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دراسة على منتجات النمو لميكروب السل الكاذب من مختلف المصادر مع اشارة خاصة الى انتاج التوكسين

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لقد تم دراسة مقارنة على منتجات السل الكاذب المعزول من الأغنام والبقر والجاموس والماعز والمعامل بالبلورات البنفسجية.

من هذه الدراسة لوحظ أن أعلى انتاج في حالة العترة المعاملة بالبلورات البنفسجية والأغنام تم الحصول عليها في اليوم الثالث وفي اليوم الخامس في حالة كل من الجاموس والماعز وفى اليوم السابع في العترات المعزولة من الأبقار.

وقد تم الفصل الكهربائي لافرازات الخلايا الخارجية واختبار كل جزء منفصل من كل عترة من العترات عن مختلف الأنشطة. وقد لوحظ أن بعض هذه الأجزاء ليس لها أية نشاط بينما اجزاء أخرى لها تأثير جلدى فقط أو جلدى وتكسير كلي لكرات الدم الحمراء.

وتبين من هذه الدراسة أن العترات المعاملة بالبلورات البنفسجية فقدت كل من التأثير الجلدى وتكسير كرات الدم الحمراء بالمقارنة بالعترات الغير معاملة.

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STUDIES ON THE GROWTH PRODUCTS OF C. OVIS OF DIFFERENT SOURCES WITH SPECIAL REFERENCE TO TOXIN PRODUCTION

(With 3 Tables & 1 Fig.)

By
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SUMMARY

Comparative studies on the growth products of C. ovis isolated from sheep, cow, buffaloes, goat and (C.V.T) strains were carried out. From the results obtained it was noted that maximum products were obtained after 3 days in case of C.V.T. and sheep strains, 5 days in case of buffaloes and goats strains and 7 days in case of cow strain. Electrophoretic separation of extracellular products was also performed and eluted portions of each of the produced bands were tested for different activities. It was noted that one of the bands showed no activity while other bands may only produce dermo-necrotic activity (D.N.A) or both D.N.A. and hemolytic activities. C.V.T. loss its lethality as well as its dermo-necrotic and hemolytic activity in comporative to the other strains.

INTRODUCTION

C. ovis is one of the commonest agents responsible for suppurative lesions in sheep and the condition is known as caseous lymphadenitis (STABLEFORTH and GALLOWAY, 1959; JOKLIK and WILLETT, 1976). In horses C. ovis caused a some what localized infection, ulcerative lymphangitis (JOKLIK and WILLETT, 1976), although a fatal generalized infection was reported by HUGHES, et al. (1962). The organism was also isolated from cattle affected with ulcerative lympangitis, caseous bronchopneumonia by some authors (TRAUM, 1923; PURCHASE, 1944 and SOLIMAN, 1962). In goats C. ovis was reported to cause caseous lymphadenitis (MISSENARD, 1935; BARAKAT, et al. 1970, 1972). Some authors reported that the hemolytic activity of staph. B.lysin was inhibited by C. ovis whether using living organism (FRASE, 1962, 1964; ZAKI, 1965; SEDDIK, 1980) or bacterial free supernatant (LOVELL and ZAKI, 1966; SEDDIK, 1980). The inhibitory agent of C. ovis was neutralized by C. ovis antitoxin (LOVELL and ZAKI, 1966 and SEDDIK, 1980). The aim of the present work is to study the products of C. ovis isolated from different hosts and the inter-relations of these products to the incubation period and the other activities.

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MATERIAL and METHODS

a) Media used:

- Modified Carn's media (LOVELL and ZAKI, 1966).
- LEANARD and HOLMS, 1935 used for production of staphylococcal B.lysin.
- 5% sheep blood agar plate.
- Christensen's medium (CHRISTENSEN, 1946).

Blood cells:

a) sheep, rabbits, chicken and human blood cells 2% in physiological saline.
b) A cetate buffer. pH 5.6 (CRUICKSHANK, et al. 1975).
c) The apparatus used for electrophoresis was hanging strip electrophoresis, OE - 202.

Strain used:

- 5 selected strains, from each of sheep, goat, cow, buffaloes and C.V.T. (Crystal Violet Treated).
- Staphylococcal var. albus. B.lysin isolated from bovine milk and staphylococcal hemolysin was prepared and titrated as described by SEDDIK (1980).
- Different silica gel activated at 110°C, solvent system which include, chloroform-amyl alcohol-acetic acid (50:50:3) for amino acid and detective reagents as Nitrous oxide gas followed by B-naphthol in alcohol 5% or ninhydrin in acetone 0.2%.

Methods:

The strains were identified by biochemical method as described by SEDDIK (1980). Each strain was inoculated into a tube of modified carn's medium and incubated at 37°C for 48 hours. After checking its purity, 2 drops from each strain were inoculated into the same sterile medium using Maccartiney's bottle and incubated in the slanting position at 37°C for different periods (3, 5, 7 and 10 days). Each culture was centrifuge at 400 r.p.m. for 30 minutes and the supernatant was pipetted into sterile MacCartiney's and the sediment was cultured to check purity. The total nitrogen was estimated by Kjeldahl method (WOTTON, 1974), and from each high nitrogen portion electrophoresis was applied using different buffer systems and 3 mm filter paper 36 cm long, 14 cm wide. The apparatus used for electrophoresis was hanging strip electrophoresis (OE-2.2). The supernatant of each strain was applied using standard micropipette each as stripat in the middle between the cathode and the anode. Running time was 2.5 hors, voltage adjusted from 200 V to 1500 V. After electrophoretic separtation each paper was allowed to dry in the air. A narrow strip 1 cm was cut longitudinally then sprayed with ninhydrin the corresponding area was cut with a pair of scissors and soaked with saline about 1.5 ml and left for 24 hours and tested for dermonecrotic, hemolytic activities as well as its action on staph. B.lysin by the well-method. Thin layer chromatography was used (SEDDIK, and EL-EMARY, 1981) for proteins fractionation in the studied strains.

RESULTS

From Table I it appeared that optimmum nitrogen content was obtained with the cow strains after 7 days incubation period, while the sharp decline phase detected at the 10th day. C.V.T. as well as sheep strains showed the high nitrogen content after 3 days which decreased with further incubation. Buffaloe and goat strains showed similar nitrogen content

after the 5th day which began to decrease with further incubation period. As regards the control, no change was noted with the incubation period.

To test for toxigenicity, 4 doses were used (0.7, 1, 2 and 3 ml) from the high nitrogen content of each strain and it was noted that C.V.T. had no lethal effect; a weak lethal activity was noted with goat strains at high dose while all other supernatants were lethal at the 3 ml dose with the exception of cow strains which showed lethality at the 2 ml dose but not at the 1 ml. As regards the electrophoretic seperation (Table 3) it was noted that there were some similarity between most species in hands migration e.g. C.V.T., sheep and cow. All the 5 bands of C.V.T. showed no effect either separated or in a mixture. The eluted portion of 1st and 4th bands of sheep showed dermo-necrotic activity in addition to the lytic activity of the 5th band on chicken RBC$_s$. There were also similarity between the activity of 2nd band in both buffaloe and cow strains. Bands migration in case of goat strains were more or less differents in its activity as well as migration distance.

Fig. 1: shows the use of TLC for seperation of protein frations. 4 bands can be distinguished in C.V.T., buffaloes and goat supernatant, while sheep as well as cow supernatants showed more complex bands.

The eluted portion from each band of the different species showed no effect on staph. B.lysin by the well Technique.

DISCUSSION

From the results, it was observed that maximum products were reached after 3 days with C.V.T. and sheep; while buffaloe and goat strains in the 5th day and the 7 day in case of cow strains. Further incubation lead to decrease in the nitrogen content which may be used for bacterial multiplication or need more explanation. The crystal violet treated strains showed no toxic effect on 1/p injection to G.pigs even at high dose while all other strains lead to toxic manifestation on 1/p injection. From our studies it was appeared that cow strain more toxic than the other species. C.ovis toxin was found to be lathal to G.pigs by many workers (WATSON, 1920; CARNE, 1940; MAXIMESCU, et al. 1973; SEDDIK, 1980). Also ZAKI (1965) proved that C.ovis supernatant was lethal to mice and had a dermonecrotic activity on guinea pigs.

The eluted portion of some band show no effect on skin reaction or RBC$_s$ while other bands showed more than one activity. Collection of different bands of the same species lead to sever dermo-necrotic activity.

The conclusion of this work is the identification of certain protein fractions inthe toxic substances obtained from the strains studied that can lead to sever dermo-necrotic activity and inhibitory effect on Staph. B.lysin.

The actuial nature of these matters will be the title of another extended work.

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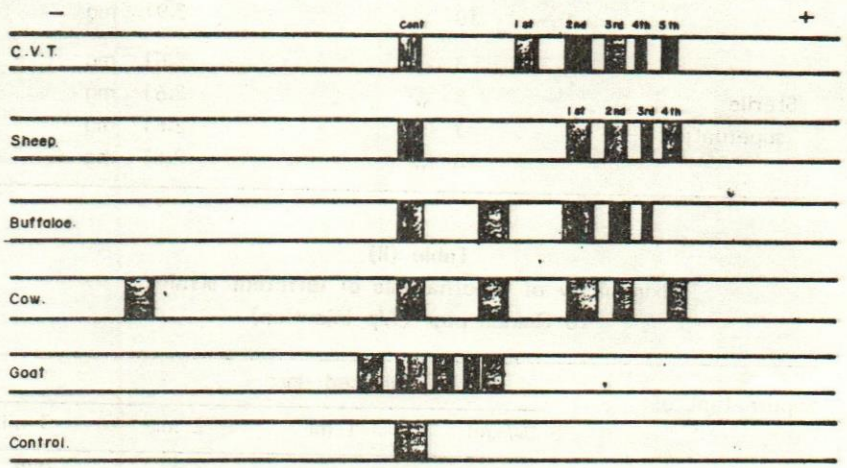

Fig.(1): The electrophoretic separation of the toxic fraction of C.V.T. sheep, Cow, goat and buffaloe strains.

C.OVIS TOXIN

Table (I)

Estemation of nitrogen content of bacterial free supernatant by Kjeldahl method (Wotton, 1974)

Supernatant of	Incubation period	Nitrogen content per 1/2 ml
C.V.T. 1(a) strains	3 days	4.35 mg
	5 „	4.30 mg
	7 „	3.48 mg
	10 „	2.92 mg
Sheep (2) strains	3 „	4.35 mg
	5 „	3.19 mg
	7 „	2.90 mg
	10 „	2.12 mg
Buffaloe (3) strains	3 „	3.34 mg
	5 „	4.35 mg
	7 „	2.32 mg
	10 „	1.45 mg
Cow (C.S. 4) strains	3 „	3.84 mg
	5 „	4.90 mg
	7 „	5.60 mg
	10 „	1.80 mg
Goat (5) strains	3 „	2.90 mg
	5 „	4.30 mg
	7 „	3.46 mg
	10 „	2.91 mg
Sterile supernatant	3 „	2.61 mg
	5 „	2.61 mg
	7 „	2.61 mg
	10 „	2.61 mg

Table (II)

Toxigenicity of supernatants of different strains to Guinea-pigs (1/p injection)

Supernatant of	Injected dose			
	0.7 ml	1 ml	2 ml	3 ml
C.V.T.	0/2	0/2	1/2*	0/2
Sheep	0/2	0/2	0/2	2/2
Buffaloe	0/2	0/2	0/2	2/2
Cow	0/2	0/2	2/2	2/2
Goat	0/2	0/2	0/2	1/2

Abbreviation:

* : The death occur after 20 minutes which may be due to a naphylactic chock.

\- : The numerator gives the number of dead animals, and the denominator the number of animals inoculated.

Table (III)
Band electrophoresis and activity of each eleuted band

Supernatant of	Band No.	Migration in cm	Length in cm	Dermo necrotic activity	hemolytic activity on RBC 2% of sheep	human	Rabbit	Chicken	Alternative activity on D.N.A.
C.V.T.	1st(+)	10	2	-	-	-	-	-	Collection of all band show no effect
	2nd(+)	13	3	-	-	-	-	-	
	3rd(+)	17	2	w	-	-	-	-	
	4th(+)	20	1.5	-	-	-	-	-	
	5th(+)	24	1.5	-	-	-	-	-	
	All			-	-	-	-	-	
Sheep	1st(+)	13	2	+	-	-	-	-	1+2+3---++
	2nd(+)	17	3	w	-	-	+	-	1+3+4---+
	3rd(+)	21	2	-	-	-	-	-	3+4--w
	4th(+)	24	2	+	-	-	-	-	2+4--w
	All			++	-	-	+	+	
Buff.	1st(+)	8	3	w	-	-	-	-	1+2+3--+
	2nd(+)	14	3	+	-	-	+	-	2+3+4--+
	3rd(+)	17	3	-	-	-	-	-	3+4--- -
	4th(+)	20	2	-	-	-	+	-	2+4-- w
	All			++	-	-	+	+	
Cow	1st(+)	8	2	w	-	-	-	-	1+2+3+4+5---- ++
	2nd(+)	13	2	+	-	-	+	-	2+3+5 -- +
	3rd(+)	17	2	-	-	-	-	-	3+5 --- -
	4th(+)	24	2	w"	-	-	-	w	
	5th(+)	21	2	-	-	-	+	+	
	All			++	-	-	+	+	
Goat	1st(+)	2	1.5	-	-	-	-	-	1+2+3--- +
	2nd(+)	3	1.5	w	-	-	+	+	1+3 --- w
	3rd(+)	8	2.5	w	-	-	-	-	1+4 --- -
	4th(+)	2	1.5	-	-	-	-	-	
	All			+	-	-	+	+	

Abbreviation:

w = week effect. (+) = in positive charge.
++ = strong dermonecrotic activity.
+ = mild dermo-necrotic activity.
- = No. effect.