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دراسة حيوية ميكروبي الكلوستريديم شوفياي
والكلوستريديم سبتكم في التربة

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أضيفت كمية معلومة من الخلايا الخضرية والحويصلات لكل من ميكروبي كلوستريديم سبتكم (6×10^6 خلية خضرية أو حويصلية / جرام من التربة) الى ثلاثة أنواع مختلفة من التربة (تربة زراعية وتربة رملية وتربة مزارع الألبان) .

تم اجراء الفحص البكتريولوجي لكل نوع من التربة على حده وفي اوقات محددة بعد العدوى الصناعية لأنواع التربة المختلفة . ولقد وجد أن حوالي ٨٠ - ٩٠% من الخلايا الخضرية لكل من الميكروبين قد تحوصلت خلال ٧٢ - ٩٦ ساعة من العدوى الصناعية للتربة وكان الكلوستريديم شوفياي أسرع في التحوصل قليلا عن الكلوستريديم سبتكم .

هذا بالاضافة الى أن حويصلات الميكروبين قاومت في مختلف أنواع التربة لمدة تتراوح بين ١٠ - ١٦ شهر .

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SURVIVAL OF CLOSTRIDIUM CHAUVOEI AND CLOSTRIDIUM SEPTICUM IN ARTIFICIALLY INFECTED SOIL

(With 2 Tables)

By

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SUMMARY

A known viable population of the washed inoculum of either Cl.chauvoei or Cl.septicum (6×10^6 spores or vegetative cells/gm soil) was added to three types of soil (dairy farm soil, cultivated soil as well as sandy soil).

At predetermined times, bottles representing each soil and control were subjected to bacteriological examination.

About 80-90% of the inoculated vegetative cells of Cl.chauvoei and Cl.septicum sporulated within 72-96 hours. The rate of Cl.chauvoei sporulation was slightly rapid than Cl.septicum. Moreover the spores of the two organisms persisted in the different soil from 10-16 months.

INTRODUCTION

Like many of other clostridia, Cl.chauvoei and Cl.septicum have two principal habitats, soil and the intestinal tract of mammals. The persistence of these pathogenic organisms in soil has been largely considered as a problem in disease control.

MINETT and DHANDA (1941) found that Cl.chauvoei was not able to grow in soil when artificially inoculated unless the soil contained large amounts of organic matter. SHALKO and KORNILOVA (1955) were able to isolate Cl.chauvoei after 5 years in soil artificially infected. KOVALENKO (1956) found that in loamy soil Cl.chauvoei remained alive for 13 months. POPESCU (1970) worked on the effect of some wild and cultivated plants on the persistence of Cl.chauvoei spores in soil, and found that it survived for 2-6 years according to the plant used. FARRAG (1972) isolated Cl.chauvoei from farm soil during an outbreak of blackleg in 40% of the samples examined and in 2.7% from those with two years previous history of the disease, while soil of non infected farms were free from the organism.

GARCIA and MCKAY (1969) inoculated Cl.septicum into soil treated by different ways and reported that vegetative forms persisted in both sterile and unsterile soil for up to 30 days in incubated samples, and that glucose, peptone and mannose enhanced the growth and metabolic activity of the organism.

GARCIA and MCKAY (1970) considered Cl.septicum and Cl.chauvoei as pathogenic bacteria that could grow in soil. SMITH and HOLDMAN (1968) reported that Cl.septicum had been

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found in soil from all parts of the world, being present in greatest numbers in relatively fertile loam but being found even in sandy soil. POL NILKOU (1982) was able to isolate Cl.septicum deposited at depth of 5-10 cm in experimentally infected soil for 180 days but not after 12 months.

The aim of this work is to study the behaviour of Cl.septicum and Cl.chauvoei under different circumstances such as the effect of moisture, pH value and organic matter in different types of Egyptian soil.

MATERIAL and METHODS

Soil:

Three types of soil were used in this study. Soil samples were obtained from a dairy farm, cultivated soil and sandy soil located at Giza Governorate. The samples contained organic matter 31%, 7% and 6% pH values were 7.5, 6 and 6 and moisture 22%, 16% and 3% respectively. The soil were dispensed in screw capped bottles each containing 25 grams of soil. These were sterilized by autoclaving for 30 minutes and kept at room temperature ready for use.

Vegetative cell suspension:

A pure 24 hours thioglycolate broth cultures of Cl.chauvoei and Cl.septicum were prepared. The cells were harvested by centrifugation, washed twice with saline and then resuspended in saline. The percentage of vegetative cells was 100% when tested microscopically just before soil infection.

Spore suspension:

It was prepared according to COOPER, et al. (1960). The suspension was heated at 80°C for 15 minutes to kill any vegetative forms.

Soil treatment:

A known viable population of the washed inoculum of each organism (6×10^6 spores or vegetative cells/gm) was added to the bottles of sterilized soil. A set of un inoculated soil was left as control.

At predetermined times, bottles representing each soil and a control were subjected to bacteriological examination. Ten fold dilutions were prepared from each bottle under test. Each soil dilution was shaken thoroughly before samples were withdrawn. Plating was done by spreading 0.1 ml portion of appropriate dilution on blood agar. Stiff blood agar plates were used for Cl.septicum (5% agar). Colonies were counted after 48 hours anaerobic incubation. The resulting counts correspond to the total viable clostridia (vegetative and spores) present in soil at the time of sampling. The proportion of spores in the total population was ascertained by counts made after heat treating the soil dilutions at 80°C for 10 minutes before plating. The heating technique was only done in soil dilutions infected with the vegetative cells to detect the percentage of sporulations at different times.

RESULTS

The rate of Cl.chauvoei and Cl.septicum sporulation in soils infected with vegetative cells:

Total viable counts of soil samples infected with vegetative organisms were made at 24, 48, 72 and 96 hours after soil infection. During this period the total viable count of both

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organisms was approximately the same as the original inoculum. The proportion of spores to the total population increased rapidly in all the types of soil and was more rapid in sandy soil than cultivated soil than dairy soil. The rate of Cl.chauvoei sporulation was slightly rapid than Cl.septicum.

The results are illustrated in Table (1).

Survival of Cl.chauvoei and Cl.septicum spores in different types of soil:

Total viable counts of soil samples infected with spores of Cl.chauvoei and Cl.septicum were made every two months after soil infection.

Cl.chauvoei decreased slightly at 2 months in the dairy farm soil. At 4 months the number decreased markedly until disappeared after 10 months. In cultivated and sandy soil, the spores started to decrease at 6 months and disappeared after 12 and 14 months respectively.

Cl.septicum spores remained approximately unchanged in sandy and cultivated soil for 6 months after which it declined to disappear completely at 16 month in cultivated soil while still persisted in small number in sandy soil. The number of spores in dairy farm soil started to decrease after 4 months until it disappeared after 12 months.

Results are illustrated in Table (2).

DISCUSSION

Epidemiological investigation have shown that the excretion of Clostridia by animals leads to contamination of the environment. These anaerobes are believed to occur as spore forms and could cause diseases under appropriate conditions. Little is known, however, about which soil conditions favour the predominance of either vegetative cells or spores. The preceding results give some clarification on the survival and sporulation rate of Cl.chauvoei and Cl.septicum in certain soils. Table (1) indicates that vegetative cells when inoculated into sterile soil sporulated rapidly and in 96 hours most of the cells of both organisms were in spore form. This observation does not agree with GARCIA and MCKAY (1969) who suggested that Cl.septicum could multiply and survive in the vegetative form as it did not immediately sporulate when they inoculated it into the soil. The difference between our results and these authors may be due to that they added to their soil readily utilizable organic matter (glucose and manure) and incubated it at anaerobic condition which tended to delay the rate of sporulation.

Under farming conditions where turnover of readily available organic material like manure is frequent, the increase of the clostridial population might be maintained for a longer period.

There is a great discrepancy in the literature about the survival period of Cl.chauvoei and Cl.septicum spores. Our results (Table 2) indicate that Cl.septicum spores were more resistant than Cl.chauvoei in the three types of soil tested, but the unexpected results were that the spores of both organisms survived for longer periods in sandy soil than the cultivated and dairy farm soil. Also it was astonishing that the spores of both organisms particularly that of Cl.chauvoei did not persist for long time in dairy farm soil.

It may be suggested that this was likely due to some proliferation of the spores to the vegetative forms (heat sensitive) in the dairy farm soil according to the presence of suitable pH (7.5), higher percent of organic matter and humidity.

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However, it could be concluded that the behaviour of clostridia under the condition of our investigation may be different than the natural environmental conditions which is usually affected by different seasons, sun light, dryness and other factors.

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Table (1)
The percentage of spores to the total viable Clostridia
in soils infected with vegetative cells

Hours after infection	Dairy farm soil		Cultivated soil		Sandy soil	
	Cl.ch.	Cl.sept.	Cl.ch.	Cl.sept.	Cl.ch.	Cl.sept.
24	10%	5%	10%	10%	20%	15%
48	20%	15%	20%	20%	35%	30%
72	60%	50%	70%	60%	80%	75%
96	80%	80%	85%	80%	90%	85%

Cl.ch. = Cl.chauvoei

Cl.sept. = Cl.septicum

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Table (2)

Survival period of Cl.chauvoei and Cl.septicum spores in different types of soil

Period after incubation	Dairy farm soil		Cultivated soil		Sandy soil	
	Cl.ch.	Cl.sept.	Cl.ch.	Cl.sept.	Cl.ch.	Cl.sept.
2 months	5.5×10^6	6×10^6	6×10^6	6×10^6	6×10^6	6×10^6
4 months	3.0×10^5	6×10^6	6×10^6	6×10^6	6×10^6	6×10^6
6 months	2.0×10^4	4×10^5	5×10^5	6×10^5	5×10^6	6×10^6
8 months	1.0×10^4	3×10^4	4×10^4	6×10^4	4×10^5	5×10^5
10 months	2.0×10^3	1×10^4	3×10^4	5×10^4	6×10^5	4×10^5
12 months	-	2×10^2	1×10^3	4×10^4	3×10^5	4×10^5
14 months	-	-	-	2×10^2	2×10^3	3×10^4
16 months	-	-	-	-	-	2×10^2