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TRANSFERABILITY OF TOXIN PRODUCTION IN CLOSTRIDIUM HAEMOLYTICUM IRP-135

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Summary

Transfer of a toxin production from a toxigenic donor strain to a nontoxigenic recipient strain was demonstrated in Clostridium haemolyticum (Clostridium novyi type D). A high frequency transfer (Hft) characteristics of a conjugative plasmid was observed.

INTRODUCTION

Bacterial entrachromosal elements (plasmids) are known to contain a variety of properties such as resistance to antibiotic (Falkow 1975), toxin production (Gyles et al 1974, Jacob et al 1975), surface antigens (Bak et al 1972) and bacteriocin (Clowes 1972). Plasmids of bacteria commonly carry these genitic factors. Few investigations have been made concerning plasmids in Clostridia. The emergence of antibiotic resistant strains was reported in Clostridia in 1968 (Johnston and Cockeraft 1968). Recently the role of a prophage was demonstrated in synthesis of Cl. botulinum type C and D toxins (Inous and Iida 1970).

A plasmid was shown to be responsible for the production of a bateriocin (Ionesco et al 1976), and antibiotic resistance (Berfort et al 1978) in Cl. perfringens. Plasmids have been studied in bacterioides fragilis (Tinnel and Macrina (1976).

This paper reports the transfer of the toxigenic character of a donor strain to a recipient by means of a conjugative plasmid having a Hft characteristic.

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MATERIALS AND METHODS

Organisms- Clostridium haemolyticum IRP-135 was obtained from the—National Animal Disease Center, Ames, IOwa.

Media- Cooked meat medium was used for maintanence medium TPYG broth, Trypticase peptone (BBL) 5%, Proteose peptone (Difco) 0.5%, Yeast extract (BBL) 0.5%, glucose 0.5%, sodium thioglycolate 0.1% and cysteine hydrochloride 0.05% was used for toxin production. The TPYG broth was adjusted to a final pH of 7.6 prior to use. Solid medium was prepared from the TPYG broth by addition of 2% agar. Culture plates and tubes were incubated anaerobically at 37°C using the Gas pak system (BBL).

Assay of toxicity

The culture was initially grown in cooked meat medium for 24 hours. Then 5% (V/V) of the culture was inoculated into TPYG broth and incubated for 18 hours. The culture supernatant fluid was tested for toxin by the intravenous inoculation.

The serum neutralization test also was used to identify the presence of toxin in the culture supernatant fluid. Pairs of mice were inoculated with 0.5 ml of mixture composed of 0.3 ml of toxic fluid and 0.2 ml of the Cl. haemolyticum antitoxin. Control mice were inoculated with 0.3 ml of the toxin.

Selection of toxigenic and nontoxigenic cultures.

Clostridium haemolyticum IRP–135 was grown in cooked meat medium for 24 hours, inoculated into the surface of the solid medium and incubated for 48 hours. Then 5% (V/V) of the culture was transferred into TPYG broth for toxin production. Contents of each tube were centrifuged at 6000 xg for 10 minutes and 0.3 ml supernatant of each tube inoculated intravenously into two mice for the toxin detection. Toxin assay was performed on the culture supernatant of 30 selected colonies. Two isolates, No 6 nontoxigenic and No 13 toxigenic, were selected for further studies. These two cultures were inoculated on the surface of plates containing TPYG agar with 100 mg/ml streptomycin to determine sensitivity.

Isolation of streptomycin resistant mutation of nontoxigenic cultures.

Ten ml of a culture of nontoxigenic strain No 6 was centrifuged at 6000 xg for 10 minutes, the supernatant fluid was discarded and the packed cells were resuspended in 10 ml of 0.85% NaCl solution. The washed cells were harvested and suspended in 10 ml of 0.85% NaCl solution and equal aliquots dispensed into petri dishes and exposed to ultraviolet irradiation for 30 and 60 seconds

respectively, at a distance of 45 cm. The irradiated cells from each dish were inoculated into a double strenght of TPYG broth and incubated fo 24 hours. A loopful of cells were transferred to the surface of TPYG agar containing 100 mg/ml streptomycin and incubated for 3 days. Ten colonies from each plate were isolated, inoculated into cooked meat medium and then transferred into TPYG broth. The supernatant of each tube was injected into two mice by the intravenous route. One streptomycin resistant mutant culture was used in the experiment.

Transfer of the toxigenic characteristic

A single colony culture of strain IRP–135 was streptomycin sensitive and toxigenic (T.S-)used as the donor in test for transfer of the toxigenic factor. The recipient strain in this experiment was streptomycin resistant and did not produced toxin (NT,S+). 0.5 ml of 24 hours culture of donor strain grown in cooked meat medium was mixed with 0.5 ml culture of the recipient strain. For control 0.5 ml of donor culture was placed in the test tube, the control and recipient consisted of 0.5 ml of culture and 0.5 ml of the broth. An additional control consisted of 0.5 ml of the nontoxigenic streptomycin resistance culture mixed with 0.5 ml of filtrate prepared by passing the supernatant of donor culture through a membrain (Millipore corporation) filter of 0.45 micron per size. All tubes were incubated in Gas pak system for 4–6 hours. A loopful of the culture mixture inoculated on surface of the streptomycin agar. The plates were inoculated on surface of the streptomycin agar. The plates were incubated at 37°C for 3 days. Ten colonies were picked and transfered to TPYG broth and tested for toxicity.

RESULTS

Culture supernatant of 30 colonies isolated of Cl. haemolyticum IRP–135 were tested for toxicity by inoculation into mice. Two colonies isolated were nontoxigenic. All 30 colonies isolated were sensitive to streptomycin at 100mg/ml. One nontoxigenic culture was exposed to UV irradiation and plated on TPYG streptomycin containing agar. Ten nontoxigenic streptomycin resistant organisms were isolated. Mutant colony isolated was used as recipient in further studies.

The donor strain (T,S-) and the recipient strain (NT,S+) were used in the genetic transfer. After incubation of the mixture of the culture of donor strain with the culture of recipient strain and then inoculated in streptomycin containing agar plates, 3 of 10 isolated colonies were demonstrated to be toxigenic and streptomycin resistant. The experiment using culture filtrate of the donor strain (T,S–) incubated with the culture of the recipient strain (NT,S+) and plated on streptomycin containing TPYG agar did not produce organisms

with the combined characteristics of toxigenicity and streptomycin resistance. The electron microscopic examination of the pellet of ultracentrifugated culture supernatant did not reveal any bacteriophage.

DISCUSSION

The Cl. novyi is divided into 4 types A,B,C and D in accordance of their different antigen. Eklund et al (1976) reported the relationship of bacteriophage to the production of the alpha toxin in Cl. novyi type A and B. When they removed the specific bacteriophages of type A, it ceased to produce alpha toxin and the reverted strain was closely resembled Cl. botulinum type C and D. Similarly when the specific phage of type B was eliminated, the alpha toxin production ceased. The reverted strain resembled Cl. novyi type D. In fact because of the similarity of Cl. novyi type D with type B, Oakley and Warrack (1959) referred to Cl. haemolyticum as Cl. novyi type D. These two strains are similar with the beta toxin, but as described by Rutter and Collee (1969) the beta toxin produced is grater in Cl. haemolyticum than Cl. novyi type B. Eklund et al (1976) isolated the phage from the supernatant of 18-h TPYG broth culture of the toxigenic parent strain. They made plaque formation by inoculation of diluted filtrate of supernatant culture on agar over layed plates.

Nakamura, Takematsu and Nishida (1975) did not succeed in demonstrating plaque formation in Cl. novyi type D. They demonstrated phage-like particles in some Mitomycin C lysates of some type D strains.

In our studies on Cl. haemolyticum IRP-135 we did not observed any plaque forming on agar over layed plates by the different diluted supernatant of toxigenic strain. In concentrated supernatant culture by dialysis bag against polyethylen glycol and by ultra centrifugation we did not observed any phages or phage - like particles by the use of Electron Microscopy.

It is sugested that in some type D strains there may be or may not be phages or phage-like particles as reported by Nakamura, Takematsu and Nishida.

The transfer of toxigenic character from donor strain (T,S–) to recipient (NT,S+) in our study showed that there were not any phages or phage-like particles mediated to transfer this character. The failure to transfer this property by the use of strile filtrated supernatant of donor (T.S–) to recipient (NT, S+) confirmed this matter. Indeed in this experiment cell to cell contact could transfer the toxigenicity proposed. It means that a plasmid or plasmids could transfer this property.

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