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Bacteriocin Production by Strains of *Neisseria meningitidis*[1]

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ABSTRACT

KINGSBURY, DAVID T. (Naval Medical Research Institute, Bethesda, Md.). Bacteriocin production by strains of *Neisseria meningitidis*. J. Bacteriol. **91**:1696–1699. 1966.—Strains of *Neisseria meningitidis* produce substances inhibitory to other strains of meningococcus. These substances are nontransmissible and show a high degree of strain specificity. The properties of one of these substances resemble those of the class of bacterial inhibitors called bacteriocins. Synthesis of this "meningocin" can be increased as much as 200-fold by induction with mitomycin C. It shows a high degree of heat stability and is sensitive to proteolytic enzymes. Six bacteriocins from strains of *N. meningitidis* have been used to type meningococci. By use of this procedure, strains that were identical serologically were placed into distinct bacteriocin groups.

Bacteriocins are antibacterial substances of a protein nature that are produced by a wide variety of different genera of bacteria and act on bacteria of the same or related species (10). Bacteriocins resemble bacteriophages in their mode of action (8) and, in fact, colicin 15 may well be a defective bacteriophage (11).

The analogy between bacteriocins and bacteriophages may be carried further. It has been clearly demonstrated that the capacity to synthesize colicins is determined by a genetic factor that resembles a prophage in lysogenic cells; this genetic factor has been placed among the episomes (6). Many agents used to induce temperate bacteriophages are effective in the induction of bacteriocins; among these are ultraviolet light (7) and mitomycin C (9). Furthermore, as is the case with temperate bacteriophages, only some bacteriocins are inducible.

The most important differentiating characteristic between bacteriocins and bacteriophages is that bacteriocins are not capable of reproduction. Another important difference is the resistance of bacteriophages to proteolytic enzymes which generally inactivate bacteriocins.

The genus *Neisseria* is one for which no bacteriocin-like substance has been reported. This paper deals with the properties of an inducible bacteriocin-like substance from *N. meningitidis* and the use of this and other noninducible "meningocins" in the grouping of the meningococcus.

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MATERIALS AND METHODS

N. meningitidis strains used in this study were recent isolates of both case and carrier strains from several widely separated geographical areas. Positive identification was made on the basis of cultural, morphological, and serological properties. Stock cultures were kept in less than the fifth passage from original isolation in a frozen state at −60 C. Table 1 gives the source and serological type of the six strains used in the bacteriocin typing of the meningococcus.

The microorganisms were grown in Trypticase Soy Broth or on Mueller Hinton agar. Broth cultures were grown in a reciprocal shaker at 37 C. Plates and slants were incubated in a moist incubator (37 C) with an atmosphere of 10% CO_2 in air.

Mitomycin C was added to broth cultures at a level of 1 μg/ml. Ultraviolet induction was carried out by exposing 3 ml of a bacterial suspension of approximately 10^9 cells per milliliter to an ultraviolet lamp (General Electric 15-w "germidical" lamp at 50 cm) for 60 to 90 sec. A fivefold dilution was then made into fresh broth, and the cultures were shaken at 37 C.

Bacteriocin activity was titered on agar plates by use of standard procedures (4), and titers were expressed as the reciprocal of the highest dilution clearly showing a zone of lysis.

RESULTS

Demonstration of bacteriocins. Bacterial inhibitors produced by strains of *N. meningitidis* were first observed during attempts to isolate

TABLE 1. *Source and properties of strains of Neisseria meningitidis used in bacteriocin typing*

Bacteriocin no.	Strain no.	Source	Location	Serogroup
1	L-1	Case	Lafayette, La.	B
2	6-B	Carrier	Arlington, Va.	B
3	SD-6	Case	San Diego, Calif.	C
4	2-B	Carrier	Arlington, Va.	B
5	NOR-8	Carrier	Norfolk, Va.	B
6	CL-4	Case	Camp Lejeune, N.C.	B

temperate bacteriophages by spotting culture supernatant fluids on homologous and heterologous strains, following the procedure of Fisk (3). Many strains of meningococci produced antibacterial substances which exhibited strain specificity. No inhibitory activity was transferred when agar cores taken from areas of inhibition were suspended in 1 ml of broth and retested. These preparations were tested both by spotting and by incorporation of samples in soft agar overlay with the indicator strain.

To determine the incidence of production of these bacteriocins by strains of *N. meningitidis*, randomly selected strains were grown from stab inocula on agar plates. After 48 hr, the colonies were sterilized with chloroform vapor and overlaid with possible indicator strains in soft agar overlay (4). After overnight incubation, the plates were examined for zones of inhibition in the bacterial lawn around the colonies being tested. Of the 32 strains tested in this manner, 28 showed bacteriocin-like activity on one or more of the indicator strains.

Production and properties of the bacteriocin. After the preliminary studies, a single strain, L-1, was chosen for further study. Irradiation of strain L-1 of *N. meningitidis* with ultraviolet light resulted in a fourfold increase in the production of bacteriocin. The addition of mitomycin C to a young broth culture of strain L-1, followed by a 4-hr incubation period, gave a 200-fold increase in the production of bacteriocin (Table 2).

A mitomycin C-induced bacteriocin preparation from strain L-1 was subjected to various physical and chemical treatments. Since this bacteriocin did not cross a dialysis membrane, it was treated as a macromolecule, presumably similar to other bacteriocins. As summarized in Table 3, the bacteriocin was very heat-stable, showing no detectable loss of titer even after 15 min of boiling. The bacteriocin activity is completely destroyed by proteolytic enzymes but not affected at all by either deoxyribonuclease or ribonuclease.

Action and specificity of the bacteriocins. A large number of strains of the meningococcus

TABLE 2. *Effect of ultraviolet light and mitomycin C on production of bacteriocin by Neisseria meningitidis strain L-1*

Treatment	Bacteriocin titer*
None	4–8
Ultraviolet light†	16–32
Mitomycin C‡	1,000

* When tested on strain 174.

† Irradiation for 60 sec followed by a 5-hr incubation period.

‡ The mitomycin C was added to a young broth culture, and the culture was incubated for an additional 4 hr.

TABLE 3. *Effects of various chemical and physical treatments on bacteriocin activity*

Treatment	Conditions	Bacteriocin titer
Heating	100 C for 15 min	500
	120 C for 15 min	0
Trypsin	0.2%, 2 hr at 37 C	0
Pronase	0.2%, 2 hr at 37 C	0
Deoxyribonuclease	10 μg/ml, 2 hr at 37 C	500
Ribonuclease	10 μg/ml, 2 hr at 37 C	500
Control	Untreated	500

were tested to determine the range and specificity of the bacteriocins and to check their suitability for possible typing of meningococci. In addition, three other species of *Neisseria* were tested. Table 4 summarizes the reactions of various species of *Neisseria* to each of six bacteriocins. The bacteriocins of *N. meningitidis* did not affect any of the non-*Neisseria* species tested.

On the basis of the reaction of these strains to different bacteriocin preparations, it has been possible to differentiate between strains of the meningococcus that are otherwise identical. The formation of divisions, bacteriocin types, is obvious, and may be of some value in epidemiological studies. The data in Table 4 are not adequate to demonstrate any correlation or lack of correlation between bacteriocin type and the antigenic nature of the organisms. It was not possible to correlate bacteriocin type with any other property, such as virulence for the mouse, the source of the strain, or the geographical origin of the strain.

DISCUSSION

The data presented here show that strains of *N. meningitidis* produce substances which inhibit the growth of other *Neisseria* strains. One of these inhibitors is protein in nature as shown by sensitivity to proteolytic enzymes.

TABLE 4. *Reaction of representative Neisseria strains to selected bacteriocins*

Strains	Serological group	Bacteriocin no.						Bacteriocin type
		1	2	3	4	5	6	
1027A, CL-4	A	+*	+	−	−	−	−	C_1
EUR-2	A	−	−	−	−	−	−	C_0
6-B, O-3, O-4, CL-31, SD-8, SD-10, CL65-75, 6799, SD-11	B	−	−	−	−	−	−	C_0
8-B, NOR-10, 2-B, 9-B	B	−	−	+	−	−	+	C_2
L-1	B	−	+	+	+	+	+	C_3
NOR-8	B	−	+	+	+	−	+	C_4
NOR-7	B	−	+	+	−	+	+	C_5
174	B	+	+	−	+	−	+	C_6
236, SD-6	C	−	−	+	−	−	+	C_2
1628, PTS-5	C	−	−	−	−	−	−	C_0
SD-18, SD-23, NOR-29, KC661, KC662	Untypable	−	+	−	−	−	−	C_7
SD-22, NOR-25, NOR-26, NOR-28, KC660	Untypable	−	−	−	−	−	−	C_0
N. perflava		+	−	+	−	−	−	
N. subflava		+	−	−	−	−	−	
N. flavescens		+	−	−	−	−	−	

* Symbols: + = sensitive to bacteriocin; − = resistant to bacteriocin.

The inhibitors do not appear to be bacteriophages, since they cannot be replicated, and are sensitive to enzymes which do not generally inactivate bacteriophages. The inhibitory materials also show a greater heat stability than reported for most bacteriophages.

The inducible nature of at least one of these bacteriocins by ultraviolet irradiation and by mitomycin C suggests that the bacteriocins of *N. meningitidis*, or "meningocins," are episomal elements similar to those found in other bacteria. Although no other episomes have been reported for the meningococcus, bacteriophages have been reported in other *Neisseria* species (12; Phelps and Kellogg, Bacteriol. Proc., p. 115, 1965).

The use of bacteriocins in typing bacteria has been suggested by several authors (1, 2, 5). The bacteriocins of *N. meningitidis* show the specificity required to establish a typing system, and in the absence of a phage-typing system could serve as a valuable adjunct to the serological typing system which at present is only capable of placing isolates in one of the four established broad groups.

Of the 37 strains studied in this investigation, 17 were serological group B. Bacteriocin typing placed these 17 organisms into six bacteriocin types. The large number of tested strains not sensitive to any of the bacteriocins suggests that further subgrouping may be possible. There is no doubt that, as more strains are tested, bacteriocins will emerge which will be of additional value in grouping these organisms.

The bacteriocin-typing scheme reported here is not to be considered the best available, but serves to illustrate the possible uses of bacteriocins in the subgrouping and study of the meningococcus. It may now be possible to monitor the bacteriocin type of case organisms and to correlate it with the incidence of that bacteriocin type in the population at that time. It may also be possible for the first time to study the epidemiology of particular bacteriocin types in closed populations where the meningococcus can become a problem.

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