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# ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF CLOSTRIDIUM SPECIES FROM SPOILED FOOD MATERIALS.

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**ABSTRACT**

The Clostridium sp. were isolated from the rotten food materials, and found that the culture is anaerobic in nature, spore forming, thermophilic species; Cellulolytic bacteria, saccharoclastic. The colonies obtained after spreading consists a diameter of 3 to 6 mm, cream colored, Glossy and smooth, entire and umbonate property. The specific epithet refers to the initial isolate source, which is rotten in nature (a pile of compost).

**Key words:** Clostridium, Thermophilic, Spore, Rotten, Cellulolytic.

## INTRODUCTION

Processes cheeses are made from hard cheese such as gouda or cheddar **[1]**. Other ingredients are butter fat, salt, seasonings, cream, emulsifier, powdered milk etc. spores of clostridium Species can survive in the production of processed cheese, and can germinate and grow. One of the sources of the *Clostridium spp.* is hard cheese **[2]**.

Typical symptoms of spreading cheese spoilage because of the development of these clostridial species involves excessive gas forming and off – smelling caused by butyric acid, carbon di oxide and acetic acid production **[3]**. The flaw is the most common one called butyric or late blowing one found during spreading of dried cheese. A couple of cases of botulism of cheese products have been registered. A botulism outbreak was related to mascarpone cheese in 1996 **[4,5]** and a commercial processed cheese in 1993 outbreak sauce **[6-9].** Studies of inoculation with the causative strain has shown raise in the *clostridium botulinum* and the formation of toxins in the food materials and cause the spoilage. If the people consume such spoiled food, they have to suffer lots of infections due to consumption of the clostridium such as diarrhea, food poising etc. The aim of the study was to isolate, identify and characterize spore-forming bacteria from spoiled food materials **[10-13]**.

## METHODOLOGY

### Sample collection

Different spoiled food materials were collected from different location to obtain the desired species **[14]**.

**Isolation of bacteria from sample:**

The samples were serially diluted in 0.85% NaCl solution and then the samples were spread on sterilized nutrient agar pates. Once the colonies were obtained, the cultures were shortlisted on the basis of their morphological parameters and then converted to pure cultures using streak plate method **[15]**.

**Identification of bacteria:**

**F**or bacterial culture identification, various staining's and biochemical tests were carried out which were based on Bergay's manual **[16]**.

## RESULTS

### Collection of sample:

The samples were collected from various spoiled food materials.

### Isolation of bacterial culture:

The bacterial cultures were selected on the basis of their morphological parameters and then pure cultures were performed. As the clostridium sp. survives in anaerobic conditions, one culture S3C5 was selected for further biochemical analysis.

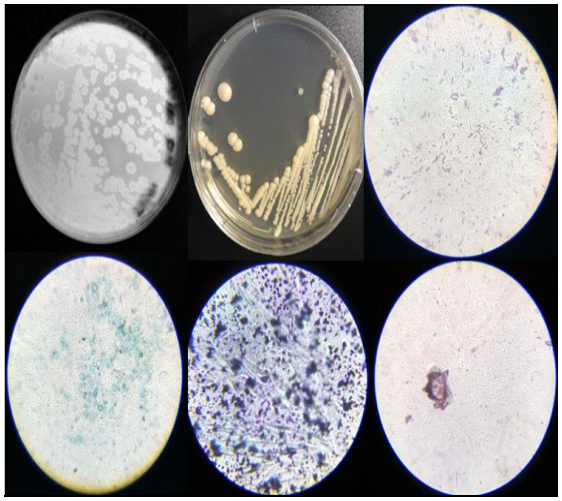

**Fig: 1.** a & b- colony morphology on blood agar plate, c- gram staining; d- spore staining;, e- capsule staining, f- biofilm

### Biochemical characteristics

*Clostridium* species are very diverse in biochemical characteristics. They are reported well for their Photolytic, Biolytic and Hemolytic activity.

### Protease

Casein milk agar plate assay was performed for protease activity. Clearance around the colonies indicated that culture is photolytic (Fig 2a). Proteases are group of 25 enzymes that hydrolyze peptide bonds of proteins and break them into polypeptide or free amino acids. Both *C. botulin* belonging to class 1 and *C. sporogenes* are proteolytic in nature. It was reported that nutritional and biochemical requirements of proteolytic *C. botulin* and *C. sporogenes* are indistinguishable. Extracellular protease production by *C. sporogenes* was reported during end of active growth phase or stationary phase under energy-limiting conditions (Allison & Macfarlane, 1990). *Clostridium* species like *C. botulin* and *C. tetani* belonging to proteolytic class are pathogenic, whereas *C. sporoenes* is considered as non-toxic variant of *C. botulin* (Sebaihia et al, 2007).

**Lipase**

Plate assays performed for lipase were positive with a clear halo zone in Oil agar plate (Fig 2b). A zone of oil hydrolysis is indicative of either lipase or esterase activity. They hydrolyze lipid to release free fatty acids and glycerol .Among *Clostridium* species, *C. sporogenes* and *C. botulinum* are reported for lipase activity. Lipase assay is generally used to distinguish *C. sporogenes* and *C. botulinum* from other *Clostridium* species (Barrow & Feltham, 1993). *C. sporogenes and C. botulinum* are very closely related and possess almost similar biochemical characteristics except for minor differences.

**Hemolysis**

Hemolysis is determined by streaking for isolation on a blood agar plate. From the lipase test and protease test analysis, it was indicative that the isolate could be either the *C. botulinum* or *C. sporogenes*. The Blood Agar Plate reaction for Hemolysis helps in distinguishing *C. botulinum* from *C. sporogenes* (Barrow & Feltham, 1993). *C. botulinum* gives a positive reaction for hemolysis with cleared under growth, show beta hemolysis (fig 2c).

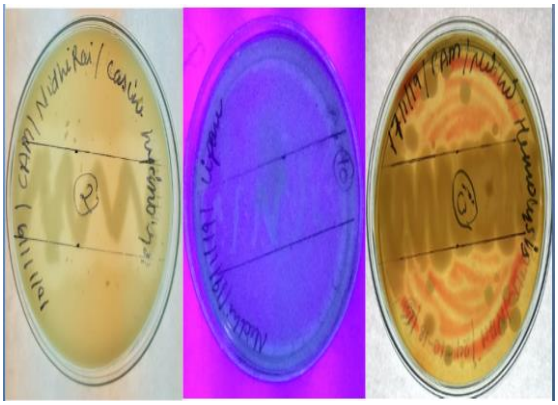

***Fig: 2.*** A: Protease, B: Lipase, C: Hemolysis

Biochemical tests for the isolate also gave positive result for H2S production, methyl red test, and the bacteria was negative for urease, Catalase, Peroxidase, Indol test, citrate utilization and nitrate reduction. Few *Clostridium* species are known for Stickland reaction in amino acid metabolism by using pairs of amino acids as electron donors and acceptors. Hydrogen sulphide production is reported for few sulfates reducing *Clostridium* species like *C. perferingens, C. sporogenes, C. botulinum*, *C. pasteurianum* etc. In *C. botulinum*, group I and II produce H2S whereas group III and IV are negative for H2S production.

Strains belonging to I and II are very different from III and IV (Oquma et al, 1986). It was reported that many of the *Clostridium* species follow inducible dissimulator type sulfate reduction pathway (Harrison, 1984). Acetoin production is reported in just few species of *Clostridium* and acids production (positive for methyl red test) is common with all *Clostridium* species.

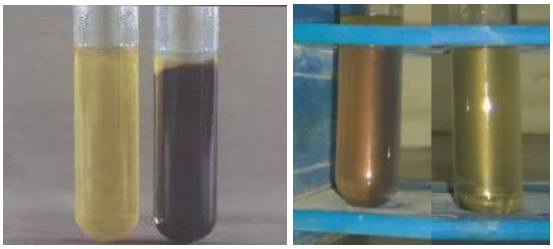

**Fig: 3.1.** Hydrogen sulfide test, Methyl Red Test

**Production, Isolation and Purification of *Botulinum* Toxin A**

The biologically active botulin toxin A can be produce by the fermentation method. The fermentation of toxin can be done by providing the suitable media under anaerobic condition. Crude enzyme obtained after 24hr of fermentation was purified by ammonium sulphate precipitation followed by dialysis of the salt precipitated protein.

*Fig: 4.1. Production of enzyme by the Fermentation process in different culture broth*

*Fig: 4.2.Salt precipitation*

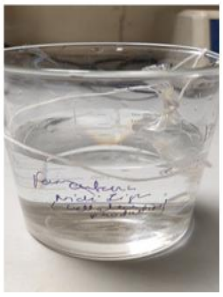

*Fig: 4.3. Dialysis*

**Enzyme assays of Crude and Purified Enzyme:**

The bacteria *C. botulinum* was able to grow in starch, CMC and casein rich broth. Growth was more profound in casein broth.

## DISCUSSION AND CONCUSION

*Clostridium botulinum,* was isolated and then screening was done for confirmation then biochemical testing was done for assessment of *clostridium*. Different enzyme was extracted from that bacteria like amylase protease, and cellulose. *Clostridium botulinum* produced maximum amylase enzyme. Substrate was made up of starch and that was coated on matrix. Then bacterial samples were inoculated on matrix and iodine test were done to check the hydrolysis of starch.

Pathological Grade increases mean Il-10 concentration in serum increases. Mean IL-10 concentration of Moderately Differentiated (30.0%), well differentiated (70.0%), respectively.

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