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Bacteriocin of a Group B Streptococcus: Partial Purification and Characterization

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The production of bacteriocin-like inhibition by two of 135 strains of group B streptococci was demonstrated during the growth of these organisms on solid nutrient media. Although a variety of gram-positive organisms was susceptible, none of a wide range of different gram-negative strains was inhibited. The active substance produced by one of these strains was extracted from cultures grown on solid media (Todd-Hewitt agar) and was shown to be bactericidal in action. This bacteriocin (streptocin B_1) was partially purified by ammonium sulfate precipitation followed by gel filtration and ion-exchange chromatography. Production of the bacteriocin was repressed by addition of certain fermentable carbohydrates to the basic medium and enhanced by the addition of yeast extract. The bacteriocin was shown to exist in two distinct and interconvertible physical forms: a basic unit of molecular weight 10,000 and an aggregate having a molecular weight of over 200,000. The bacteriocin was inactivated by proteolytic enzymes and, although labile in alkali, it was stable to boiling in mild acids.

Studies of the antagonistic activity of streptococci have indicated that bacteriocinogenicity may be widespread within the genus (2, 13-15, 19, 20, 22, 25, 27). Very few of the streptococcal inhibitory agents have yet been characterized, largely due to difficulties experienced in isolating and purifying the substances from liquid cultures (13, 15, 23, 29). It has become apparent that the composition of the medium and conditions of incubation are critical factors and that these may vary from one antagonistic strain to another.

In earlier studies (24-26) we reported the partial purification and characterization of streptocin A, a bacteriocin produced by a group A streptococcus. More recently we have examined bacteriocinogenicity within the group B streptococci (J. R. Tagg and A. S. Dajani, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, m111, p. 85). The present report deals with the isolation and preliminary characterization of one of these bacteriocins, which we refer to as streptocin B_1.

MATERIALS AND METHODS

Bacterial strains. One hundred and five strains of group B streptococci from human sources were selected from culture collections at the University of Minnesota and the Children's Hospital of Michigan. An additional 30 strains of group B streptococci from cases of mastitis in cattle were obtained by courtesy of R. Farnsworth of the College of Veterinary Medicine, University of Minnesota. Serological identification of the streptococci was confirmed by capillary tube precipitation tests using group- and type-specific rabbit antisera. The two streptocin B_1-producing organisms are identified as strains 74-628 and 74-629 of the culture collection in the Department of Pediatrics at the University of Minnesota. Strains of the other bacterial species tested for susceptibility to streptocin B_1 were obtained from culture collections of the Departments of Microbiology and Pediatrics at the University of Minnesota and from the Diagnostic Microbiology laboratories at the Children's Hospital of Michigan. Organisms in regular use were subcultured weekly on blood agar and stored at 4 C. Stock cultures of all strains were stored at −70 C and in the lyophilized state.

Media and chemicals. Liquid media tested as substrates for the production of bacteriocin included Todd-Hewitt broth (THB), tryptic soy broth, tryptose phosphate broth, and brain heart infusion, all obtained from Difco. Purple broth base, used in preparation of carbohydrate broths for fermentation studies, and tryptose blood agar base were also from Difco. Fetal calf serum was from Grand Island Biological Co., Grand Island, N.Y., and sheep blood was obtained from Brown Laboratories, Topeka, Kan. Mannitol, maltose, sucrose, glucose, fructose, iodoacetic acid, and bovine serum albumin (fraction V) were from Sigma Chemical Co., St. Louis, Mo. Solid media were prepared by addition of 1.5% agar (Difco) to the various liquid media.

Detection and assay of bacteriocin activity. Screening tests for the demonstration of bacteriocin

production utilized the methods of either simultaneous or deferred antagonism, outlined previously (26). Similarly, the method used for the critical dilution spot-test assay of inhibitory activity in solutions has also been described before (24). This inhibitory activity of the bacteriocin, as measured in the spot-test assay, was expressed as arbitrary units (AU) per milliliter. "Inhibitory activity" in the present sense is taken to indicate the prevention of growth of the indicator strain and not necessarily the killing of the organisms. The highest twofold dilution of the test preparation to give definite inhibition of a standard indicator lawn was arbitrarily defined as containing 1 AU/ml. The titer of bacteriocin was the reciprocal of this dilution. The standard indicator lawn was freshly seeded on well-dried blood agar plates by pouring on and then decanting off a 1:100 dilution of an 18-h THB culture of *Micrococcus luteus*. Specific bacteriocin activities were expressed in terms of AU per milligram of protein, after quantitation of the amount of protein in the samples by the method of Lowry et al. (18). The bactericidal activity of the bacteriocin was determined by measurement of the decrease with time in the number of colony-forming units of a suspension of a susceptible indicator strain after mixing the cells with a bacteriocin preparation. The procedures adopted have been described previously (24).

Preparation of streptocin B_1. The bacteriocin was prepared from lawn cultures of the producer strain grown on Todd-Hewitt agar (THA) supplemented with 1% yeast extract. The plates were seeded by flooding with and then decanting a logarithmic-phase culture, diluted 1:1,000 in THB. After incubation at 35 C for 18 h, the contents of a batch of plates (2 to 3 liters) were removed to a bag of cotton gauze. This was then frozen at −20 C and later allowed to thaw while the bag was suspended over a beaker. The crude extract obtained by this procedure was then clarified by filtration through Filter Cel (Hyflo Filter Cel, Johns-Manville, N.Y.) and adjusted to pH 6.5 with 5 N sodium hydroxide. Heat-labile streptococcal enzymes were inactivated by heating the extract at 98 C for 15 min.

Streptocin B_1 was precipitated from the crude extract by adding solid ammonium sulfate to 60% saturation at 4 C and was recovered by collection on Filter Cel and elution into a minimal volume of M/15 phosphate-buffered saline (PBS; pH 6.5). This preparation was then fractionated by chromatography on Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). A column (bed size, 3 by 90 cm), prepared with M/15 PBS (pH 6.5), was used to process samples of up to 15 ml at a flow rate of 15 to 20 ml/h. Eluant fractions of 5 ml were collected and assayed for inhibitory activity and for protein content (optical density at 280 nm). Fractions containing inhibitory activity were combined into two pools. The first contained high-molecular-weight bacteriocin, which was excluded from the gel at the column void volume and thus had an apparent molecular weight in excess of 200,000. The second pool contained low-molecular-weight bacteriocin, which was eluted from the column just ahead of the bulk of peptide material from the growth medium. Void volumes were determined with Blue Dextran (Pharmacia).

Further purification of these two forms of bacteriocin involved ion-exchange chromatography on carboxymethyl-cellulose (carboxymethyl-Sephadex C-25). It was found that only the low-molecular-weight species of the bacteriocin was adsorbed to the resin in M/15 PBS (pH 6.5). Samples (50 ml) were applied to carboxymethyl-cellulose columns (2.5 by 9.0 cm). Then, after washing with 2 to 3 column volumes of PBS, low-molecular-weight bacteriocin was eluted with 0.5 M sodium chloride in the same buffer. Inhibitor activity and optical density were determined as described. Concentration and dialysis of fractions containing the bacteriocin could be achieved by use of an ultrafiltration cell (model 52) fitted with a UM-2 membrane (Amicon Corp., Lexington, Mass.). Highly purified bacteriocin remained active when stored in the lyophilized state if 0.5% bovine serum albumin (Sigma) was first added as a protective agent.

Miscellaneous procedures. The demonstration of bacteriocin adsorption to washed whole cells followed previously outlined procedures (4).

RESULTS

Incidence of bacteriocin production in group B streptococci. Two of 105 group B streptococci isolated from human sources were found to produce a substance(s) which interfered with the growth of the majority of the other strains of group B streptococci when interactions among all of these strains were studied in deferred and simultaneous antagonism tests on blood agar medium. Serological typing of 66 of these strains indicated a distribution of 11 type Ia, 5 type Ib, 29 type Ic, 9 type II, 10 type III, and 2 not typable. None of 30 group B streptococci isolated from cattle appeared to have any antagonistic activity. A variety of tests, also used in a previous study (26), excluded the possibility that the interference was due to the release of bacteriophage or hydrogen peroxide or to the development of an inhibitory pH. It was considered most likely that the inhibition was due to the production of streptococcal bacteriocins (streptocins) by these two organisms.

The antagonistic strains were both serologically type II and appeared to have identical inhibitory spectra (see below). Bacteriocin(s) produced by these strains is referred to as streptocin B_1. The bacteriocin of strain 74-628 was selected for detailed investigation in the present study.

Inhibitory spectrum of streptocin B_1. Various gram-positive and gram-negative bacteria were tested for susceptibility to streptocin B_1 by using strain 74-628 in simultaneous and in deferred antagonism tests. The plate shown in Fig. 1 illustrates for demonstration purposes

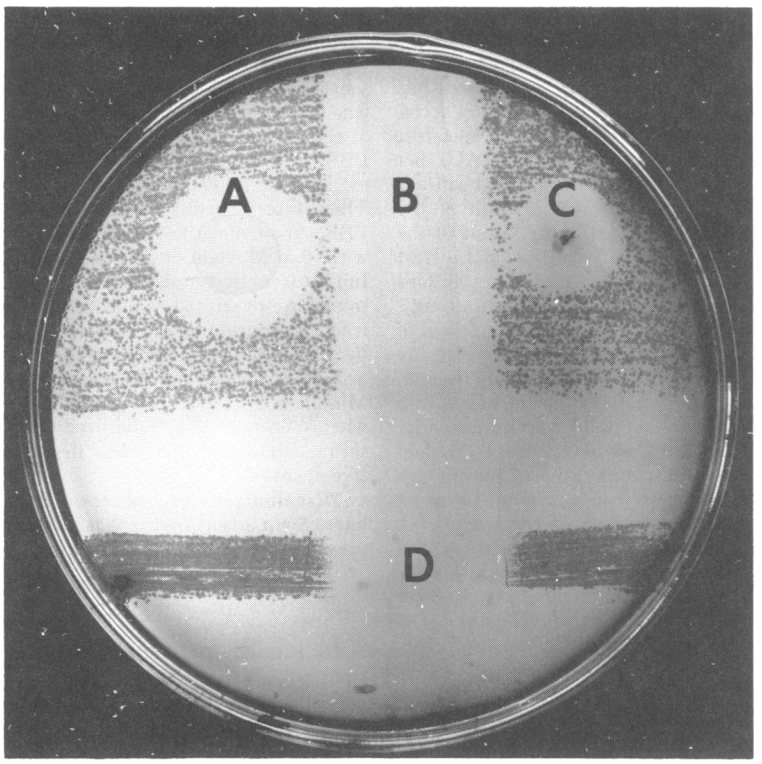

FIG. 1. *Inhibition of M. luteus by group B streptococcus strain 74-628. Strain 74-628 was inoculated as a drop (A), a vertical streak (B), or a stab culture (C) onto an indicator lawn, freshly seeded on the upper half of the plate. Formation of wide zones of inhibition indicated the production and release of a diffusible inhibitor by strain 74-628. For demonstration of deferred antagonism, the lower half of the vertical streak culture of the producer strain was removed. A cross-streak inoculum (D) of M. luteus failed to grow near the original growth line of strain 74-628.*

several different testing procedures; *M. luteus* was the indicator culture. Some of the organisms tested in deferred antagonism tests and those determined to be susceptible to streptocin B_1 are listed in Table 1. None of the gram-negative strains examined were susceptible to streptocin B_1, but the growth of many gram-positive strains was inhibited. Antagonism of streptococci belonging to groups A, B, C, D, F, and G was variable; however, the proportion of susceptible strains was greatest in groups A (66%) and B (57%). The susceptibility or resistance of these strains to streptocin B_1 did not appear to be determined by the serological type of the organisms. Certain species of *Bacillus* were susceptible, whereas other species appeared resistant. Staphylococci were in general resistant, with only 14% susceptible.

The most susceptible organisms include *M. luteus* and *Staphylococcus aureus* strain CIT, the latter being a lemon-pigmented strain formerly known as *S. citreus*. Both of these strains were regularly used as indicators when testing for streptocin B_1 activity. It was of interest to note that *Streptococcus pyogenes* strain FF-22, a producer of streptocin A (26), was susceptible to streptocin B_1, whereas *S. aureus* strain C 55, a producer of a staphylococcin (3), appeared to be resistant. Although strain 74-628 did not appear active when tested against itself in either deferred or simultaneous antagonism tests, crude streptocin B_1 preparations having a titer of 64 or greater were inhibitory when spot tested on lawns of the producer strain. Neither streptocin A nor the staphylococcin seemed to interfere with the growth of strain 74-628 in deferred or simultaneous antagonism tests.

Conditions affecting the production of streptocin B_1 on solid media. A number of culture parameters were systematically varied to determine the effect on the manufacture of streptocin B_1 by the producer organism. It was of importance to identify optimal conditions for the production of high-yield preparations of the

bacteriocin for use in subsequent purification studies. Production of the bacteriocin on solid media was assessed both by simultaneous and by deferred antagonism and also by testing the liquor obtained by freeze-thaw elution from cultures of strain 74-628 on solid media.

Preliminary assessment of streptocin B_1 production indicated that THA was a more suitable substrate than either brain heart infusion agar or tryptic soy agar. Production of the bacteriocin was significantly greater at 35 C than at either 30 or 39 C. Incubation at 42 C resulted in complete suppression of bacteriocin production, whereas growth of the producer strain was only slightly reduced. By use of the deferred antagonism test (Fig. 2), it was demonstrated that incubation at 42 C inhibited the production of the bacteriocin and did not merely interfere with the killing activity of bacteriocin produced at this temperature. In addition, it was found that the ability to produce an inhibitory effect returned to these organisms when cross-streaked and reincubated at 35 C. Other experiments (unpublished observations) have also indicated that there is no apparent spontaneous curing of the bacteriocinogenic property of strain 74-628 during growth at elevated temperatures.

Crude liquor obtained by the alternate freezing and thawing of solid media was tested for inhibitory activity to determine the effect of variations in the density of the producer lawn cultures and in the length of incubation of these cultures upon the yield of streptocin B_1. A series of cultures of strain 74-628 was prepared by using decimal dilutions of an 18-h THB culture to seed lawns on the THA supplemented with 1% yeast extract. The greatest yields of bacteriocin were obtained from lawns sown with a 1:1,000 dilution of the producer strain. Studies of the dynamics of production indicated no detectable activity in the first 6 h of incubation. Peak yields, having a titer of 32 to 64 AU/ml, were obtained between 18 to 24 h, with a fall to about 75% of this activity at 72 h.

Prior treatment of strain 74-628 with either mitomycin C (0.5 to 2.0 μg/ml) or ultraviolet irradiation (15 to 30 s), using previously described procedures (26), did not appear to enhance the production of bacteriocin when the cells were subsequently grown on solid or in liquid media.

The influence of various additives to the basic media (tryptic soy agar and THA) on the yield of streptocin B_1 was investigated. Eighteen-hour cultures of strain 74-628 were diluted 1:1,000 in tryptic soy broth and in THB and then were used to seed lawns on tryptic soy agar- and THA-based media, respectively. Bacteriocin activity was determined in the freeze-thaw eluted supernatants prepared after incubation of the lawn cultures for 18 h at 35 C. By reference to Table 2 it can be seen that greater yields were obtained from THA-based media, with the greatest amount obtained by use of THA containing 1% yeast extract. An interesting effect was the apparent suppression of bacteriocin production by glucose, fructose, and sucrose, all of which were shown to be fermentable carbohydrate sources by the successful

TABLE 1. *Susceptibility of various organisms to streptocin B_1*

Susceptible organisms	No. susceptible/ no. tested	Resistant organisms	No. susceptible/ no. tested
Group A streptococcus	98/149	Gram-positive	
Group B streptococcus	27/47	α-Streptococci	2/2
Group C streptococcus	7/23	*Corynebacterium diphtheriae*	2/2
Group D streptococcus	5/29	*Bacillus cereus*	
Group F streptococcus	1/5	*B. licheniformis*	
Group G streptococcus	3/16	*Mycobacterium smegmatis*	
Streptococcus salivarius	2/2	*Listeria monocytogenes*	
S. lactis		*Streptococcus sanguis*	
S. pneumoniae	2/4	Gram-negative	
Staphylococcus epidermidis	1/6	*Klebsiella pneumoniae*	6/6
S. aureus	3/23	*Enterobacter cloacae*	
Micrococcus cyaneus		*E. aerogenes*	
M. luteus	2/2	*Pseudomonas aeruginosa*	13/13
M. roseus		*P. fluorescens*	
Bacillus coagulans		*P. maltophilia*	
B. cereus var. *mycoides*		*Escherichia coli*	
B. anthracis		*Serratia marcescens*	
		Shigella sonnei	

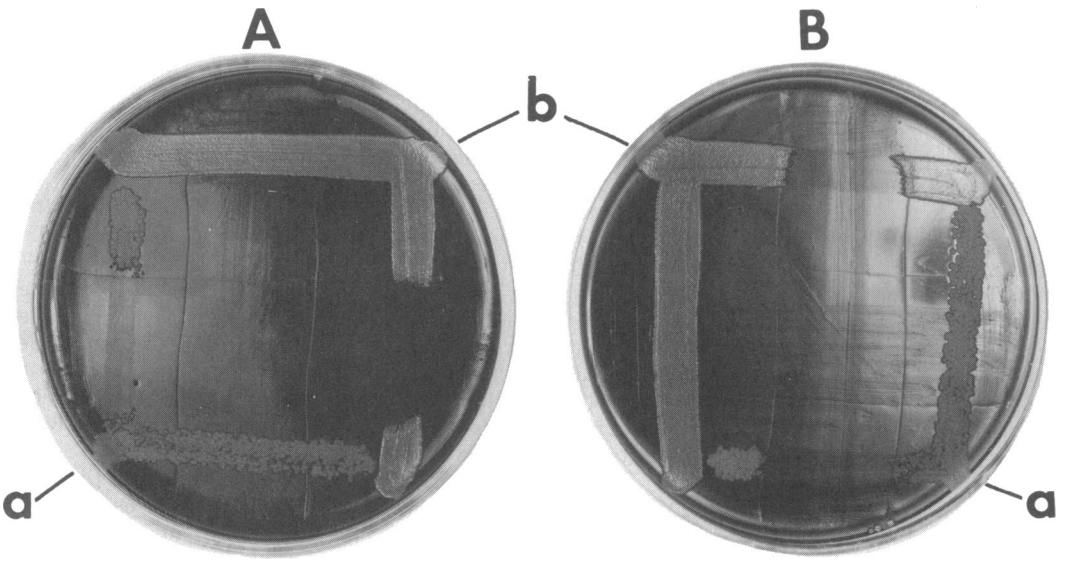

FIG. 2. *Inhibition of indicator strains S. aureus strain CIT (a) and M. luteus (b) by group A streptococcus strain 74-628 in deferred antagonism tests conducted at 35 and 42 C. (A) Strain 74-628 was first incubated at 42 C as a vertical streak culture. A portion of this growth was then streaked horizontally (at right angles to the original streak) and incubated at 35 C. This growth was then removed and indicator cultures were grown as peripheral streaks. Bacteriocin appears to have been produced at 35 C, but not at 42 C. (B) Strain 74-628 was first incubated at 35 C (vertical streak) followed by incubation at 42 C (horizontal streak). Again, active bacteriocin was formed only at 35 C.*

TABLE 2. *Yield of streptocin B_1 by use of different growth media*

Supplement (1%)	Titer (AU/ml) of streptocin B_1 in extract of solid media	
	THA base	TSA base[a]
Nil	16	4
Maltose	16	4
Mannitol	16	4
Glucose	2	0
Fructose	2	0
Sucrose	2	0
Neopeptone	16	2
Yeast extract	32	4
Sheep blood (5%)	16	4

[a] TSA, Tryptic soy agar.

growth and acid production (pH 4.2 to 4.8) by strain 74-628 in purple broths (Difco) containing 1% concentrations of the individual sugars. Maltose, although fermented by strain 74-628 to give a terminal pH of 4.2, did not appear to interfere with the production of the bacteriocin. Mannitol was neither an inhibitor of bacteriocin nor was it an available substrate for the producer strain. Active preparations of streptocin B_1 showed no loss of activity when the sugars were added to 1% concentration.

Bacteriocin production in liquid cultures. Numerous attempts were made to recover active streptocin B_1 from liquid cultures of the producer strain when grown at temperatures between 30 to 37 C. Media tested included THB, tryptic soy broth, brain heart infusion, and tryptose phosphate broth. Supplements tested in various combinations included 1% yeast extract, neopeptone, glucose and mannitol, and 5% fetal calf serum.

Broth cultures were incubated for various intervals, and the supernatant fluids were tested following centrifugation (15,000 $\times$ *g* for 20 min) and filtration through a membrane filter (0.45-μm pore size; Millipore Corp., Bedford, Mass.). In addition, all culture supernatants were tested for bacteriocin after 10-fold concentration by freeze drying. No culture supernatants had inhibitory activity when tested directly; however, trace amounts of the bacteriocin could be detected in the 10-fold concentrate from THB supplemented with 1% neopeptone. This low level of activity was considered inadequate for purification trials.

It was impossible to detect bacteriocin activity from washed cells of broth cultures, whole washed cells killed with chloroform or by heating at 80 C for 10 min, or cells disrupted in a Mickle disintegrator (Mickle Laboratory, Gomshall, Surrey) or after extraction (18 h at 4 C) of the packed cells with an equal volume of 6 M urea or 1 M sodium chloride.

Purification of streptocin B_1. Results of the

purification of streptocin B_1 are summarized in Table 3. Chromatography on Sephadex G-200 (Fig. 3) indicated that the inhibitory activity was associated with two distinct molecular species. The smaller inhibitory substance appeared to have a molecular weight of about 10,000, as determined by its position of elution from the G-200 column relative to the standard marker proteins myoglobin, cytochrome *c*, and streptocin A (24).

Properties of streptocin B_1. It appeared that the two different inhibitory substances isolated from the crude extract represent a single bacteriocin that occurs as either a monomeric form or a high-molecular-weight aggregate of this basic unit. The two forms seemed to have identical inhibitory spectra when spot tested against organisms susceptible to the crude bacteriocin. The proportion of each form was largely dependent upon the ionic strength of the solution, the high-molecular-weight form being favored by low salt concentrations and the low-molecular-weight unit occurring in high salt solutions. Interconversion of the two forms could be demonstrated by variation of the ionic strength. High-molecular-weight bacteriocin excluded from Sephadex G-200 was dialyzed against 1.0 M PBS using a UM-2 membrane. Chromatography of this dialyzed material on Sephadex G-200 equilibrated with 1.0 M PBS indicated that over 50% of the residual activity now occurred in the low-molecular-weight form. By contrast, dialysis of the high-molecular-weight form against M/15 PBS did not result in any detectable conversion to low-molecular-weight bacteriocin. In a similar study, dialysis of low-molecular-weight bacteriocin using 5 mM PBS was found to give almost complete conversion to

TABLE 3. *Stepwise purification of streptocin B_1*

Step	Vol (ml)	Bacteriocin activity (AU)	Protein (mg)	Sp act (AU/mg)	Recovery (%)	Times purified
Crude filtrate	300	19,200	6,720	2.86		
Ammonium sulfate precipitate						
0–40%	10	6,400	77	83	33	29
40–60%	20	6,400	533	12	33	4.2
60–80%	30	1,920	1,267	1.5	10	0.5
Sephadex G-200 chromatography						
Large form	288	1,440	40	36	7.5	12.6
Small form	690	4,140	248	16.7	21.5	5.8
Carboxymethyl-cellulose chromatography and Diaflo concentration						
Large form	30	480	9.6	50	2.5	17.5
Small form	70	280	26	10.8	1.5	3.8

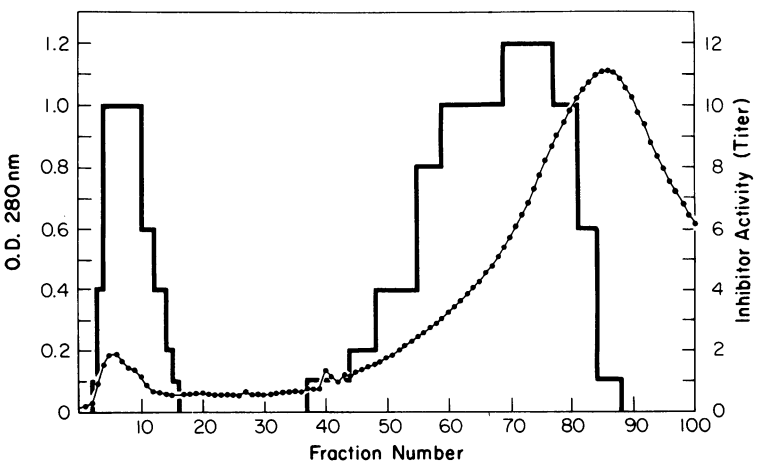

FIG. 3. *Gel filtration of a 5-ml sample of 60% ammonium sulfate-precipitated streptocin B_1 on Sephadex G-200. Each fraction was assayed for streptocin B_1 activity (——) and protein (●).*

high-molecular-weight bacteriocin. Prolonged dialysis of the crude bacteriocin against 6 M urea solution did not appear to favor dissociation into low-molecular-weight bacteriocin.

The crude bacteriocin, when extracted from agar, was an extremely stable substance and could be stored at 4 C for at least 6 months with little loss in activity. By comparison, the purified bacteriocin was considerably less stable, particularly the low-molecular-weight form, which became inactive when held at 4 C for 1 week. Some protection was afforded, however, by addition of 0.5% (wt/vol) bovine serum albumin to the purified preparations.

The crude bacteriocin (at pH 6.5) showed no detectable loss in activity when boiled for 15 min and retained approximately 75% of activity on autoclaving at 120 C for 15 min. Although quite stable in acid (pH 2 to 6.5), crude streptocin B_1 became progressively less stable in increasing alkalinity. Boiling for 5 min at pH 13 resulted in complete inactivation of the bacteriocin.

A crude streptocin B_1 preparation (pH 7.0) having a titer of 10 was completely inactivated on incubation with Pronase (1 mg/ml) for 60 min at 37 C. The bacteriocin was less susceptible to trypsin; incubation with 5 mg of trypsin per ml for 1 h at pH 8 was required to effect a 50% reduction in activity. Enzyme action was terminated by heating at 75 C for 10 min. Both large and small forms of the bacteriocin were susceptible to proteolytic inactivation.

Additional tests indicated no loss of activity of crude streptocin B_1 on incubation for 30 min at 35 C with 5 mM iodoacetate and thus indicated that free sulfhydryl groups were not important for activity. Also the crude bacteriocin seemed stable to mechanical agitation, since vigorous bubbling of N_2 through a preparation did not decrease the titer.

Studies of the inhibitory action of streptocin B_1. Although the inhibitory spectrum of streptocin B_1 appears rather specific, in that it is confined to only certain gram-positive organisms, the bacteriocin lacked adsorption specificity. Washed cells from 18-h, 35 C cultures of *S. aureus* strain CIT and of the streptocin B_1-resistant organisms *Escherichia coli* and *S. aureus* 502A were found equally effective in removing streptocin B_1 activity from solution. Adsorption could be demonstrated using either viable or heat-killed (96 C/30 min) cells.

Investigations of the nature of the inhibitory action of streptocin B_1 (Fig. 4) indicated a bactericidal effect. Logarithmic-growth-phase cultures of *S. aureus* strain CIT were treated with a preparation of crude streptocin B_1 (titer 32), and subsequent viable counts showed a substantial decrease in the number of colony-forming units. It can be seen that this lethal effect was markedly temperature dependent, with less killing at lower temperatures. Moreover, the lack of any change in the optical density of the cultures associated with death of the cells indicated that the lethal effect was unaccompanied by cell lysis.

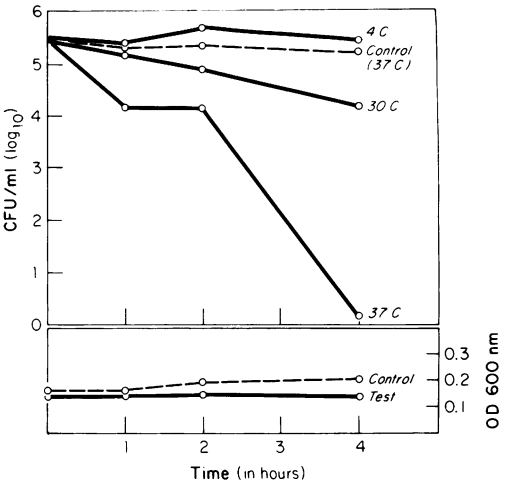

FIG. 4. *Effect of crude streptocin B_1 (titer 32) (solid line) or M/15 PBS (dashed line) on the optical density ($OD_{600\ nm}$) at 37 C and viability (colony-forming units [CFU]/ml) of a suspension of cells of S. aureus strain CIT. The bactericidal effect of streptocin B_1 was determined at 37, 30, and 4 C.*

DISCUSSION

The interference exhibited by two of 135 strains examined in this study appeared to be caused by an antibiotic material(s) with properties consistent with those of the loosely defined class of bacterial inhibitors called the bacteriocins (21). Little information is available concerning the production of bacteriocins by group B streptococci. As early as 1949 Sherwood and associates (23) had described an antibiotic, named streptostasin, which was produced by various beta-hemolytic streptococci, including one of nine strains of group B streptococci tested. In another study, Kuttner (15) reported that three of eight group B streptococci produced bacteriocin-like inhibition of a group A streptococcus indicator strain. This same indicator was used by Prakash et al. (20), who found that five of 14 strains of group B streptococci were antagonistic. None of these studies reported the isolation or characterization of the inhibitory substances.

The most comprehensive study to date of the

group B streptococcal bacteriocins is that of Kramer and Brandis (14), who reported that eight of 121 group B streptococci interfered with the growth of indicator lawns of group B streptococci in simultaneous antagonism tests. One of these bacteriocins (B73) was isolated from the supernatant fluid of liquid SK-medium cultures and was partially purified by differential centrifugation and Sephadex gel filtration. This bacteriocin appears to have some properties in common with streptocin B_1. The activity spectra of the two bacteriocins appear remarkably similar, with widespread activity against group A and B streptococci and relatively less activity against staphylococci, *Corynebacterium diphtheriae*, and *Listeria monocytogenes*. Inhibitory activity directed against organisms of a number of different species is a characteristic of many of the bacteriocins of gram-positive species (8).

Streptocin B_1 did not appear to be inhibitory against its own producer strain when tested by deferred antagonism. However, as was found for streptocin A (26), this "auto" immunity is not absolute and high-titer preparations of crude streptocin B_1 interfered with the growth of the homologous strain.

Difficulties in obtaining satisfactory yields of bacteriocins from liquid media are common with bacteriocins of gram-positive species (6, 7, 9, 13, 15). In the present study the greatest yields of bacteriocin were obtained by growing the producer strain on THA supplemented with 1% yeast extract.

Glucose, sucrose, and fructose when added in 1% concentration to solid nutrient media suppressed production of streptocin B_1. This effect was not attributable to the production of low pH or to interference by these sugars in the inhibitory action of the released bacteriocin. In contrast, glucose enhances the production of streptocin A (26). Similarly, mannitol may suppress the production of staphylococcin 414, but it will enhance the production of staphylococcin 462 (7). Several other studies have also described the suppressive effects of glucose on the production of certain colicins (10, 17). Such effects have been attributed to repression of the bacteriocinogenic factor associated with the catabolism of some fermentable carbohydrates (17). The possibility of repressive effects such as these occurring should be kept in mind when selecting appropriate media for bacteriocin production. The medium of choice needs to be determined empirically for each particular bacteriocin.

Other factors found to be important for the demonstration of production of streptocin B_1 and streptocin A (26) on solid media include the density of the lawn and the period of incubation. As with most of the bacteriocins of gram-positive organisms, streptocin B_1 synthesis did not appear to be inducible by treatment with either mitomycin C or ultraviolet irradiation.

The bacteriocin was not detected in whole washed cells in significant amounts and presumably is released from the cells soon after being synthesized. Various methods of releasing cell-bound bacteriocins which have proved successful in other studies, including extraction with 5% sodium chloride (12), 6 M urea (7), or cell fragmentation (6), all proved unsuccessful in the current study.

Streptocin B_1 was found to occur in two distinct molecular forms, the relative proportion of each being dependent upon the ionic strength of the suspending solution. The occurrence of a bacteriocin in two different molecular species is not uncommon, particularly for bacteriocins of gram-positive species (1, 5–7, 12, 16, 22, 28). In a study by Schlegel and Slade (22) it was demonstrated that, as for streptocin B_1, the extent of dissociation-association was partially dependent upon the ionic strength of the solution.

Unlike staphylococcin 1580 (12), the high-molecular-weight form of streptocin B_1 could not be dissociated into subunits by treatment with 6 M urea. The molecular weight of this form of streptocin B_1 appeared to be greater than 200,000, since it was excluded from Sephadex G-200, and was probably in excess of 300,000 as it was retained by an XM300 ultrafilter (unpublished observation). The low-molecular-weight form seemed to be about 10,000 from its position of elution from Sephadex gels relative to marker proteins. This agrees well with the size of the B73 bacteriocin studied by Kramer and Brandis (14); however, there was no report of a high-molecular-weight form of this substance.

Like many other bacteriocins of gram-positive species (3, 5, 11, 14, 28), streptocin B_1 was extremely heat stable in acid, whilst labile in alkali. Though susceptible to trypsin and Pronase, streptocin B_1 seemed somewhat less susceptible to proteolytic digestion than streptocin A. Preliminary attempts to elicit the production of neutralizing antibody have failed, as with streptocin A (24).

Streptocin B_1 has no bacteriolytic effect on susceptible organisms. The rate of its bactericidal activity is markedly temperature dependent. Although the inhibitory action of streptocin B_1 was specific for certain organisms, adsorption of the bacteriocin to cells appeared to lack

specificity. Immunity to streptocin B_1 must operate at some stage after adsorption. Other bacteriocins shown to lack adsorption specificity include lactocin 27 (28) and staphylococcin 414 (6).

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