ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1978, p. 886-892
0066-4804/78/0014-0886$02.00/0

Vol. 14, No. 6
Printed in U.S.A.

Comparative Study of Ten Bacteriocins of *Clostridium perfringens*

D. E. MAHONY* AND A. LI

Department of Microbiology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7

Received for publication 10 July 1978

Bacteriocins of *Clostridium perfringens* were prepared by ammonium sulfate precipitation of supernatant broth from 10 bacteriocinogenic strains. These bacteriocins were compared with respect to their ability to produce spheroplasts in a sensitive indicator strain; their inducibility; sensitivity to pH, proteolytic enzymes, and boiling; and their effect on macromolecular synthesis. Two bacteriocins were stable over a wide range of pH values and resisted boiling, and three bacteriocins were resistant to trypsin. Five bacteriocins shut down DNA, RNA, and protein syntheses; three bacteriocins had varying effects on DNA and RNA synthesis; and two bacteriocins had little effect on macromolecular synthesis. Antiserum prepared against one bacteriocin highly neutralized three bacteriocins with partial neutralization of five others; two bacteriocins were unaffected. Mutant strains selected for resistance to bacteriocin 28 also demonstrated coresistance to two other closely related bacteriocins and partial resistance to five others.

Bacteriocins, which are substances produced by one organism having antibiotic-like activity against other members of the same species, have been reported for many species of bacteria. Bacteriocins of *Clostridium perfringens* have been described since 1959 (9), but very few reports concern the modes of action of these bacteriocins. In 1971 (7), we described a bacteriocin, named 28, which acted on the cell wall of *C. perfringens*, allowing production of spheroplasts and L-form growth of the treated culture. Macromolecular synthesis was not markedly affected by this bacteriocin. In 1975, Wolff and Ionesco (10) succeeded in purifying a bacteriocin, N_5, of *C. perfringens* strain BP6K-N_5, and Ionesco and Wolff (4) described the mode of action of this bacteriocin.

We (5) described a bacteriocin typing scheme for *C. perfringens* with 10 different bacteriocins and subsequently (6) showed the inducibility of 6 of these with mitomycin C. In this paper, we present data on the nature of the 10 bacteriocins and their action on macromolecular synthesis in a common indicator strain.

MATERIALS AND METHODS

Bacteriocin preparation. The 10 bacteriocins (nos. 4, 28, 43, 48, 55, 63, 73, 75, 78, and 96) were prepared by growing the producing strain of *C. perfringens* (designated by the same number) in freshly boiled brain heart infusion broth (BHIB) (Difco Laboratories, Detroit, Mich.) for 5 h at 37°C. The cultures were centrifuged at 6,000 × *g* for 10 min; the supernatant fluid was removed, and ammonium sulfate was added to the fluid (28 g/100 ml). This was slowly stirred at 4°C for 18 h, after which precipitated protein was collected by centrifugation at 6,000 × *g* for 10 min. The precipitate was suspended in a small volume of BHIB, and this served as the source of bacteriocin used in the experiments described below.

To titrate a bacteriocin, the common indicator strain of *C. perfringens*, strain no. 2, was grown as follows. One milliliter of an overnight cooked-meat culture (Difco) was subcultured in BHIB for 3 h at 37°C, diluted 1:100 in BHIB, and swabbed onto the surface of a blood agar plate (BHI base plus 10% human blood). Ten microliters of serially diluted bacteriocin was spotted on the seeded plate. After incubation of the plate under an atmosphere of nitrogen at 37°C for 18 h, the highest dilution of bacteriocin causing a zone of growth inhibition was considered the titer, the inverse of which was referred to as units (U) of bacteriocin.

Spheroplast production as a function of bacteriocin activity. Indicator strain 2 was grown overnight in cooked-meat medium. Volumes of 0.5 ml of this culture were added to 5-ml volumes of boiled BHIB containing 10% sucrose (wt/vol; to protect spheroplasts from disruption). These were incubated for 1.5 h at 37°C, after which 0.5 ml of the respective bacteriocins was added. After an additional 2 h of incubation, the cells were examined by phase-contrast microscopy to note any morphological changes. A control culture did not receive any bacteriocin.

pH sensitivity of bacteriocins. The 10 bacteriocins of *C. perfringens* were exposed to a variety of pH values ranging from pH 2 to 11. The buffer solutions used for these determinations were phosphate-citrate buffer (0.1 M:0.05 M, pH 2 to 7), tris(hydroxy-

methyl)aminomethane-hydrochloride buffer (0.05 M, pH 8) and glycine-NaOH buffer (1 M, pH 9 to 11). Bacteriocin (0.1 ml) was added to 0.9 ml of the respective buffer, incubated for 30 min at 37°C, and then assayed for activity.

Susceptibility of bacteriocins to trypsin, Pronase, and boiling temperature. To determine the susceptibility of the 10 bacteriocins to trypsin and Pronase, 0.25 ml of each bacteriocin, adjusted to 128 to 256 U/ml, was treated with 0.25 ml of trypsin (2.5 mg/ml) or Pronase (2.5 mg/ml) in phosphate-buffered saline, pH 7, and was incubated for 1 h at 37°C. Trypsin soybean inhibitor (0.5 ml of a 20-mg/ml solution) was added and incubation continued for 30 min. Bacteriocin activities were then assayed by placing drops of serially diluted bacteriocin solution onto blood agar plates seeded with *C. perfringens* strain 2. Controls included the experiment performed with untreated bacteriocin and buffer (positive control); trypsin and Pronase alone; trypsin inhibitor alone; and bacteriocin plus trypsin inhibitor (negative controls).

The temperature sensitivity of the 10 bacteriocins was determined by boiling 0.5-ml volumes of bacteriocin solution for 10 min. Bacteriocin activities were titrated before and after boiling.

Incorporation of ^{3}H-labeled amino acids, [^{3}H]-thymidine, and [^{3}H]uracil by bacteriocin-treated strain 2. Stock solutions of ^{3}H-labeled amino acid mixture, [^{3}H]thymidine, and [^{3}H]uracil (New England Nuclear Canada) were prepared by adding the respective isotopes to BHIB to obtain a final activity of 0.6 μCi/ml. These were distributed to test tubes in 8-ml volumes and boiled for 10 min to drive off dissolved oxygen. Strain 2 was grown for 2.5 to 3 h in BHIB and diluted 1:5 in BHIB, and 0.8 ml of this dilution was added to the radioactive broth. After 1 h of incubation at 37°C, 1 ml of bacteriocin was added; a control tube received 1 ml of broth instead of bacteriocin. At 0, 30, 60, 75, 90, 105, 120, and 150 or 180 min, 0.2-ml duplicate samples were taken and added to equal volumes of ice-cold 10% trichloroacetic acid. After 20 min of precipitation at 4°C, the samples were filtered on 3 MM Whatman filter papers and washed three times with ice-cold 5% trichloroacetic acid and once with ether. The filter papers were dried, placed in 5 ml of PPO/POPOP scintillation fluid (2,5-diphenyl-oxazole/*p*-bis[2-(5-phenylozaxolyl)]-benzene), and counted in a scintillation counter (Nuclear-Chicago, Isocap/300).

Preparation of antiserum against bacteriocin 28. Anti-bacteriocin serum was prepared from rabbits immunized with ammonium sulfate-precipitated bacteriocin, injected subcutaneously four times at weekly intervals. Bacteriocin 28 (40,960 U/ml) emulsified in an equal volume of Freund complete adjuvant was used for the initial inoculation (1.5 ml at two sites). Subsequent injections were done in Freund incomplete adjuvant. Following this procedure, a month was allowed to elapse before the animals were boosted with another injection. Blood was collected by cardiac puncture 1 to 4 weeks after the last injection, and, after clotting, the serum was removed.

Assay for cross-reactivity of antiserum. Each of the 10 bacteriocins was assayed as follows. The antiserum was serially diluted in 0.85% saline, and an equal volume of bacteriocin, adjusted to 64 U/ml, was added to each dilution. The dilutions were incubated at 37°C for 30 min, and 10 μl of each was spotted onto a blood agar plate seeded with a 3-h culture of the indicator strain. The plates were read after an overnight incubation at 37°C, and the absence of inhibition zones indicated neutralization of the bacteriocin by antiserum. Normal rabbit serum was used as a control.

Isolation of strain 2 (indicator) mutants resistant to bacteriocin 28. To isolate spontaneous mutants resistant to bacteriocin 28, blood agar plates were swabbed with bacteriocin 28 (5,120 U/ml). Subsequently a 1/100 dilution of a 3-h culture of strain 2 was swabbed onto the same plates and incubated overnight at 37°C. Any surviving colonies found on the plates were subcultured again onto bacteriocin-containing plates to confirm their resistance.

RESULTS

Spheroplast production as a function of bacteriocin activity. When strain 2 was treated with bacteriocins 28, 55, 63, and 96, almost 100% of the culture was converted to spheroplasts (Table 1). Bacteriocins 4, 43, 48, 73, 75, and 78 induced little spheroplasting activity, although occasional small spheroplasts were observed. Rod-shaped ghost cells were sometimes seen in the latter case and were particularly evident in those cultures exposed to bacteriocins 75 and 78. These two bacteriocins also suppressed visible growth of the cultures more than the other bacteriocins did.

pH sensitivity of bacteriocins. There were 4 basic patterns of sensitivity to different pH values demonstrated by the 10 bacteriocins (Fig. 1A to D). Bacteriocins 75 and 78 were unaffected by any pH tested. Bacteriocin 43 was sensitive to pH values below 5 but resistant to an alkaline pH of 10.6. Bacteriocins 4 and 48, although sensitive to pH values below 6 and 5, respectively, showed higher resistance to alkaline pH than did many bacteriocins, i.e., 50% survival at pH 10. The fourth pattern of sensitivity shown by bacteriocins 28, 55, 63, 73, and 96 was a marked drop in activity at both high and low pH values on either side of a plateau of stability. The stability values varied for the respective bacteriocins but fell within the range of pH 5 to 9. The stability ranges are summarized in Table 1.

Susceptibility of bacteriocins to trypsin, Pronase, and boiling temperature. The susceptibilities of the 10 bacteriocins to trypsin and Pronase are summarized in Table 1. Bacteriocins 28, 55, 63, 73, 75, 78, and 96 were sensitive to both trypsin and Pronase, although bacteriocin 78 showed a trace of residual activity after trypsin treatment; bacteriocin 4 was sensitive to Pronase but not trypsin; and bacteriocins 43 and

TABLE 1. *Summary of properties of 10 bacteriocins of C. perfringens*

Property	Bacteriocin[a]									
	4	28	43	48	55	63	73	75	78	96
Inducibility[b]	−	+	+	−	+	+	+	−	+	−
Spheroplast production	−	+	−	−	+	+	−	−	−	+
pH stability range	6–9	5–7	5–10.5	5–8	5–7	6–7	5–9	2.5–10.5	2.5–10.5	5–8
Inactivation by trypsin	−	+	−	−	+	+	+	+	+	+
Inactivation by Pronase	+	+	−	−	+	+	+	+	+	+
Destruction by boiling	+	+	+	+	+	+	+	−	−	+
Inhibition of protein synthesis	+	−	+	+	−	+	+	+	+	+
Inhibition of DNA synthesis	+	−	+	+	−	−	−	+	+	+
Inhibition of RNA synthesis	+	−	+	+	−	−	+	+	+	−
		(+)			(+)	(+)				(+)

[a] +, Positive response; −, negative response; (+), positive with high concentration of bacteriocin.
[b] Cited from reference 6.

48 were completely resistant to both enzymes. Neither trypsin, Pronase, nor trypsin inhibitor inhibited the growth of the indicator strain. Trypsin activity was inhibited by trypsin inhibitor, whereas Pronase was unaffected.

The sensitivity of these bacteriocins to boiling is also summarized in Table 1. All bacteriocins except 75 and 78 were inactivated by such treatment.

Effect of bacteriocins on macromolecular synthesis. Bacteriocins 28 and 55 did not completely inhibit protein synthesis in *C. perfringens* strain 2, although the incorporation of radioactive amino acids was less than that of controls. Bacteriocins 4, 43, 48, 63, 73, 75, 78, and 96 completely inhibited protein synthesis within 15 min of addition to the culture (Fig. 2A and B). Although the titers of the bacteriocins used in these experiments differed, very similar results were obtained when all the bacteriocins were adjusted to 256 U before addition to the bacterial culture (data not shown).

The inhibition of DNA synthesis, as measured by the incorporation of [^{3}H]thymidine, occurred after addition of bacteriocins 4, 43, 48, 75, 78, and 96 to strain 2 (Fig. 2A and B). There was a rapid drop in labeled precipitable material during the first 15 min after the addition of bacteriocin, followed, in most cases, by an increase in labeling of the DNA, suggesting initial DNA damage and recovery had occurred. All of these bacteriocins also had an immediate effect upon protein synthesis.

Bacteriocins 28, 55, 63, and 73 (Fig. 2A) did not affect incorporation of thymidine as described for the other bacteriocins, but the amount of incorporation was lower than that of control cultures. Protein synthesis was inhibited by the treatment of strain 2 with bacteriocins 63 and 73, whereas DNA synthesis did not cease.

To further examine the suppressive effect of bacteriocin 28 on protein and DNA synthesis in strain 2, the above experiments were performed by using a lower titer of 2,560 U of bacteriocin 28 and a 16-fold higher titer of 40,960 U. As shown in Fig. 3, the amount of isotope incorporated into DNA and protein is somewhat suppressed at the lower concentration of bacteriocin and is further suppressed when 40,960 U of bacteriocin is used. Even at such a high concentration of bacteriocin, however, the synthesis of these macromolecules is not shut off, especially during the first critical 30 min of the experiment.

The incorporation of [^{3}H]uracil into bacteriocin-treated bacteria was taken as an indicator of RNA synthesis. For these experiments, the strength of the bacteriocin stock solutions was standardized to 256 U, except for bacteriocins 48 (512 U), 73 (80 U), and 78 (32 U). The results shown in Fig. 2A and B indicate that RNA synthesis is shut off by bacteriocins 4, 43, 48, 74, 75, and 78 but is not by bacteriocins 28, 55, 63, and 96, although the total incorporation of isotope under the influence of these latter bacteriocins might be lower than control values. To further investigate the effect of bacteriocin concentration on RNA synthesis, these four bacteriocins were utilized at much higher concentrations: bacteriocin 28 (40,960 U), 55 (20,480 U), 63 (10,240 U), and 96 (40,960 U). At these higher concentrations, RNA synthesis was greatly suppressed if not entirely shut off. The data from all these experiments are summarized in Table 1.

Antiserum and cross-reactivity. Antiserum to bacteriocin 28 prepared in rabbits neutralized 256 U of bacteriocin 28 at a dilution of 1:8. When 64 U of the 10 bacteriocins was added to serial dilutions of the antiserum, bacteriocins 28 and 55 were completely neutralized and bacteriocin 63 was largely neutralized, but bacteriocins 73, 75, and 78 were unaffected. Partial

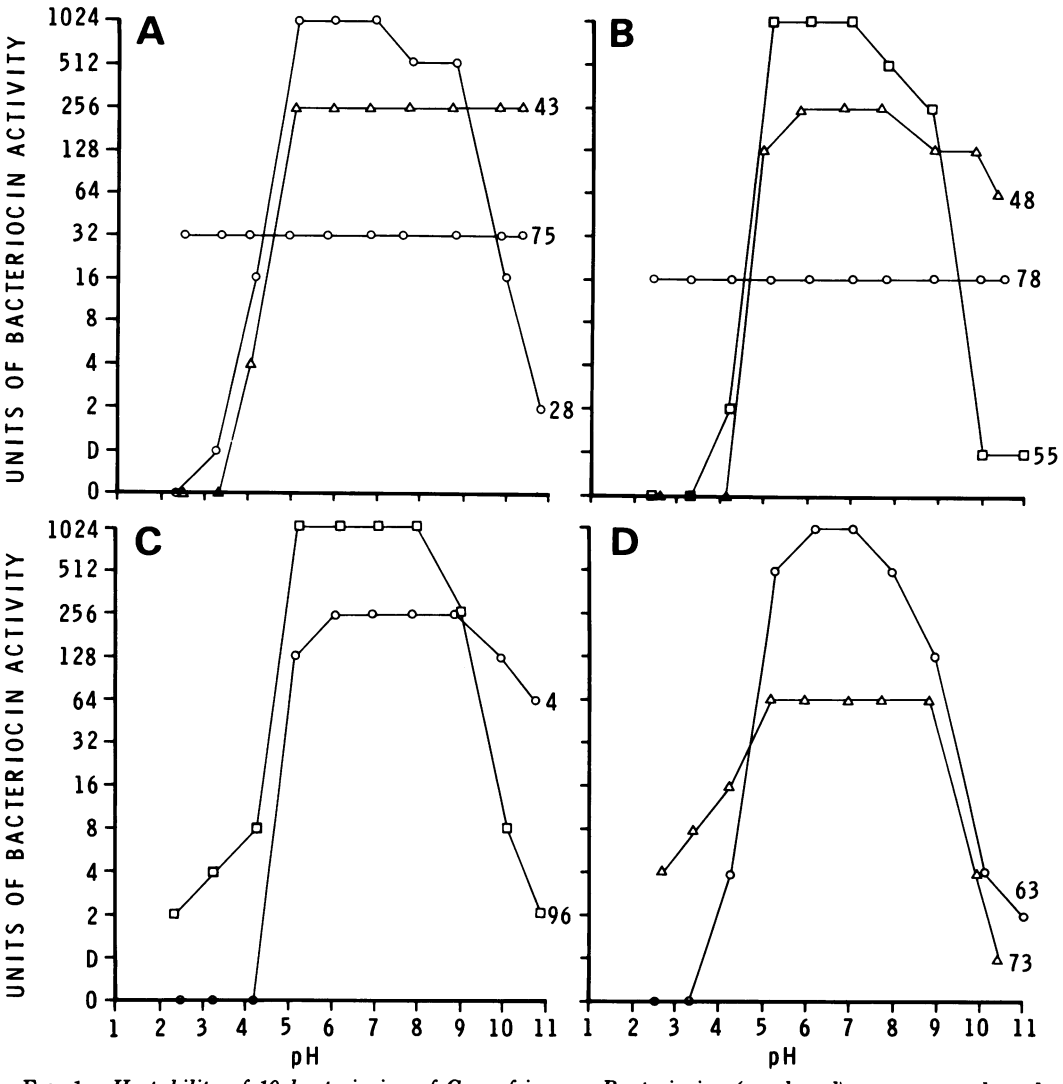
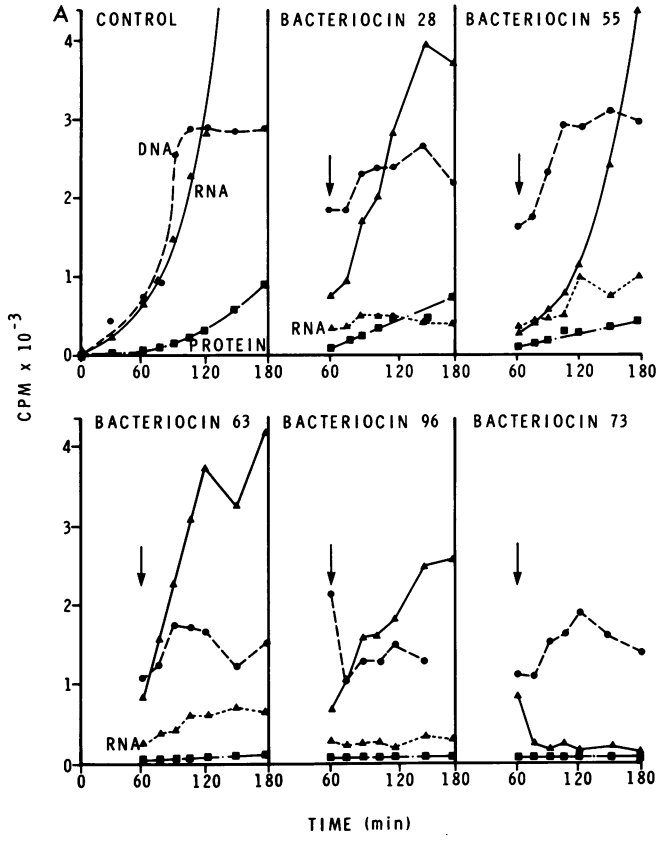


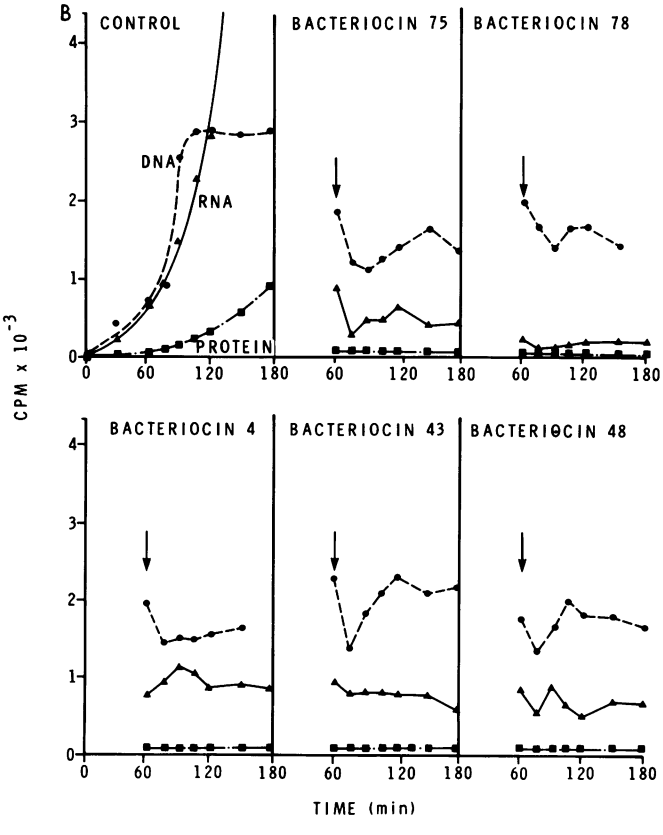
FIG. 1. *pH stability of 10 bacteriocins of C. perfringens. Bacteriocins (numbered) were exposed to the indicated pH for 30 min at 37°C and then assayed for biological activity.*

neutralization of bacteriocins 4, 43, 48, and 96 occurred. Normal rabbit serum had no neutralizing effect on any of the bacteriocins.

Isolation of bacteriocin-resistant mutants. Four mutants of the indicator strain 2 were isolated that were resistant to bacteriocin 28. These mutants had altered sensitivity to bacteriocins, did not themselves produce bacte-

FIG. 2. *Effects of 10 bacteriocins of C. perfringens on macromolecular synthesis in strain 2. [^{3}H]thymidine, [^{3}H]uracil, and ^{3}H-labeled amino acids were added, respectively, to BHIB at a final activity of 0.6 μCi/ml. Strain 2 was inoculated (final cell number about 1 × 10^7 bacteria/ml), and, after 1 h at 37°C, 1 ml of bacteriocin was added (↓). Samples (0.2 ml) were taken as indicated, precipitated by cold 10% trichloroacetic acid as described in the text and counted in a scintillation counter. Curves are not shown up to 60 min to provide clarity in these figures. The control panel represents average curves based on several experiments. DNA (●---●); RNA, low-titer bacteriocin (▲——▲); RNA, high-titer bacteriocin (▲---▲); protein (■——■). Units of bacteriocin used (bacteriocin number/DNA/RNA/protein): 28/40,960/256 low, 40,960 high/20,480; 55/20,480/256 low, 20,480 high/10,240; 63/20,480/256 low, 10,240 high/320; 96/10,240/256 low, 40,960 high/2560; 73/64/80/128; 75/512/256/256; 78/512/32/16; 4/1280/1280/256; 43/128/1280/256; 48/128/-512/128.*


A
CONTROL
BACTERIOCIN 28
BACTERIOCIN 55
DNA
RNA
PROTEIN
RNA
BACTERIOCIN 63
BACTERIOCIN 96
BACTERIOCIN 73
RNA
CPM x 10^-3
TIME (min)


B
CONTROL
BACTERIOCIN 75
BACTERIOCIN 78
DNA
RNA
PROTEIN
BACTERIOCIN 4
BACTERIOCIN 43
BACTERIOCIN 48
CPM x 10^-3
TIME (min)

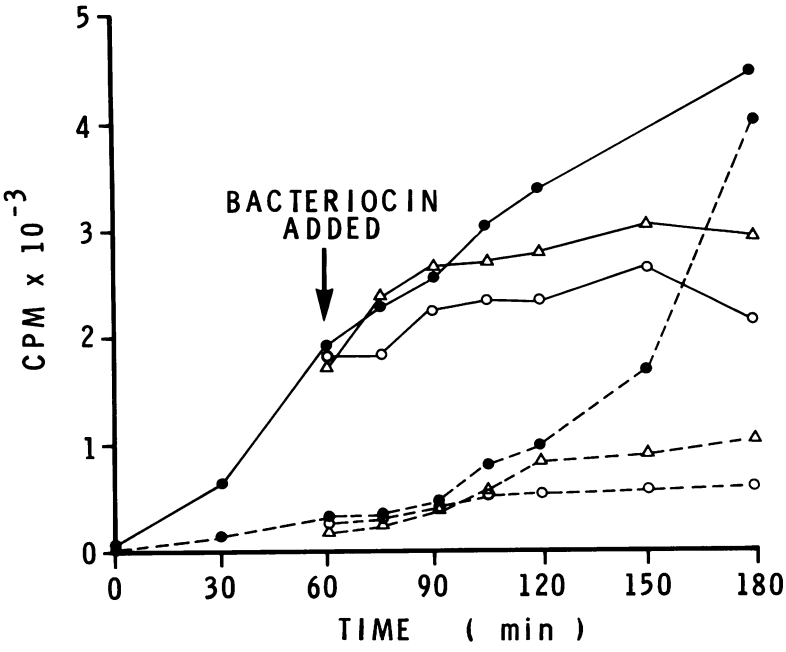


FIG. 3. *[^{3}H]thymidine and ^{3}H-labeled amino acid incorporation in strain 2 treated with two concentrations of bacteriocin 28. Thymidine average control (●——●); amino acid control (○---○); thymidine + bacteriocin (2560 U, △——△); thymidine + bacteriocin (40,960 U, ○——○); amino acid + bacteriocin (2560 U, △---△); amino acid + bacteriocin (40,960 U, ○---○).*

riocin, and produced smaller colonies than their parent strain 2 when grown on blood agar. These mutants, parent strain 2, and bacteriocin-producing strain 28 were typed with bacteriocins as described elsewhere (5). The results (Table 2) indicated that acquired resistance to bacteriocin 28 led to coresistance to bacteriocins 55 and 63. These mutants were also partially protected in some instances from the action of bacteriocins 43, 48, 73, and 96. No alteration in sensitivity to bacteriocins 75 and 78 was observed.

TABLE 2. *Bacteriocin typing of mutants of C. perfringens resistant to bacteriocin 28*

Strain no.	Bacteriocin[a]									
	4	28	43	48	55	63	73	75	78	96
28	±	−	−	−	−	−	±	+	+	−
2	+	+	+	+	+	+	+	+	+	+
Mutant 2 (1)	+	−	±	±	−	−	±	+	+	±
Mutant 2 (2)	+	−	+	+	−	−	+	+	+	+
Mutant 2 (3)	+	−	±	±	−	−	+	+	+	+
Mutant 2 (4)	+	−	±	±	−	−	±	+	+	+

[a] +, Sensitive to bacteriocin; −, resistant to bacteriocin; ±, partially sensitive to bacteriocin; ±, slightly sensitive to bacteriocin.

DISCUSSION

Although all the bacteriocins of *C. perfringens* described in this paper inhibit the common indicator strain, the nature of the inhibitors differs as does their mode of action. Bacteriocins 75 and 78 are similar with respect to their resistance to inactivation by extremes of pH and boiling. Their lack of cross-reactivity with antiserum to bacteriocin 28 also makes them unique. From our preliminary observations, the only differentiating feature is inducibility; however, this may indicate a strain difference or a technical failure in our ability to induce this strain. The host range of these bacteriocins differs (5). Sasarman and Antohi (8) have reported heat-resistant bacteriocins of *C. perfringens* as have Hirano and Imamura (2) and Clarke et al. (1). The latter two investigators also demonstrated stability of their bacteriocins through extremes of pH.

A distinguishing feature of bacteriocins 43 and 48 is their resistance to both trypsin and Pronase. Bacteriocin 4 is resistant only to trypsin. Hirano and Imamura (2) previously described trypsin and pepsin resistance of the heat- and pH-tolerant bacteriocins already cited. The pH stability of bacteriocins 4, 43, and 48 is similar; all are unstable at low pH but relatively stable at alkaline pH values, although bacteriocin 43 is the most resistant to inactivation at alkaline pH values.

Bacteriocins 28, 55, and 63 have very similar

properties except that bacteriocin 63 inhibits protein synthesis. The effect on cell wall by these bacteriocins is obvious by the conversion of the bacteria to spheroplasts. Bacteriocins 28 and 55 have little or no direct effect on macromolecular synthesis unless extremely high concentrations are used, and both are completely neutralized by antiserum to bacteriocin 28. Bacteriocin 63 is highly neutralized with this antiserum. Since cell wall is very much involved in the mode of action of these bacteriocins, the effect on macromolecular synthesis that does occur may be secondary to cell wall damage. Bacteriocin 96 causes spheroplast formation in strain 2 but also interferes with protein and DNA synthesis (and RNA synthesis at high concentrations of bacteriocin). A more complex mode of action may exist with this bacteriocin, or possibly the strain produces more than one bacteriocin.

Bacteriocins 4, 43, 48, 75, and 78 shut off protein, DNA, and RNA syntheses very soon after addition to the indicator strain. It is not possible from the data presented to determine whether there is a sequence to this suppression since all pathways are equally affected by the time of the first sampling. The lowered incorporation of [^{3}H]thymidine obtained by treating the indicator strain with bacteriocins 63 and 73 may be secondary to the early shut-off of protein synthesis by these bacteriocins.

Hirano and Imamura (3) described a lytic type of bacteriocin and compared its action to that of lysozyme, since the lytic factor caused a loss of the Gram-staining property and disintegration of sensitive cells. However, it acted only on strains of *C. perfringens,* and both living and dead cells were so affected. Clarke et al. (1) indicated a lytic action of their perfringocin on cells of *Clostridium pasteurianum.* Ionesco and Wolff (4) described the mode of action of their purified bacteriocin N_5 from *C. perfringens* in which simultaneous inhibition of DNA, RNA, and protein syntheses occurred in sensitive bacteria treated with this bacteriocin.

The isolation of mutants resistant to bacteriocin 28 has reinforced some of our other findings. The coresistance of mutant strains to bacteriocins 55 and 63 suggests a common receptor or target site for these bacteriocins, whereas partial resistance of the mutants to bacteriocins 43, 48, 73, and 96 may indicate a relatedness with respect to these bacteriocins. Sensitivity to bacteriocins 4, 75, and 78 did not change, suggesting a lack of relatedness to acquried resistance to bacteriocin 28.

Complete neutralization of bacteriocin 28 and 55 and a high degree of neutralization of bacteriocin 63 by the antiserum prepared against bacteriocin 28 suggest that these bacteriocins have common antigenic determinants associated with their active sites. Partial neutralization of bacteriocins 4, 43, 48, and 96 indicates a sharing of some common antigenic determinants with bacteriocin 28. An aggregation of bacteriocin molecules by antiserum might result, preventing efficient entry into the bacterium or binding to receptor sites.

It would seem that many of our bacteriocins behave as the N_5 bacteriocin, although others are clearly different. It is our intention to purify our bacteriocins to assure that only one inhibitor is involved in each case and to further investigate their modes of action.

ACKNOWLEDGMENTS

We are indebted to the technical support of K. A. Patrick and the financial support of the Medical Research Council of Canada (grant no. MA 5581).

LITERATURE CITED

1. **Clarke, D. J., R. M. Moyra, and J. G. Morris.** 1975. Purification of two *Clostridium* bacteriocins by procedures appropriate to hydrophobic proteins. Antimicrob. Agents Chemother. **7**:256-264.
2. **Hirano, S., and R. Imamura.** 1972. Two types of bacteriocins produced by *Clostridium perfringens.* Acta Med. Univ. Kagoshima **14**:115-118.
3. **Hirano, S., and T. Imamura.** 1972. A phage-associated lysin of *Clostridium perfringens.* Acta Med. Univ. Kagoshima **14**:91-101.
4. **Ionesco, H., and A. Wolff.** 1975. Sur le mode d'action de la bactériocine N_5 purifiée de *Clostridium perfringens.* C. R. Acad. Sci. Paris Ser. D **281**:2033-2036.
5. **Mahony, D. E.** 1974. Bacteriocin susceptibility of *Clostridium perfringens:* a provisional typing schema. Appl. Microbiol. **28**:172-176.
6. **Mahony, D. E.** 1977. Induction of bacteriocins from *Clostridium perfringens* by treatment with mitomycin C. Antimicrob. Agents Chemother. **11**:1067-1068.
7. **Mahony, D. E., M. E. Butler, and R. G. Lewis.** 1971. Bacteriocins of *Clostridium perfringens.* II. Studies on mode of action. Can. J. Microbiol. **17**:1435-1442.
8. **Sasarman, A., and M. Antohi.** 1971. Une nouvelle classe de welchicines. Rev. Can. Biol. **30**:183-189.
9. **Smith, H. W.** 1959. The bacteriophages of *Clostridium perfringens.* J. Gen. Microbiol. **21**:622-630.
10. **Wolff, A., and H. Ionesco.** 1975. Purification et characterisation de la bactériocine N_5 de *Clostridium perfringens* BP6K-N_5 type A. Ann. Microbiol. (Paris) **126**:343-356.