Induction of Bacteriocins from *Clostridium perfringens* by Treatment with Mitomycin C

D. E. MAHONY

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

Received for publication 4 February 1977

Six of ten bacteriocinogenic strains of *Clostridium perfringens* were inducible by treating the cultures with mitomycin C. Induction did not occur in the continued presence of mitomycin C but only when excess mitomycin C was removed. Cell death was associated with induction of bacteriocin.

Some bacteriocins of *Clostridium perfringens* are inducible by ultraviolet light (2, 3, 5), and there is one report (1) of induction by mitomycin C. In this latter report, a fourfold increment in bacteriocin titer resulted from a 2-h treatment of *C. perfringens* cultures with 2 μg of mitomycin C per ml. We (4) previously described a typing scheme for *C. perfringens* based on the susceptibility of various strains of the species to 10 bacteriocins. This paper describes the inducibility of these 10 bacteriocins and the technique that was found valuable for detecting induction.

The 10 bacteriocin-producing strains of *C. perfringens*, their origin, and their cultivation have been described (4). Mitomycin C was obtained from Sigma Chemical Co., St. Louis, Mo. This antibiotic was suspended in brain heart infusion (BHI) broth (Difco) as a stock solution of 400 μg per ml. A range of mitomycin C concentrations was tested for induction capability as follows. One hundred milliliters of BHI broth was inoculated with 6 ml of an 18-h cooked-meat culture of the bacteriocinogenic strain to be tested. This 100-ml culture was referred to as the "primary culture." After this culture was incubated for 2 h at 37°C, mitomycin C was added (final concentrations ranging from 0.25 to 8 μg per ml). The culture was incubated for 1 more h and then centrifuged at 6,000 × g in an International B-20 centrifuge for 10 min. The bacterial pellet was resuspended in 5 ml of BHI broth and added to 100 ml of freshly boiled and cooled BHI broth. This culture, referred to as the "secondary culture," was incubated for up to 24 h, and 2-ml samples were removed for the assay of bacteriocin titer or other characteristics of growth at various times. Viable counts of bacteria were done by plating duplicate 0.1-ml volumes of serially diluted samples onto blood agar plates. These were incubated anaerobically at 37°C for 18 h. The remainder of the samples were centrifuged to remove bacteria, and the supernatant fluids were titrated against indicator strain no. 2 in our collection. The method of assay has been described (4), but, essentially, it involves spotting 16 μl of serially diluted bacteriocin onto blood agar plates that have been seeded with a 1:100 dilution of a 3-h culture of strain no. 2.

Six of the ten bacteriocinogenic strains were inducible with mitomycin C (strains 28, 43, 55, 63, 73, and 78). The usual inducing dose was 2 to 4 μg/ml, although strain 43 was more efficiently induced with 1 μg/ml. Higher concentrations inhibited growth and bacteriocin production. Exposure of bacteria to mitomycin C for less than 1 h decreased the final yield of bacteriocin, whereas exposures of more than 1 h did not increase the yield obtained.

*C. perfringens* strain no. 28 was further studied as a model for the induction phenomenon. Bacteriocin production by strain no. 28 is shown in Fig. 1. These results are representative of at least four such experiments. The time scale refers to time after the centrifugation step and subculturing to the second 100-ml volume of broth (secondary culture). There was a 32- to 64-fold increment in bacteriocin titer in mitomycin C-induced cultures compared with that of control cultures treated exactly the same way but without the addition of mitomycin C. The somewhat lower titer shown for the control culture at time zero in Fig. 1 is not a consistent finding and probably represents differences in bacteriocin carryover from the primary to the secondary culture. The titer of the induced culture rapidly increased 15 to 30 min after subculturing, until 2 h, when the maximum titer was achieved. No increase in bacteriocin titer beyond control values was observed in the pri-
primary culture in the continued presence of mitomycin C, even after several hours of incubation. Therefore, the centrifugation and subculturing steps were required for the expression of induction.

The viabilities of induced and control cultures were compared to determine whether bacteriocin production or release or both resulted in death of the producing bacteria (Fig. 1). The mitomycin C-induced secondary culture showed a rapid 1-log drop in viability during the first 30 min, which was followed by a recovery of growth and survival throughout the rest of the experiment. Control cultures showed a slight decline in viability during the first few minutes of the secondary culture, after which the count increased slightly and then remained stationary. The production and/or release of bacteriocin in induced cultures would, therefore, seem to be related to cell death.

The effect of mitomycin C on alpha- and theta-toxin production was briefly examined, and there was no marked difference in toxin titers obtained in induced or control cultures during the primary culture. In the secondary culture, toxin levels were either slower appearing or generally lower in induced cultures when compared with control cultures. Table 1 relates these data with respect to the period of incubation when the highest bacteriocin level is achieved in both the primary and secondary cultures. Rescuing the induced bacteriocin at 2 h in the secondary culture provided high bacteriocin titers with relatively low amounts of the two toxins assayed.

In conclusion, we have shown that some bacteriocin-producing strains of C. perfringens are inducible by treating with mitomycin C and that mitomycin C must be removed from the culture for the subsequent expression of induction.

I would like to acknowledge the technical assistance of K. A. Patrick and the financial support of the Medical Research Council of Canada (grant no. MA5681).

LITERATURE CITED