Lecithinase Production by Clostridium perfringens in Chemically Defined Media

MITSURU NAKAMURA, JUDY ANN COOK,¹ AND WILLIAM R. CROSS²
Department of Microbiology, University of Montana, Missoula, Montana 59801

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The factors that influence lecithinase production by Clostridium perfringens have not yet been elucidated. Routine laboratory media do not support the production of high yields of lecithinase, and special media are required for lecithinase production by this organism (7). Lecithinase production in complex media was studied by various workers (1, 4, 5, 8).

Chemically defined media are more suitable for studies involving enzyme precursors and factors that stimulate enzyme production. Boyd et al. (2) cultivated C. perfringens in a defined medium. The medium consisted of amino acids, glucose, vitamins, and inorganic salts. However, the organism did not produce lecithinase in this medium. Jayko and Lichstein (3) added glycyl-L-asparagine to the medium of Boyd et al. and reported that slight amounts of enzyme were produced.

This report presents data on the stimulation of lecithinase production in C. perfringens grown in chemically defined media fortified with various synthetic peptides. The basal medium to which the peptides were added was Medium NCTC 109 (Grand Island Biological Co., Grand Island, N.Y.). This is a chemically defined medium routinely used for tissue cultures. The medium was sterilized by pressure filtration through a DeLaval (Model L-14) filter and aseptically dispensed into sterile screw-capped test tubes. One mg of sterile L-ascorbic acid per ml was added to the tubes. Synthetic peptides and amino acids were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. These were added individually to the basal medium at a concentration of 50 μg/ml to determine whether they would support lecithinase synthesis.

Strains BP6K and A48 of C. perfringens were used in these experiments. Strain BP6K is a classical gas gangrene strain and an active producer of lecithinase. Strain A48 was isolated from food and is a weak producer of lecithinase. These organisms have been maintained in medium NCTC 109 for more than 20 subculture transfers.

A modification of the method of van Heyningen (9) was used to determine lecithinase activity. This method was described in detail by Nakamura and Cross (6). The enzyme activity is expressed in terms of micrograms per milliliter of lecithinase as determined from a standard assay curve using commercial lecithinase (Nutritional Biochemicals Corp.).

Medium NCTC 109 supported excellent growth of C. perfringens but did not permit the organisms to produce lecithinase. Lecithinase was produced by strain BP6K in the complex medium (6). Of the 40 peptides studied, 21 stimulated lecithinase production by strain BP6K in the chemically defined medium. The peptides that stimulated maximal production of lecithinase are listed in Table 1. The complex medium supported a lecithinase activity of 95 μg/ml. Some of the peptides stimulated intermediate production of lecithinase (20 to 60 μg/ml). They were DL-alanyl-DL-methionine, DL-alanyl-DL-phenylalanine, DL-alanyl-DL-valine, glycyl-DL-leucine, DL-leucyl-DL-glycylglycine, L-leucylglycylglycine, and DL-leucyl-DL-alanine. The following peptides did not stimulate lecithinase production in strain BP6K: glycylglycylglycylglycine, glycylglycyl-DL-phenylalanine, glycylglycyl-L-alanine, glycyl-L-threonine, DL-alanyl-DL-norvaline, DL-alanyl-DL-norleucine, glycyl-L-tyrosine, glycyl-DL-asparagine, and glycyl-DL-α-amino-n-butyric acid.

In contrast, strain A48 of C. perfringens produced only 26 μg of lecithinase per ml in complex media. Only two of the peptides tested stimulated lecithinase production by strain A48 in the chemically defined medium. Glycyl-L-tryptophan supported a lecithinase activity of 10 μg/ml and DL-benzoylalanine supported an activity of 15 μg/ml. All of the other peptides were without activity in supporting lecithinase production by this strain.
The free amino acids glycine, L-leucine, DL-alanine, DL-norvaline, DL-phenylalanine, and L-serine did not stimulate lecithinase production by either strain.

From the data presented, it is evident that conditions suitable for growth of C. perfringens are not necessarily suitable for lecithinase production. Strain BP6K was able to produce lecithinase in a chemically defined medium that contained selected dipeptides. A criticism of previous reports indicating that peptides stimulated lecithinase production is that the organisms were not maintained in the defined medium before lecithinase activity was assayed. In our studies, the organisms were subcultured over 20 times in the basal chemically defined medium, thus eliminating the possibility that materials carried over from the complex medium may have stimulated lecithinase production.

Strain A48 was unable to produce high levels of lecithinase even with the addition of various synthetic peptides. However, this strain did not produce much lecithinase in the complex medium either. These results suggest that there are considerable differences in the abilities of various strains to produce lecithinase.

We believe that this is the first report of an unequivocal nature indicating that C. perfringens is capable of producing lecithinase in a chemically defined medium.

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**Literature Cited**