RESEARCH PAPER

Asian Journal of Bio Science, Vol. 4 Issue 2 : 226-229 (October, 2009 to March, 2010)

Optimization and comparative analysis of meat infusion in muller medium for the production of effective tetanus toxin

C.S. SIVA PRASATH AND S. PRAKASH

Department of Biotechnology, Udaya School of Engineering, Udaya Nagar, Ammandivilai, KANYAKUMARI (T.N.) INDIA

(Accepted : August, 2009)

Optimizations of fermentation medium for *Clostridium tetani* (MTCC 449) in the effective production of tetanus toxin were studied. Modified Muller Media with different concentration of Meat infusion has been used for the production of tetanus toxin . In this present work, the comparative study was carried out to determine the efficacy of tetanus toxin with the commercially prepared meat infusion broth compared and the meat infusion powder supplemented in Muller medium. The observed results showed the higher titer value of toxin in Muller media supplemented with meat infusion with higher concentration. The OD of the cell growth and pH changes due to buffering capacity were also studied. The better growth of the *C.tetani* was observed in the MM medium which contain higher rate of meat infusion broth. The level of the tetanus toxin was measured by Lf test. Minimal lethal dose was performed to check the presence of tetanus toxin.

Key words : Tetanus toxin, *Clostridium tetani*, Fermentation medium

INTRODUCTION

Tetanus is a life-threatening disease caused by infection with *Clostridium tetani* (Wassilak *et al.*,1994). *C. tetani* is a gram positive, spore forming, motile, anaerobic bacillus. The most common source of the environmental exposure to *C.tetani* are bacilli and spores in the soil, where the organism is widely but variably distributed. Typically measuring 0.3 to 0.5 micro meter in width and 2 to 2.5 micro meter in length, the vegetative form often develops long filament like cells in culture. Toxin has been traditionally prepared by growth of *C.tetani* in media containing animal and dairy products as a source of proteins, peptides and amino acids need for good growth. The growth medium traditionally used to produce tetanus toxin is MM (Mueller and Miller,1954),which contain glucose, beef heart infusion, a pancreatic digest of glucose, some amino acids and vitamins, uracil and inorganic salts. Mueller and Miller (1955) isolated three types of acid- labile components from casein digests by resin treatment, all of which were necessary for good toxin production, they were not chemically identified, however, meat infusion broths are good broths but the amount of peptone in them makes them expensive compared with digest broths.

The type of meat used is an important factor in determining the quality of the broth. It should be fresh, not frozen. Commercially available meat is used for the preparation of meat infusion broth. It makes costly and time consuming process. When the meat infusion broth is used in the media, the toxin production will vary depending upon the meat quality and health of the animal. So in order to maintain the consistency, use readymade meat infusion powder in the medium. The meat infusion powder contain 12% total nitrogen content. It reduce the time, cost and it make standard quality of meat protein. Since an infusion of beef heart is an essential component of the culture medium, the possibility exists that variations in different batches of this material may account for differences in toxin produced. Earlier work on the growth requirements of *C. tetani* (Feeney *et al.*, 1943) have shown the need for a fairly complex assortment of vitamins and extractives. The toxin is one of the most potent known poisons on a weight basis. As little as 1 nano gram / kg may kill a mouse; and 0.3 nano gram/kg will kill a guinea pig. The estimated minimum human lethal dose is less than 2.5 nano gram.

C. tetani produces two exotoxins, tetanolysin and tetanospasmin. Tetanus vaccines are based on tetanus toxoid. Conventional production includes growth of toxigenic strains of *C.tetani* in a liquid medium that favors toxin production, toxin harvest by filtration, detoxification by formaldehyde and several steps of purification and sterilization. The efficacy of the toxin has been analyzed by the antitoxin and the MLD test.

MATERIALS AND METHODS

Seed strain:

The strain of *Clostridium tetani* (MTTC 449) was obtained from Microbial Type Culture collection (MTTC)