bacteriocin is attempted. Such studies, further characterization of the bacteriocin, and investigation of its mode of action are in progress.

ACKNOWLEDGMENT

This work was supported by a grant from the Matilda Wilson Fund, Detroit, Mich.

LITERATURE CITED

1. Buchanan, R. E., and N. E. Gibbons. 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
2. Crowe, C. C., W. E. Sanders, Jr., and S. Longley. 1973. Bacterial interference. II. Role of the normal throat flora in prevention of colonization by Group A streptococcus. J. Infect. Dis. 128:527-532.
3. Dajani, A. S. 1972. A bacteriocin from phage type 71 *Staphylococcus aureus*: its effect on Group A streptococci, p. 157-168. *In* L. W. Wannamaker and J. M. Matsen (ed.), Streptococci and streptococcal diseases. Recognition, understanding and management. Academic Press Inc., New York.
4. Dajani, A. S., E. D. Gray, and L. W. Wannamaker. 1970. Bactericidal substance from *Staphylococcus aureus*. Biological properties. J. Exp. Med. 131:1004-1015.

5. Dajani, A. S., E. D. Gray, and L. W. Wannamaker. 1970. Effect of a bactericidal substance from *Staphylococcus aureus* on group A streptococci. I. Biochemical alterations. Infect. Immun. 1:485–490.
6. Dajani, A. S., and L. W. Wannamaker. 1969. Demonstration of a bactericidal substance against beta-hemolytic streptococci in supernatant fluids of staphylococcal cultures. J. Bacteriol. 97:985–991.
7. Dajani, A. S., and L. W. Wannamaker. 1973. Kinetic studies on the interaction of bacteriophage type 71 staphylococcal bacteriocin with susceptible bacteria. J. Bacteriol. 114:738–742.
8. Gagliano, V. J., and R. D. Hinsdill. 1970. Characterization of a *Staphylococcus aureus* bacteriocin. J. Bacteriol. 104:117–125.
9. Hale, E. M. and R. D. Hinsdill. 1973. Characterization of a bacteriocin from *Staphylococcus aureus* strain 462. Antimicrob. Agents Chemother. 4:634–640.
10. Hale, E. M. and R. D. Hinsdill. 1975. Biological activity of staphylococcin 462. Bacteriocin from *Staphylococcus aureus*. Antimicrob. Agents Chemother. 7:74–81.
11. Jetten, A. M., and G. D. Vogels. 1972. Nature and properties of a *Staphylococcus epidermidis* bacteriocin. J. Bacteriol. 112:243–250.
12. Jetten, A. M., G. D. Vogels, and F. DeWindt. 1972. Production and purification of a *Staphylococcus epidermidis* bacteriocin. J. Bacteriol. 112:235–242.
13. Reeves, P. 1972. The bacteriocins. Molecular biology biochemistry and biophysics, vol. 11. Springer-Verlag, New York.
14. Sanders, E. 1969. Bacterial interference. I. Its occurrence among the respiratory tract flora and characterization of inhibition of Group A streptococci by viridans streptococci. J. Infect. Dis. 120:698–707.
15. Sprunt, K., G. A. Leidy, and W. Redman. 1971. Prevention of bacterial overgrowth. J. Infect. Dis. 123:1–10.
16. Sprunt, K., and W. Redman. 1968. Evidence suggesting importance of role of interbacterial inhibition in maintaining balance of normal flora. Ann. Intern. Med. 68:579–590.
17. Tagg, J. R., A. S. Dajani, and L. W. Wannamaker. 1975. Bacteriocin of a group B streptococcus: partial purification and characterization. Antimicrob. Agents Chemother. 7:764–772.
18. Tagg, J. R., A. S. Dajani, L. W. Wannamaker, and E. D. Gray. 1973. Group A streptococcal bacteriocin: production, purification and mode of action. J. Exp. Med. 138:1168–1183.
19. Tagg, J. R., R. S. D. Read, and A. R. McGiven. 1973. Bacteriocin of a group A streptococcus: partial purification and properties. Antimicrob. Agents Chemother. 4:214–221.