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A STUDY ON ISOLATION, IDENTIFICATION AND THE DISTRIBUTION OF *CLOSTRIDIUM* SPECIES FROM IRAQI SOIL SAMPLES

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ABSTRACT

One thirty eight soil samples from ten locations in Iraq were collected in November/December 2017 and inspected for presence of the variety Clostridium species. Direct Immune-fluorescent measure (IFA) examination demonstrated that five Clostridium species were distinguished in the dirt of particular locations in Iraq. A sum of 26 identified samples of five diverse toxigenic *Clostridia* species was segregated. From the results, soil sample from S1, S4, S7 and S8 have relatively the most elevated aggregate *Clostridium perfringens* counts compared with the other five areas. These investigations add to the comprehension of pervasiveness of toxigenic *Clostridia* species and phylogeny inside the species and aid advancement of enhanced diagnostics and therapeutics for the treatment of *Clostridia* diseases. A part of the tainting could be ascribed to extra cellular and in partly decayed cells.

Keywords: Soils samples, *Clostridium* species, Isolation, Identification

INTRODUCTION

The obligatory anaerobic bacteria have two principal habitats in nature, the alimentary tract of animals and man and the soil. It has been shown in the extraction of *Clostridia* from soil that strains of a given microbial varieties change in toxigenicity [1]. The genus *Clostridium*, comprises of an extensive number of species with a wide range in biochemical and physiological characteristics and given in Bergey's

manual [2]. The presence of the organism in soil has made it an important soil pathogen. Soil borne diseases might be viewed as a major issue in an extensive livestock production, in spite of the absence of up-to date records and data on their the study of disease transmission. Microbial cultures in soil depends of different elements, for instance, temperatures, salts obsession, pH, carbon sources, etc., while developing in soil, microorganisms contend with one another for development focal points and advance a system to prevail upon different organism [3]. Significant paper is done to discover new antimicrobial producing microscopic organisms confined from soil [4]. *Clostridia* are widely available microbe in nature especially in soil as well as in freshwater and marine sediments throughout the world. *Clostridium* contains about hundred species, further sub-divided into an immense number of non-pathogenic species, almost 25-30 minor pathogens and nearly thirteen significant pathogens [5-6]. Based on the literature study, different methods available for the isolation of bacterial strains from soil samples viz., bacterial respiratory activity [7], Electron microscopic studies [8], DNA analysis [9], fluorescent antibody study [10] etc. Furthermore, the immaculateness of bacterial portions was not explored in a quantitative manner. The fundamental point of the work depicted in this paper was to examine the likelihood of isolating specified bacterial strains from a soil sample of Iraq, hence empowering us to perform investigations in the selected area. To acquire strains of *Clostridium* Sp. having an assortment of speculating capacities, an attempt was made on isolation from soil samples collected from ten location of Iraqi regions by standard method (Yamagshi, 1964) [1], it is important to remember that the toxigenicty and the biologic nature of sixteen isolates had been investigated from 138 soil samples.

MATERIALS AND METHODS

The samples of the soil had been taken from the soil surfaces at a separation far from cavity stream at a profundity of 0.20 cm. The profundity of zero to twenty cm was utilized on the grounds that it is trusted that contamination diminishes with increment in soil. The soils tests were gathered into marked sterile polyethylene sacks and taken in ice stuffed cooler to the research facility for biosegment. Soil samples (n = 138) were collected from 10 cultivated fields in Baghdad city and surrounding areas identified with GPS coordinate was $33.3152°N, 44.3661°E$ in Iraq. The particles sizes of the soils vary from the sand, clay and a silt fraction, as shown in Table (1).

Isolation of *Clostridium* species from Soil samples

Soil samples were suspended in TBS and diluted to 10^{-4}. A portion of diluted liquid was spread on to TSA plates. Bacteria had permitted to grow in a 35°C as shown in figure (1). After 48 hours, growth of bacteria had been studied. Bacterial colonies had been analyzed based on color and morphology, and the bacteria noticed were of different categories. Results that had been deduced coincided with the previous reports [11]. Direct immune-fluorescent assay (IFA) examination was used to make an initial identification of samples reacting *Clostridium septicum*, *Cl. novyi*, and *Cl. chauvoei*.

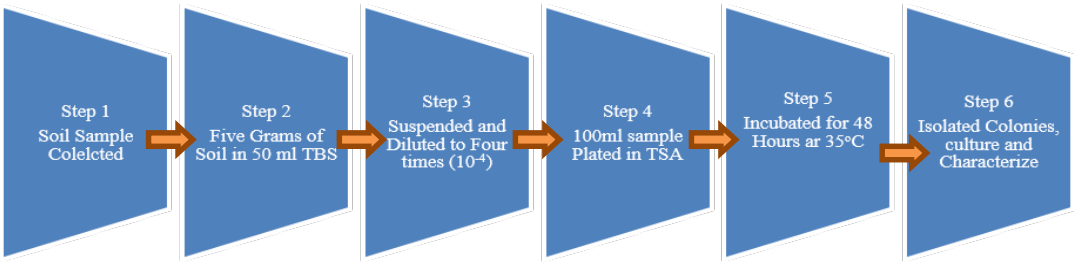

Figure 1: A diagrammatic representation of isolation of microorganism from Soil samples

Isolation of genomic DNA from bacteria

The bacteria D.N.A. had been deduced due to the procedure developed by Sambrook [12]. The genomic D.N.A. had been deduced by Doyle and Doyle's method [13]. The pure isolated D.N.A. from samples was sequenced for phylogenetic analyses.

16srRNA gene intensification

The almost entire 16S rRNA gene sequence, about 700bp - 1,500bp long, was enhanced utilizing the universal primer P930 and the universal primer P932 [14]. The P.C.R. responses were run utilizing the techniques given by Chouari et al **[15-16].** The P.C.R. result of 16srDNA enhancement had been purified with P.C.R. filtration spin unit. The purified P.C.R. samples had been then sequenced by utilizing a Taqdideoxy eliminator cycle automatic sequencer in the two bearings utilizing roughly 250ng of layout D.N.A. per reaction mixture and 6pmol/L of forward and invert primers [17].

RESULTS AND DISCUSSION

Soil around agricultural land and industrial land area is a good environmental for micro-bial growth due to abundant supply of organic material. In addition, warm temperature in the selected area in the month of November and April in Baghdad city in Iraq suitable bacterial growth. Hence this place is selected and studied bacterial population in different soil samples taken

from ten locations in and around the city. Despite a wide range available selective media for isolating specific group of bacteria from soil and clinical sample, there are relatively few media available for isolation anaerobic micro flora colonizing natural samples. The result of IFA is shown in Table 1. The presence of *C. difficile, C. sordellii, C. bifermentans, C. perfringens, C. clostridiiforme* varies with sampling location. The samples of the soil gave a prevalence of 31.15% *Clostridium* species. Among the 43 strains obtained, 5 toxigenic strains were documented up to species level which includes *C. difficile, C. sordellii, C. bifermentans, C. perfringens* and *C. clostridiiforme*. The percentage of isolation of *C. perfringens* and *C. bifermentans* being predominant were 11.59 % and 9.42 %, respectively. None of pathogen has been strained of *Clostridia* were obtained. The pH of the soils ranged from 6.37 to 7.01 with an average value of 6.80. There were no differences in the recovery of *Clostridia* strains in different pH of the soil samples.

The primer sequences, their locations on the toxin genes, and the sizes of the P.C.R. products amplified in their respective reactions are given in Table (2). The identities of approximately one-half of the amplification products generated in the P.C.R.s were verified by dot blot hybridization with the type-specific probes. The main advantage to PCR would be the toxin cannot be replaced with P.C.R., although P.C.R. may be useful as an adjunct procedure. At the end of the present paper, the sequential got from *Clostridium* species commercial strain and sequential of different species acquired from Gene Bank showed a low genetic separations with the last group showed a genetic separation of 0-06 and 89% of sequential similar among 2 *Clostridium* species strains and were grouped in this cluster in the 89% reparation, figure (3).

Since quite a while had been exhibited the utilization of 16srDNA to do the rapid diagnostic of the diseases because of various *Clostridium* species. Regardless of than the sequence of a few areas of 16srDNA gene have shown homology in a few bacterial strains, different areas have demonstrated a significant distinction [18], turning into a decent choice to distinguish the clinically vital *Clostridium* species. REA profiles delivered by detaches had uniform assimilation design with sizes extending from 25 to 170 Kb, figure (2). From the limitation designs, a framework was produced utilized, figure (3).

Table 1: Sample locations, types of samples and strains obtained from the soil samples along with its pH values

S.NO	Location of the sample (Sample Code)	Soil Type (Color)	Soil Samples Collected	Particle Size (microns)	pH	Strains Obtained (no of sites detected)	No. of cultures obtained
1	Haifa street, Baghdad (S1)	Brownish	12	70	6.84	*C. bifermentans* (5)	2
2	Al Yarmuk, Baghdad (S2),	Greyish	9	70	6.91	*C. perfringens* (3)	4
3	Alkazimayah, Baghdad (S3)	Brownish	13	100	6.67	*C. difficile* (2)	2
4	Al Hurriyah Baghdad (S4)	Reddish	11	70	6.87	*C. perfringens* (7)	1
5	Al Dawrah, Baghdad (S5)	Greyish	14	70	6.94	*C. clostridiiforme* (3)	5
6	Al Jihad, Baghdad (S6)	Colluvial brown	18	70	7.01	*C. bifermentans* (4)	4
7	Al Washash,Mansour Region (S7)	Gleyic brown	16	70	6.99	*C. sordellii* (7)	1
8	Al-Shu'ala, Baghdad (S8)	Pelogleyic brown	17	70	6.84	*C. perfringens* (6)	1
9	Al Ju`ayfir, Baghdad (S9)	Brownish	10	100	6.57	*C. sordellii* (2)	2
10	Hayy al mansour, Baghdad (S10)	Greyish	18	100	6.37	*C. bifermentans* (4)	4

Table 2: Sequences of species-specific primers for five species of *Clostridium* species

	Sequence (5'-3')	Primers	Position	Amplicon (bp)
C. difficile	CTT CAA TAT GAA AGG AGA GCC A	P930	170-193	193
	GAA CTA TCC CTG CAATGA GTA	P932	1245-1354	
C. sordellii	CCAAGC CTG CTT CAC AAC GCC	P930	167-181	957
	CGG CAC GAC TCA TCG TTC CAT	P932	1009-1094	
C. bifermentans	CTC GTC GAG CGA TCT CT AT	P930	141-157	1035
	GCA CCT CAA AAG TCT TTC G	P932	944-927	
C. perfringens	GTC TGG AGG CAT CAT TCA TGT	P930	157-179	1354
	CAT GCA GAT GTC AGA AAG AC	P932	1255-1235	
C. clostridiiforme	GTT AGG TTC GGA TGG AAT CTT GA	P930	175-194	1247
	CGA CAC GAACTT TGC CCT GCA A	P932	1264-1247	

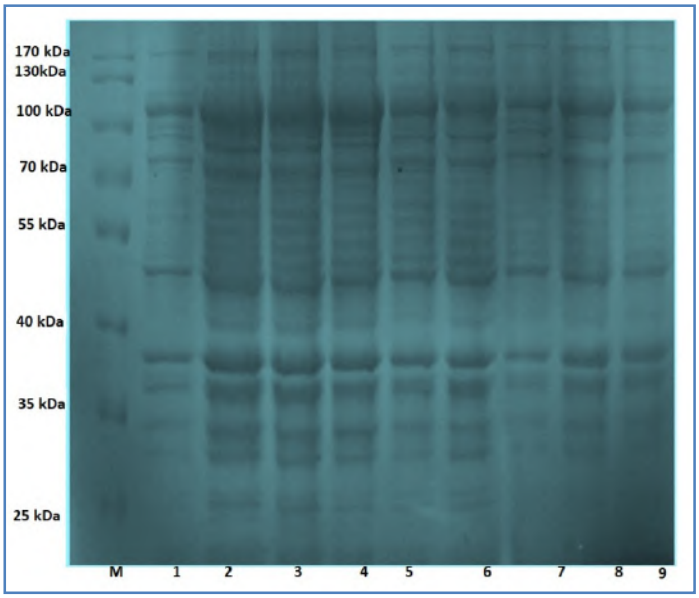

Figure 2: Comparative banding patterns of *Clostridia* isolates of whole-cell protein by SDS-PAGE Lanes: M=Molecular weight marker, 1= C. difficile, 2-3= C. sordellii, 4-6= C. bifermentans, 7-8=C. perfringens and 9= C. clostridiiforme.

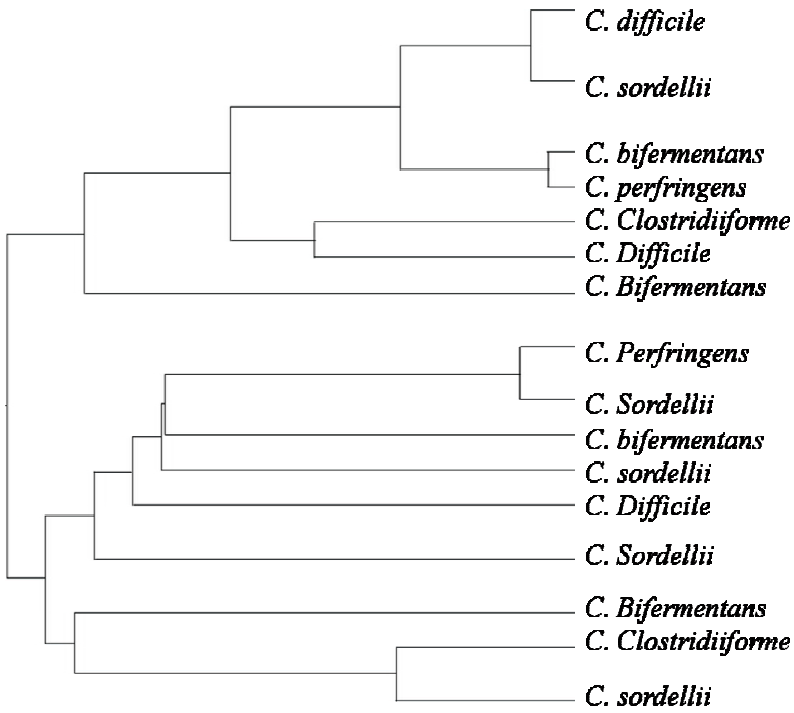

Figure 3: Dendrogram of the cluster analysis based on DNA restriction digestion banding pattern with EcoRI

CONCLUSION

The research emphasizes the need for an antimicrobial susceptibility test for anaerobic pathogenic bacteria, which transmit soil borne disease. Our data support the notion that the natural environment might be colonized by the strains which are industrially useful. The *Clostridium* pathogens secluded from soil of ranches influenced by cow-like mortality, demonstrated hereditary contrasts with reference microscopic organisms utilized by commercial research centers for the manufacturing for bacterins and toxoids. The current investigation the bio-chemical portrayal permitted recognizing bacteria species. This microbe is exceedingly pathogenic in animals, delivering an exceptionally mortality in the Baghdad city. Local characterization studies are needed to know history nature for bacteria distributed with soils.

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