Advances in Animal and Veterinary Sciences. 1 (1): 44–46
http://www.nexusacademicpublishers.com/journal/4

Short Communication

Optimization of the *In-Vitro* Growth of *Clostridium perfringens* type D

Ajay Kumar Rai, Vinod Kumar Chaturvedi*, Thangalazhy Gopakumar Sumithra, Sunita Chougule, Bincy Joseph, Murugan Shanmugam

Division of Bacteriology and Mycology, Indian Veterinary Research Institute, Izatnagar -243122, Uttar Pradesh, India
*Corresponding author: vkchaturvedi@mail.com

ARTICLE HISTORY

Received: 04-04-2013
Revised: 17-5-2013
Accepted: 20-05-2013

Key Words: Enterotoxaemia vaccine, *C. Perfringens* Type D, Sucrose, Sodium chloride, Sodium acetate, Tween-80

ABSTRACT

In order to overcome the economic difficulties associated with large scale production of enterotoxaemia vaccines, an attempt was made to optimize the *in-vitro* growth of *Clostridium perfringens* type D, so as to elevate antigenic mass of the vaccine. Maximum growth density was obtained in the production medium supplemented with 0.2% sucrose and 0.2% vitamin mixture with pH adjustment (7.5-8.0) after 8h of incubation. Growth density in these conditions was about two times more, than the growth at ordinary conditions. Among sugars tested (glucose, sucrose and dextrin), sucrose was the better growth substrate. When pH of media was controlled to 7.5-8.0 after 8h of incubation, growth was approximately one and half times more compared to same medium with uncontrolled pH. Mixture of vitamin B complex and C showed a stimulatory effect when supplemented with sugars. The optimum temperature was found to be 41°C. Sodium chloride had the utmost effect at 0.75% concentration. Tween-80 and sodium acetate were proved as inhibitors.

ARTICLE CITATION: Rai AK, Chaturvedi VK, Sumithra TG, Sunita SC, Joseph B, Murugan MS (2013). Optimization of the *In-Vitro* growth of *Clostridium perfringens* type D. *Adv. Anim. Vet. Sci.* 1(1): 44-46

Enterotoxaemia is a disease of great economical importance for sheep and goat farming worldwide (Niilo, 1980; Kriek *et al*., 1994), and has third rank among the various causes of death in small ruminants of India (Singh and Prasad, 2009). It can be well prevented by epsilon toxoid vaccine prepared from *Clostridium perfringens* type D culture (Jansen, 1967). Double initial vaccination is currently recommended for both sheep and goats, followed by a booster every year in sheep (Blood *et al*., 1983) and every 3-4 months in goats (Smith and Sherman, 1983). India has the second largest goat population and third largest sheep population in the world (Annual Report, 2010-11), so requires a large scale production of enterotoxaemia vaccine. The vaccine production can be economized to a great extent by elevating the antigenic mass which requires dense growth of *C. perfringens* type D. Therefore in the present study, attempt had been made to optimize effect of various physical and chemical variables on the growth of *C. perfringens* type D so as to have dense growth of this organism.

A highly toxigenic strain of *C. perfringens* type D procured from Division of Biological Standardization, IVRI, Izatnagar was used in the present study. Sterilized Robertson's cooked meat (RCM) media (Robertson, 1915) was used as the basal media to which various chemical agents were added to check the effect on growth. Chemicals such as glucose (20%), sucrose (20%), dextrin (20%), mixture of minerals viz. Iron, Magnesium, Zinc and Copper, mixture of vitamin B-complex and C, tween-80, sodium chloride (10%) and sodium acetate were prepared and sterilized by filtration.

Chemicals such as 0.2% glucose, 0.2% sucrose, 0.2% dextrin, 0.5% mineral mixture, 0.2% vitamin mixture (final concentration in the medium is given in %), various concentrations of tween-80 (0.1%, 0.2%, 0.3%, 0.4% and 0.5%), sodium chloride (0.5%, 0.75%, 1%, 1.25%, 1.5% and 2%) and sodium acetate (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) were added to RCM. All were inoculated with 10% actively growing overnight culture of *Clostridium perfringens* type D and incubated at 37°C for 24h.

At the same time, with the aim of finding out the optimum physical conditions, the inoculated basal media are incubated under different temperature (37°C, 40°C, 41°C and 42°C) for various incubation periods (18h, 24h, 36h and 48h). To know whether there is any importance of pH maintenance of media during the growth, pH of media was adjusted 7.5 to 8.0 with 1N NaOH at the end of 8 hrs of incubation. At the end of the incubation, O.D. of each culture was taken at 570nm.

Among the economically important bacterial diseases affecting small ruminants of India enterotoxaemia holds the first rank (Singh and Prasad, 2009) and accounts for 150 outbreaks, 1556 cases and 533 deaths only in 2009 (Annual Report, 2010-11). In order to reduce the cost for large scale enterotoxaemia vaccine production by elevating the antigenic mass in bacterial culture an attempt was made to optimize the *in-vitro* growth of *C. perfringens* under different physical and chemical conditions.

The maximum growth density was obtained in the production medium supplemented with 0.2% sucrose and 0.2% vitamin mixture with pH adjustment after 8h of incubation. The least growth was in those media supplemented with 0.2% dextrin without pH adjustment in between incubation. Further details of the results are given in Figures 1-5.

Among various sugars tested, sucrose was proved as better growth substrate which was similar to the report of Sacks (1983). But in contradictory to this, Fuchs and Bonde (1957) had reported that glucose was better than disaccharides as an energy source for *C. perfringens*. Further, vitamin also showed a

ISSN: 2307 – 8316 (online)

stimulatory effect when added with these sugars. Interestingly, when pH of these media was controlled after 8h of incubation, growth was approximately one and half times more compared to same medium with uncontrolled pH indicating the need for pH maintenance of media during the cultivation of this pathogen. On increasing the incubation period for more than 18h, there was no significant difference in growth. However, incubation temperature had marked effect on growth. Though it was reported that the optimal temperature of *C. perfringens* is in the range of 43-47^{0}C (Doyle, 2002) but the maximum growth was obtained at 41°C. On increasing the temperature up to 41°C there was a simultaneous increase in growth density, but after that further increase caused a decline in the growth. Morries *et al.* (1996) reported that sodium chloride can enhance the availability of soluble proteins as a result increase the yield of *B. thurigenesis*. However in the present study, sodium chloride was not giving a significant influence on growth of *C. perfringens*, though when used at 0.75% a slight increase could be observed. The same authors also used Tween-80 to increase the nutrient uptake of *B. thurigienesis*. While it was observed that Tween-80 was having negative effect on *C. perfringens*. Still, this effect was found to be decreasing on increasing the concentration. In order to check the effect of organic salts, sodium acetate was taken as a model. It showed negative effect on growth but, a slight increase in growth could be observed when added at 0.2% compared to control. Similar to our observation Juneja and Thippareddi (2004) also demonstrated the inhibitory effects of organic salts on growth of *C. perfringens.*

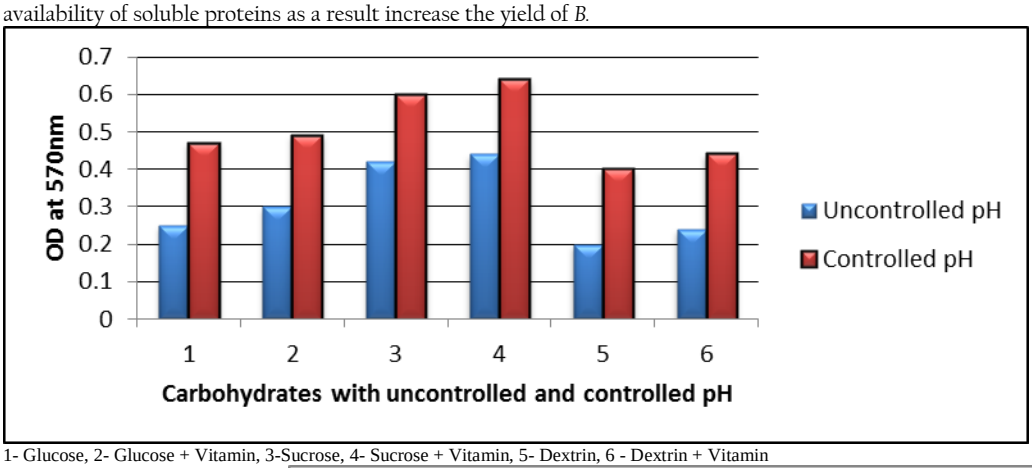
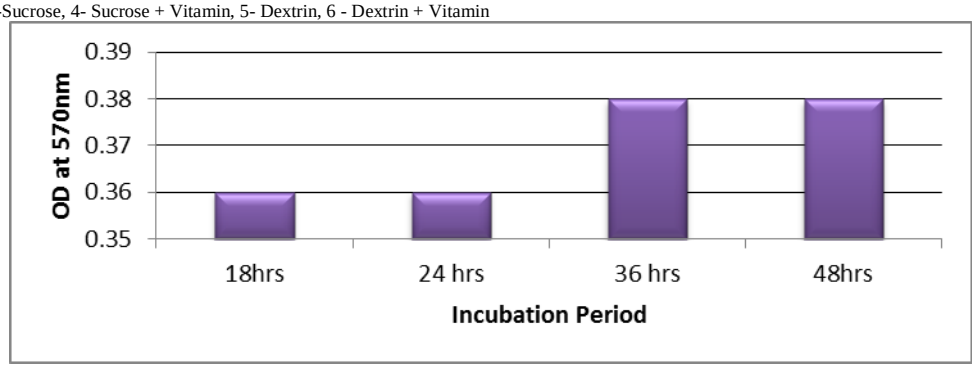


Figure 1: Comparison of growth on media with pH control and without pH control

1- Glucose, 2- Glucose + Vitamin, 3-Sucrose, 4- Sucrose + Vitamin, 5- Dextrin, 6 - Dextrin + Vitamin

Figure 2: Effect of incubation period



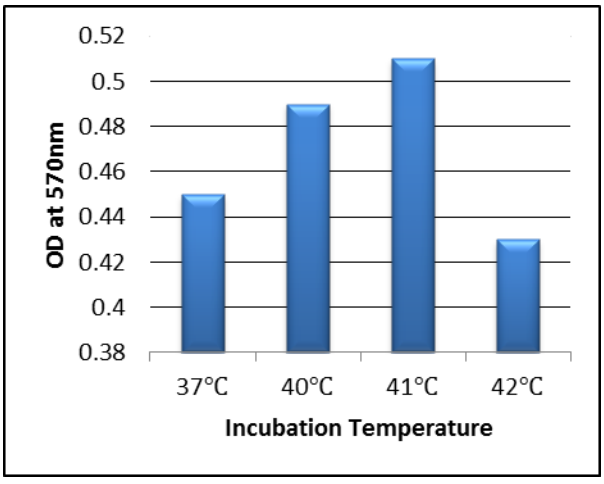


Figure 3: Effect of incubation temperature

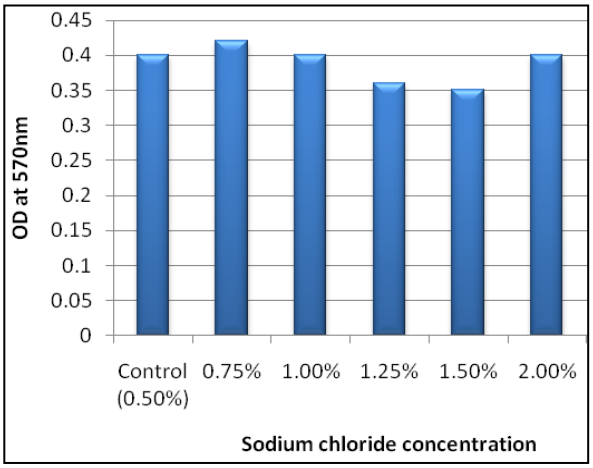


Figure 4: Effect of sodium chloride concentration

ISSN: 2307 – 8316 (online)

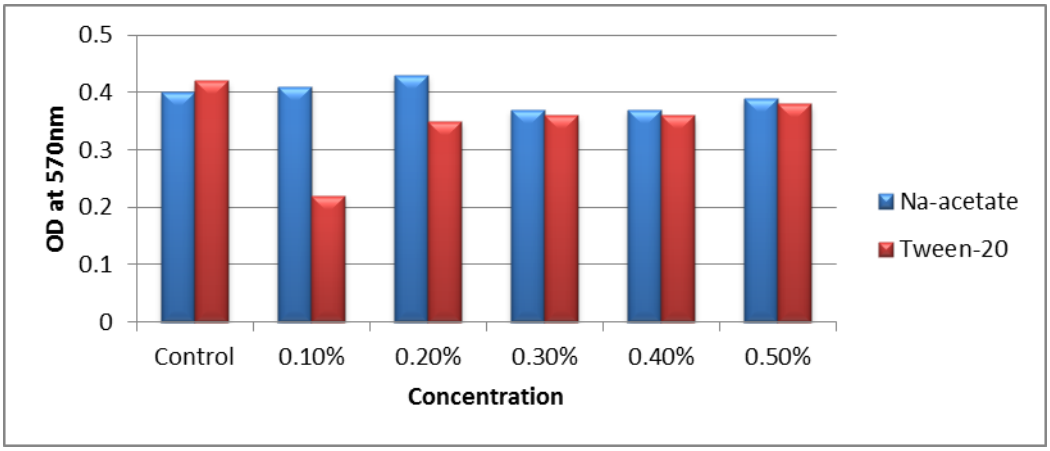


Figure 5: Effect of sodium acetate and Tween-80

In conclusion, the present study suggests that *in- vitro* growth of *C. perfringens* can be maximized by growing them in a production medium supplemented with 0.2% sucrose and 0.2% vitamin mixture and adjusting pH of the medium to 7.5-8.0 after 8h of incubation. This will help to elevate the antigenic mass during the production of enterotoxaemia vaccine hence, cut down its production cost.

ACKNOWLEDGEMENT

The authors are grateful to the Director and joint Director (Academic and Research) of the Institute for providing the necessary research facilities to carry out the present investigation. AKR thanks Indian Council of Agricultural Research (ICAR) for providing financial support in the form of Junior Research Fellowship.

REFRENCES

Annual Report (2010-11). Ministry of Agriculture, Govt. of India, Department of Animal Husbandry, Dairying and Fisheries: http://dahd.nic.in/dahd /WriteReadData/ Annual%20

Blood DC, Radostits OM and Henderson JA (1983). Veterinary Medicine: a textbook of the diseases of cattle, sheep, goats and horses. 6th Ed. Bailliere Tindall, Oxford.

Doyle E (2002). Survival and growth of *Clostridium perfringens* during the cooling step of thermal processing of meat products. A review of the scientific literature. **FRI Briefings. fri.wisc.edu.**

Fuchs AR and Bonde GJ (1957). The Nutritional Requirements of *Clostridium perfringens*. J. Gen Microbiol. 16: 317-329.

Jansen BC (1967). The prevention of enterotoxemia (pulpy kidney disease) by vaccination. *Bulletin - Office international des epizooties*, 67: 1539-1567.

Juneja VK and Thippareddi H (2004). Inhibitory effects of organic acid salts on growth of *Clostridium perfringens* from spore inocula during chilling of marinated ground turkey breast. Int. J. Food Microbiol. 93: 155-163.

Kriek NPJ, Odendaal MW and Hunter P (1994). *Clostridium perfringens* type D enterotoxaemia, In: Coetzer J.A.W., Thomson G. R. & Tustin R.C. (ed.) Infectious Diseases of Livestock with Special Reference to Southern Africa, Oxford University Press, Cape Town.

Morries ON, Converse V, Kanagaratnam P and Davies JS (1996). Effect of cultural conditions on spore-crystal yield and toxicity of *Bacillus thuringenesis* subsp. *aizawai* HD133. J. Invertebr. Pathol. 67: 426-428.

Niilo L (1980). *Clostridium perfringens* in animal disease: a review of current knowledge. Can. Vet. J. 21: 141–148.

Robertson M (1915). Notes upon certain anaerobes isolated from wounds. J. Pathol. 20: 327-349.

Sacks LE (1983). Influence of carbohydrates on growth and sporulation of *Clostridium perfringens* in a defined medium with or without guanosine. Appl. Environ. Microbiol. 46: 1169-1175.

Singh B and Prasad S (2009). Model based assessment of economic losses due to some important diseases in sheep in India. Indian J. Anim. Sci. 79: 77-80.

Smith MC and Sherman DM (1983). Goat Medicine, Lea & Febiger, Pennsylvania. pp: 620-625.

ISSN: 2307 – 8316 (online)