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CHARACTERISTICS OF A BACTERIOCIN-LIKE SUBSTANCE PRODUCED BY CLOSTRIDIUM HAEMOLYTICUM STRAIN IRP - 135

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SUMMARY

Clostridium haemolyticum IRP - 135 culture supernatant was added to cultures of 19 other Clostridial species.

Inhibitory activity was detected against 5 species as complete clear zone of growth. The fifth species contained a few cells resistant to activity of the bacteriocin-like substances. There was no evidence of bacteriophage present in the culture supernatant concentrated by ultracentrifugation and examined by electron microscopy.

The bacteriocin was demonstrated to be sensitive to trypsin digestion and heat inactivation at 60°C for 15 minutes.

INTRODUCTION

The ability of various bacteria to inhibit the growth of other bacteria was first observed by Geratia (1925). Several species of Clostridia also have been reported to produce bacteriocins (Betz and Anderson 1964; Clarke and Morris 1976; Honge et al 1968; Lou et al 1974; Mahony 1973; Mahony 1974; Mahony and Butcher 1971 ; Mahony and Swantes 1978). The studies reported in this paper concern the detection, identification and characterization fo a bacteriocin-like substance by Cl. haemolyticum strain IRP - 135.

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MATERIALS AND METHODS

Bacterial strains - The bacterial strains used in this study were Cl. haemolyticum IRP - 135 obtained from the National Animal Disease center, Ames, Iowa. Cl. bifermentans, Cl. botulinum, Cl. chauvoei, Cl. feseri Cl. haemolyticum, Cl. innocum, Ct. subterminal, Cl. terticum and cl. tetani obtained from clinical specimens submitted to the Diagnostic Laboratory Colorado State University, Fort Collins, Colorado.

Media - The cultures were maintained in chopped meat medium. The basal medium used for cultivation and bacteriocin production was designated trypticas peptone yeast glucose (TPYG) broth and consisted of trypticase peptone (BBL) 5% proteose peptone (Difco) 0. 5% yeast extract (BBL) 0. 5% glucose, 0. 5% and Agar (Difco) 1. 5% was added to the TPYG medium for preparing agar plates. The pH of the medium was adjusted to 7. 6 before autoclaving.

Inhibitory spectrum of the bacteriocin-like substance.

Various species of Clostridia were grown in chopped meat medium at 37°C for 24 hours. An aliquot of 0.5 ml. of each culture was mixed with 2.5 ml. of 0.6% plain molten agar. The mixture was poured into 60 mm plastic petri plates and allowed to solidify at room temperature.

A loopful of the culture supernatant of the reference culture of Cl. haemolyticum IRP-135 was spread on to the surface of the agar plates containing one of the Clostridia strains. The plates were incubated for 18 hours at 37°C in a Gas Pak system (BBL) and were checked daily for the inhibitory effect. Spoting method was also attemped using the sensitive strains. The sensitive strains were grown in TPYG broth for 18 hours, sample from each culture was spread to the surface of TPYG agar and allowed to dry for 15 minutes at 37°C. A drop of the supernatant of the culture of Cl. haemolyticum IRP 135 was spoted on the plate and allowed to dry for 15 minutes, then the plates were incubated for 18 hours at 37°C.

Bacteriocin titration

Ten fold dilutions were made of the bacteriocin like substance obtained from Cl. haemolyticum IRP-135. A 0.2 ml of each dilution was mixed with 0.2ml of the culture of each sensitive strain and incubated for 1-2 hours. The mixture was added to 2.5 ml molten agar at 45°C and poured on the surface of the agar plates and incubated anaerobically for 18 hours and the inhibitory effect was recorded.

Bacteriophae determination

An 18 hours culture of Cl. haemolyticum IRP-135 was centrifuged at 6000 Xg for 15 minutes. The supernatant was removed and centrifuged at 60000 Xg for 60 minutes. The pellet was collected for electron microscopic examination with the negative staining method using phosphate tangstate.

Heat sensitivity of bacteriocin - like substance

Aliquot of Cl. haemolyticum IRP 135 bacteriocin - like substance was dispensed into glass tubes and exposed to different temperature (50, 60, 70 and 94°C). At 5 minutes interval, a tube was removed from each of the controlled temperature water bath and frozen at - 70°C until evaluated.

Effect of trypsin on the bacteriocin like substance.

One ml aliquots of Cl. haemolyticum IRP 135 culture supernatant was dispended into plastic tubes and 1 ml of a 10^{-4} dilution of 1% trypsin was added to each tube. The tubes were incubated at 37°C for 5, 10, 15, 30 and 45 minutes. At appropriate time interval 0.2 ml. of the trypsinized bacteriocin was mixed with 0.2 ml. of the culture of one of the 5 indicator strains of Clostridia and incubated anaerobically 2 hours. The mixture was added to 2.5 ml. of 0.6% molten agar at 45°C and poured on to the surface of the agar plates The plates were incubated anaerobically for 24 hours and observation were recorded.

RESULTS

A substance in the culture supernatant of Cl. haemolyticum IRP - 135 completely inhibited the growth of 5 of 19 strains (Table 1).

The strains susceptible were Cl. bifermentans. Cl. butyricum. Cl. perfringens (Type C) , Cl. haemolyticum and Cl. feseri.

This inhibitory effect was due to the presence of a bacteriocin - like substance produced by Cl. haemolyticum IRP-135.The titration of the bacteriocin - like substance showed that its inhibitory effect was up to 10^{-2} dilution.

This substance was resistant to temperature of 50°C for 45 minutes but inactivated when held at 60°C for 15 minutes (table 2). The bacteriocin was inactivated when exposed to a 10^{-4} dilution of trypsin (1% solution) for 20 minutes. The presence of a bacteriohpage was not detected by the plaque formation technique using the 5 indicator strains. Also bacteriophge was not detected by electron microscopic examination of ultracentrifuged concentrated culture supernatant of Cl. haemolyticum IRP-135.

The result of spoting of the supernatant culture of Cl. haemolyticum IRP-135 on sensitive strains which seeded on the surface of agar plates showed that there were clear area around the spoted organism.

DISCUSSION

The substance which is produced by supernatant culture of Cl. haemolyticum IRP-135 inhibited the growth of some sensitive strains.

In our studies we found that the supernatant culture of Cl. haemolyticum IRP-135 did not produce any plaque on solid medium when different sensitive strains were used. In addition, the results obtained from the titration of the supernatant culture revealed that agent did not multiply inside the bacterial host.

When we exposed the supernatant culture to different temperature, it was found to be sensitive to heat. Treatment of the supernatant culture with proteolytic enzymes such as trypsin inactivated its inhibitory effect indicating that the agent was propably a protein, Further more, we were not able to observe any phages by either the plaque formating technique or the electron microscopy.

Considering the results of the experiments with regard to the properties of the inhibitory agent in the culture supernatant of Cl. haemolyticum, it can be concluded that the agent was most likely a bacteriocin.

However further experiments are requiered to islolate, purify and characterize the agent.

Table 1

Activity spectra of a Bacteriocin - Like produeced by Cl. haemolyticum IRP 135.

Clostridium species	Inbibition of growth as tested by	
	Method A (Culture supernatant)	Method B (Spot test)
C. bifermentant	+	+
C. botulinum	—	—
C. butyricum	+	+
C. chauvoei	—	—
C. feseri	+	+
C. haemolyticum	+	+
C. innocum	—	—
C. perfringens A	—	—
C. perfringens B	—	—
C. perfringens C	+	+
C. perfringens D	—	—
C. perferingens E	—	—
C. ramosum	—	—
C. sordelli	—	—
C. sporogenes	—	—
C. subterminale	—	—
C. tertium	—	—
C. tetani	—	—

+ = Indicates growth inhibition.

— = Indicates no activity.

Table 2. Heat inactivation kinetics of a Bacteriocin-Like substance produced by Cl. haemolyticum IRP 135.

Temperature	Time (Minutes)				
	5	10	15	30	45
50	+	+	+	+	+
60	+	+	—	—	—
70	—	—	—	—	—
94	—	—	—	—	—

+ = Not inactivated.

— = Inactivated.

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