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PROTEIN AND MOLECULAR CHARACTERIZATION OF *CLOSTRIDIUM SPP.* ISOLATED FROM CONTAMINATED FOOD AND SOIL SAMPLES

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ABSTRACT: *Clostridium spp.* is a common food contaminating anaerobic bacteria. Most of the packaged food contamination found to be caused due to *Clostridium spp.* Study was done on *Clostridium spp*. isolated from contaminated food and soil samples to characterize their toxic protein by SDS PAGE and to do their molecular characterization with the help of RAPD associated with restriction digestion by *EcoRI* using a random primer.

Key words: *Clostridium spp.*, food contamination, toxic protein, RAPD.

INTRODUCTION

Clostridium species is a *Gram* positive and rod-shaped microorganism. It is an obligate anaerobe, meaning that oxygen is poisonous to the cells. However, *Clostridium botulinum* tolerates traces of oxygen due to the enzyme called superoxide dismutase which is an important antioxidant defense in nearly all cells exposed to oxygen (Bengston I.A., 1924). *C. perfringens* is ever present in nature and can be found as a normal component of decaying vegetation, marine sediment, the intestinal track of humans and other vertebrates, insects, and soil. *C. botulinum* is only able to produce the neurotoxin during sporulation, which can only happen in an anaerobic environment. Other bacterial species produce spores in an unfavorable growth environment to preserve the organism's viability and permit survival in a dormant state until the spores are exposed to favorable conditions. *C. botulinum* is a lipase negative microorganism that grows between pH of 4.8 and 7 and it can't use lactose as a primary carbon source, characteristics important during biochemical identification (Suen J.C. *et. al*., 1988). *C. tetani* is found as spores in soil or in the gastrointestinal tract of animals. *C. tetani* produces a potent biological toxin, tetanospasmin, and is the causative agent of tetanus, a disease characterized by painful muscular spasms that can lead to respiratory failure and, in up to 40% of cases, death. *C. tetani* spores are extremely hardy as they are resistant to heat and most antiseptics. The spores are distributed widely in manure-treated soils and can also be found on human skin and in contaminated heroin (Warrell *et al.* 2003).

In our present study, our aim was to find out some of the indigenous food poisoning and pathogenic *Clostridium spp.* from contaminated food and soil samples and to do their protein and molecular characterization by using RAPD associated with restriction digestion. After finding the number of bands common among the DNA samples we can visually estimate the molecular relatedness of the samples.

METHODS AND MATERIALS

Isolation of *Clostridium spp.* from contaminated food and soil samples

Cooked but stable food samples and soil samples were collected and serial dilution was performed up to 10^{-8} fold and inoculated on *Clostridium* agar medium with the composition of (g/100 ml): Casein enzmic hydrolysate (1.7g), Papaic digest of soyabean meal (0.3g), Dextrose (0.6g), Sodium chloride (0.250g), Sodum thioglycollate (0.18g), L-cystine (0.025g), Sodium formaldehyde sulphoxide (0.1g), Neomycine sulhate (0.015g), Sodium azide (0.020g), Agar (1.45g), pH 7.2, which is a selective media for *Clostridium spp.* The plates were incubated at 36^{O}C for 24 hrs. The isolates were sub cultured to get pure colonies and were confirmed as *Clostridium spp.*, on the basis of various staining techniques and biochemical characteristics prescribed by Bergey's Manual of Systematic Bacteriology (Vos D. P. *et. al,* 2009).

Characterization of the endo toxin by SDS PAGE method

To isolate the endo toxin protein from the *Clostridium* broth, 1ml of broth was centrifuged at 6000rpm for 10 min. 200 µl SDS loading dye was added to the pellet and vortex gently, heated it for 15 min in water bath and vortex again to mix it. Centrifuged at 10000 rpm for 10 min and supernatant was taken to load in SDS PAGE assembly. In this system, proteins are dissociated into their constituent subunits using anionic detergent such as sodium dodecyl sulfate (SDS) or sodium lauryl sulfate. The protein mixture is denatured by heating the sample at 100^{O}C in the presence of excess of SDS. Under these conditions most polypeptide binds SDS in a constant weight ratio (1.4 gm SDS per gm of polypeptide). The intrinsic charges of the polypeptide are insignificant compared to negative charges provided by the bound detergent. Consequently SDS polypeptide complexes have essentially identical charge densities and these migrate in polyacrylamide gel of an appropriate porosity strictly according to polypeptide size or their molecular mass.

Molecular characterization of the isolates

The genomic DNA was isolated from the bacterial samples and were purified. The DNA samples were digested with EcoRI and BamHI separately. Molecular characterization was done with the help of RAPD (randomly amplified polymorphic DNA) technique associated with restriction digestion using a random primer. The DNA bands obtained were matched and the scoring results were run in a software "statistica" for the construction of linkage map to see their genetic relatedness.

RESULTS AND DISCUSSIONS

Bacterial colonies were obtained after inoculating the contaminated food samples and 1-2 feet deep soil samples on *Clostridium* agar media. Two different types of bacterial colonies were obtained at bottom of the Petri plates and their morphology was observed (Table 1). Both the colonies were identified as *Clostridium spp*., on the basis of staining techniques and biochemical characteristics (Table 2). The toxin protein was isolated and characterized by SDS PAGE method. The molecular weight of the toxic protein was found to be 48 kD. The genomic DNA was isolated (Fig 1) and digested with *HindIII* and *EcoRI* separately (Fig 2 and 3). RAPD was done with the DNA samples digested with *EcoRI* (Fig 4) and the scoring results were tabulated (Table 3) and were run in a software "statistica" for the construction of a linkage map (Fig 5) which shows the linkage distance among the samples (Table 4).

Table 1: Colony characteristics of both the isolates

Sample	Colony morphology
clostridium 1	Small yellow colored round shiny colonies were found
clostridium 2	Small colonies with elevated irregular shaped margin

Table 2: Biochemical characteristics of both the isolates

Biochemical tests and staining	Results
Gram's reaction	Positive
Endospore staining	Positive
MR	Positive
VP	Negative
Oxidase	Negative
Indole test	Negative
Glucose fermentation	Positive
Catalse test	Negative
Starch hydrolysis	Negative
Lipid hydrolysis	Positive
Gelatin hydrolysis	Negative
Casein hydrolysis	Positive
Urease test	Positive
Hydrogen sulphide test	Negative

Table 3: UPGA (Unweighted pair group average) analysis to determine the linkage distance among the DNA samples:

Variable 1	Variable 2	Variable 3	Variable 4
1	0	0	0
1	0	0	1
1	1	1	1
0	1	0	1
0	0	0	1
0	0	0	1
0	1	1	1
0	0	0	1
0	0	1	0

Hierarchical Clustering (Joining) Result:
Number of variables: 4
Number of cases: 9
Joining of variables
Missing data were case wise deleted
Amalgamation (joining) rule: Unweighted pair-group average
Distance metric is: Euclidean distances (non-standardized)

Table 4: Showing the linkage distance among the samples

Linkage distance among samples	Linkage units
CL1 AND CL2	2
CL1 AND CL3	2
CL2 AND CL3	1.4
CL2 AND CL4	2.3
CL3 AND CL4	2.3
CL1 AND CL4	2.3

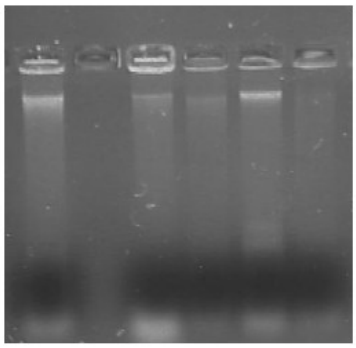

Fig 1: DNA bands of the *Clostridium* isolates

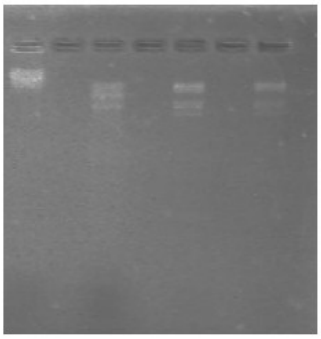

Fig 2: Restriction digestion with *HindIII*

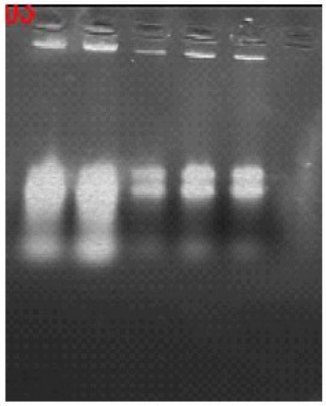

Fig 3: Restriction digestion with *EcoRI*

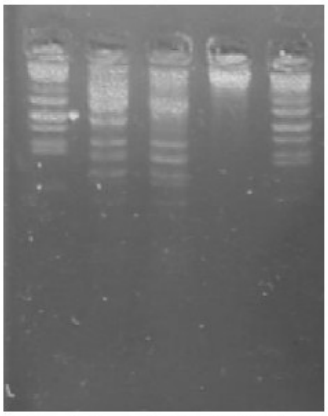

Fig 4: DNA bands after RAPD of *EcoRI* digests

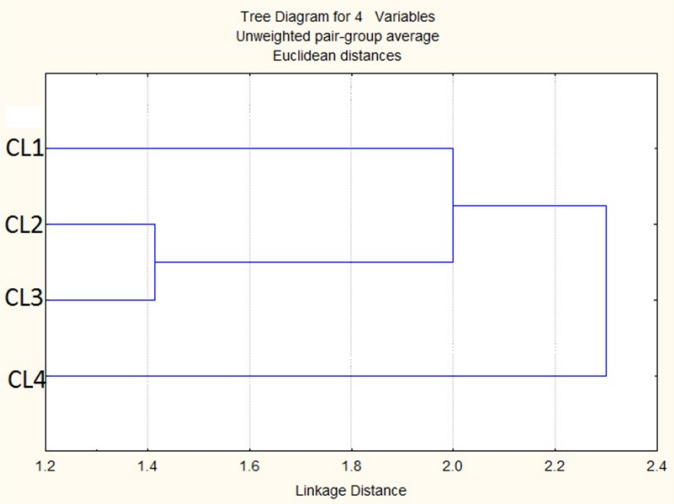

Fig 5: Linkage map showing the variation among the samples (where CL1= *Clostridium* isolate 1, CL2 = *Clostridium* isolate2, CL3 = *Clostridium* isolate3, CL4 = *Clostridium* isolate 4)

Conclusion

Identification and molecular characterization of *Clostridium species* is necessary for the detail study of it. Because of the pathogenic properties, there is a need of studying the various characteristics of the species to get a better idea about the functioning of metabolic activity of cell and which would help in finding out best applicable ways to check its growth and reduce the chances of infection in human society.

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