GENETIC ASPECTS OF CLOSTRIDIUM BOTULINUM

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ABSTRACT

The relationship of bacteriophages and plasmids to the production of neurotoxins was studied in strains of Clostridium botulinum types A through G. Neurotoxins C, and D produced by types C and D, respectively, were shown to be mediated by specific bacteriophages. Evidence is presented that strongly suggests that both neurotoxin and bacteriocin production by type G are in some manner related to a 81-MDa plasmid carried by toxigenic strains.

Antigenicity and host range of four type C and three type D converting phages were studied. The phages were classified into three groups based on their antigenicity and host range: group 1 consisted of c-st and c-468 phages; group 2 was c-203, c-d6f, and d-1873; and group 3 was d-sa and d-4947.

Nucleic acids were extracted from groups 1 and 2 phages, and nonconverting mutant phage (c)-n71 which was obtained from C-Stockholm strain as well as c-st phage. The susceptibility of phage DNAs to different types of nucleases was observed. It was concluded that the nucleic acids of all six phages were double-stranded DNA. The length of c-st, (c)-n71, c-468, and c-d6f phage DNAs was about 110 kilobase pairs and that of c-203 and d-1873 was 150 kilobase pairs. PstI digested the DNAs from two group 1 phages and (c)-n71 phage with very similar patterns, but did not digest the DNAs from group 2 phages. On the contrary, Sau3A digested only the DNAs from group 2 phages though the similarity of digestion patterns was low.

The existence of the structure genes for the toxin in these five converting phages belonging to groups 1 and 2 and (c)n-71 was confirmed by the hybridization test with phage DNAs and the oligonucleotide probe which represented the DNA sequence predicted for the N-terminal amino acids (2 to 17) of C. botulinum type C toxin. The loss of the converting ability of...
(c)-n71 phage may be caused not by the deletion of tox gene but rather by the base mutation in c-st phage DNA.

INTRODUCTION

Clostridium botulinum is divided into seven types (A to G) based upon the toxins produced. The strains of these different types of C. botulinum can be classified further into four groups based on their biochemical and physiological characteristics, and their DNA and RNA homologies. Group 1 cultures are proteolytic and include A, B, and F; group 2 are nonproteolytic and include types B, E, and F; group 3 are nonproteolytic types C and D; and group 4 is weakly proteolytic type G. The properties of the third group of C. botulinum and C. novyi type A are very similar and differ only in the type of toxins produced.

In the current study, the relationship between plasmids and phages to toxigenicity of C. botulinum was investigated.

RESULTS AND DISCUSSION

Isolation of plasmids

Toxigenic C. botulinum and nontoxigenic clostridia resembling C. botulinum were screened for plasmids. Both nontoxigenic and toxigenic strains harbored plasmids ranging in mass from 2.1 to 81 MDa. All of the proteolytic type F strains tested were found to carry a single 11.5 MDa plasmid and all of the type G harbored an 81 MDa plasmid. 8

Plasmids and the toxigenicity of C. botulinum type G

Both toxigenic and nontoxigenic derivatives were isolated from six different type G strains after sequential transfer of the cultures at elevated temperatures of incubation.

All of the 78 toxigenic isolates continued to harbor the 81-MDa plasmid and to produce type G neurotoxin. In contrast, the 81-MDa plasmid and the ability to produce type G neurotoxin were concomitantly lost in all of the nontoxigenic derivatives. In addition, all of the nontoxigenic derivatives ceased to produce a bacteriocin after they were cured of the 81-MDa plasmid. 9 This is the first evidence which suggests that the production of botulinal toxin and bacteriocin is in some manner related to the 81-MDa plasmid carried by the toxigenic strains. The data suggest that the structural gene for toxin production or a regulatory element that influences synthesis may be present on the plasmid.

Isolation of phages

The phages were induced from toxigenic cultures of C. botulinum types A to G by treatment with mitomycin C or ultraviolet irradiation. The induced lysates were filtered through a membrane filter with pore size of 450 nm, ultracentrifuged, and then the sediments were observed by electron microscopy. It was concluded that all types of toxigenic strains carried one or more phages. 1, 11

Isolation of nontoxigenic strains

Toxigenic cultures were treated with acridine orange, mitomycin C, nitrosoguanidine, or ultraviolet irradiation or allowed to sporulate. These cultures were plated on blood agar plates and incubated anaerobically for two or three days. Individual colony forming units were selected and inoculated into cooked meat medium, incubated, and checked for toxigenicity by