

OPTIMIZATION OF PHYSICO-CHEMICAL FACTORS AUGMENTING IN VITRO BIOMASS PRODUCTION OF *PASTEURELLA MULTOCIDA*

N. Sarwar, K. Muhammad, *M. Rabbani, **M. Younus, M. Sarwar, M. A. Ali, K. Hanif and M. Kamran

Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan
*University Diagnostic Laboratory, University of Veterinary and Animal Sciences, Lahore, Pakistan, ** Principal,
College of Veterinary and Animal Sciences, Jhang, Pakistan
Corresponding Author Email: drkhushi.muhammad@uvas.edu.pk

ABSTRACT

Pasteurella multocida causes hemorrhagic septicemia (HS), an economically devastating disease in buffalo and cattle. The disease is controlled effectively through mass scale vaccination of the susceptible animals. For a quality vaccine, biomass production of *P. multocida* along with its capsule (immunogen) is necessary. Physico-chemical factors such as composition of growth medium, temperature, pH, incubation time and stirring along with aeration affecting its biomass production were evaluated. In Casein yeast sucrose (CYS) broth, there was maximum growth during 18 hours incubation. Optimum conditions for maximum growth (8.2 mg/ml) of the bacterium were 35 to 40°C incubation temperature, 7.0 to 8.0 pH and 500 rpm stirring along with aeration during incubation of 24 hours. It was concluded that growth medium (CSY broth), temperature (35 to 40°C), pH (7.0 to 8.0), stirring (500 rpm) with aeration and incubation time (24 hours) are critical points to achieve high growth density (8.2 mg/ml) of *P. multocida* that is basic requirement for preparation of its cost effective vaccine.

Key words: *Pasteurella multocida*, Biomass production, Critical control points, Immunogen.

INTRODUCTION

Pasteurella multocida causes an acute and septicemic form of hemorrhagic septicemia (HS) with high morbidity and mortality in buffalo and cattle (Anonymous, 1996; De-Alwis, 1999; Horadagoda *et al.* 2001; Farooq *et al.*, 2007). The disease is highly fatal in nature and recovery is rare after onset of clinical signs. It can be controlled by mass scale vaccination in the countries where the disease is endemic in nature. Organized program of the vaccination with high quality vaccine and improvements in biosecurity are important measures to be adopted. The vaccine is highly effective especially when used before the onset of disease period.

The vaccines are prepared by using stationery culture method which is unable to produce required level of immunogen/ biomass of *P. multocida* (Shah *et al.*, 2008). As a result required amount of the immunogen is not incorporated per dose of the vaccine and hence the immune response of the animal is low and leads to failure of the immunoprophylaxis. To improve quality of current vaccines, it is necessary to work out the growth requirements (physical and chemical) of *P. multocida* to augment its biomass (Shah *et al.*, 2008). It will help to enhance the biomass production of the organism and ultimately effective vaccine preparation for the animals. It will also increase the number of vaccine doses prepared per ml of bacterial culture and decrease the cost of vaccine production.

Present study was therefore designed to investigate the factors affecting *in vitro* biomass production of *Pasteurella multocida*.

MATERIALS AND METHODS

Source of *Pasteurella multocida*: *Pasteurella multocida* (B: 2) was obtained from Department of Microbiology, University of Veterinary and Animal Sciences (UVAS), Lahore. The isolate was further characterized on the basis of cultural, morphological, biochemical and molecular characteristics (Townsend *et al.*, 1998; Kumar *et al.*, 2004).

Factors affecting biomass production of *Pasteurella multocida*: Effect of composition of the growth medium, incubation temperature, pH and stirring along with aeration on bacterial growth during *in vitro* incubation was studied and the growth was measured by dry weight method (Bratbak and Dundas, 1984).

Growth medium: Casein sucrose yeast (CSY) broth, Tryptose soya (TS) broth, Brain heart infusion (BHI) broth and nutrient broth were used to study the affect of medium on the growth of *P. multocida* (Ali *et al.*, 2000)). Each broth was prepared in separate glass flasks (one litter capacity having magnetic bar). Each of the flasks was autoclaved at 121°C for 15 minutes. Same quantity of the bacterial culture was inoculated in each flask and incubated at 37°C on magnetic stirrer for 24 hours.

Temperature: Using CSY broth, effect of temperature (10, 15, 20, 25, 30, 35, 40, 45 and 50°C) was studied on the growth of *P. multocida* (Shah *et al.*, 2008). Glass flasks with CSY broth were inoculated with the same quantity of the culture were incubated at different temperatures for 24 hours. The bacterial growth was measured at each temperature by dry weight method.

pH: Using CSY broth, effect of pH was studied by adjusting different pH (3.0, 3.4, 3.8, 4.2, 4.6, 5.0, 5.4, 5.8, 6.2, 6.6, 7.0, 7.4, 7.8, 8.2, 8.6, 9.0, 9.4 and 9.8.) of the medium (Shah *et al.*, 2008). Glass flasks (one liter capacity) with CYS broth of different pH were inoculated with same quantity of culture and incubated at 37°C for 24 hours. After incubation, the bacterial growth was measured at each pH by dry weight method.

Stirring: Using CSY broth, affect of stirring was studied at different speeds (50, 100, 150, 200, 250, 300, 350, 400, 450 500, 550 and 600 rpm). Glass flasks (one liter capacity) were prepared with CSY broth for each stirring speed (Shah *et al.* 2008), inoculated with same quantity of culture and incubated for 24 hours at 37°C. After incubation, the bacterial growth was measured at each stirring speed by dry weight method.

Commercial biofermentor: After optimizing media, temperature, pH and stirring speed, *P. multocida* was grown in commercial biofermentor. There were openings in the vessel for different sensors, air supply, sample withdrawal and inoculation etc. Temperature, pH, O₂, air flow rate and stirring were adjusted from LCD display. Culture was inoculated at the rate of 50ml/liter. Inoculation, addition of acid or base and sample removal was made through stainless steel capillaries equipped with Luer-Lock adapters.

Measurement of biomass of the bacteria: Dry mass of the grown *P. multocida* was measured by centrifugation of broth culture as described by Bratbak and Dundas (1984).

Statistical analysis: The dry mass of each flask culture was analyzed by one way analysis of variance (ANOVA) and means were compared to calculate the effect on growth of the bacteria.

RESULTS AND DISCUSSION

Pasteurella multocida causes hemorrhagic septicemia (HS) in buffalo and cattle. It is an important disease of dairy animals causing heavy economic losses (Rs 2.17 billion/annum in 1996) in Pakistan (Anonymous, 1996). It is normal inhabitant in larynx and pharynx of bovine in endemic areas and bone marrow of the dead animals. It grows well on blood agar and tryptose soya agar and shows grey, viscous mucoid, translucent and non hemolytic colonies on blood agar

after 20 hours incubation at 37°C but failed to grow on Mac Conkey's agar. Each of the isolates was Gram's negative, bipolar, coccobacilli or thin rods (De Alwis, 1996; Kayani *et al.*, 2000; Tabatabaei *et al.*, 2007; Kumar *et al.*, 2009). Different factors such as growth media, temperature, pH and agitation affected the physiological activities of *P. multocida* and had critical significance in augmenting its biomass production.

The bacteria showed growth (4.2mg/ml) in CSY broth, 1.8mg/ml in TS broth, 2.7mg/ml for BHI broth and 0.6mg/ml in nutrient broth (Fig.1). However, blood agar, MacConkey's agar, BHI broth and BHI agar are routinely used for isolation of *P. multocida* (Al-Humam *et al.*, 2004) and sucrose tryptose soya broth is used to grow *P. multocida* for preparation of inactivated dense culture (1.68mg/ml) vaccine (Ali *et al.*, 2000). Addition of white sugar (sucrose) in growth medium at rate of 0.1 to 2.0 % increases the bacterial growth (Shah *et al.*, 2008).

The bacteria showed maximum growth in CYS broth when incubated at temperature 35 to 40°C and the growth was declined when incubated at temperature above 40°C (Fig.2). Optimum growth was detected when incubated at 35±5°C while no growth was recorded at 25±5°C or above 50°C (Shah *et al.*, 2008). It showed maximum growth in CYS broth having pH range 7.0 to 7.8 (Fig.3). The organism showed optimum growth at pH range of 6.0 to 8.0 and did not grow at pH 2 and 10 (Shah *et al.*, 2008). It indicated that pH of the CYS broth is critical in its biomass production.

Agitation and aeration enormously influenced its growth (Shah *et al.*, 2008). Maximum growth of the bacterium was observed when broth was stirred at 500 to 550 rpm and growth was declined at 600 rpm agitation. The growth was 10⁸cfu/ ml in still culture, while it was 10¹⁴cfu/ ml laboratory made fermenter in 5 liter flask (agitation and aeration) and 10¹⁷cfu/ ml in commercial biofermentor (aeration and agitation were automatically controlled). A fermenter when provided with enrichment media and fresh filtered air resulted in 5×10⁹ cfu/ml and 1.68mg/ml dry weight (Ali *et al.*, 2000). The bacterial culture when incubated in a flask having BHI broth and agitated at 200 rpm shaking speed showed 10⁹ cfu/ml after 3-4 hrs incubation at 37°C (Tabatabaei *et al.*, 2007). The dry mass of the bacterial growth was 8.2 mg/ml as measured by centrifugation technique while its bacterial count was 10¹⁷ cfu/ml.

It was concluded that growth medium (CSY broth), temperature (35 to 40°C), pH (7.0 to 8.0), stirring (500 rpm) with aeration and incubation time (24 hours) are critical points in improving the biomass production of *P. multocida* in biofermentor. The dense bacterial culture is requirement for preparation of cost effective multivalent vaccines against microbial diseases of cattle and buffaloes.

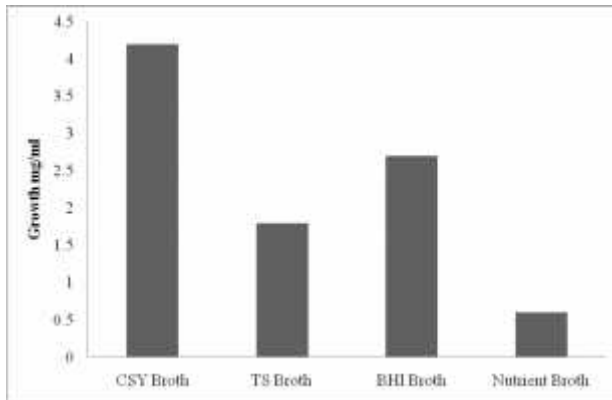


Fig.1. Growth Media VS Growth mg/ml

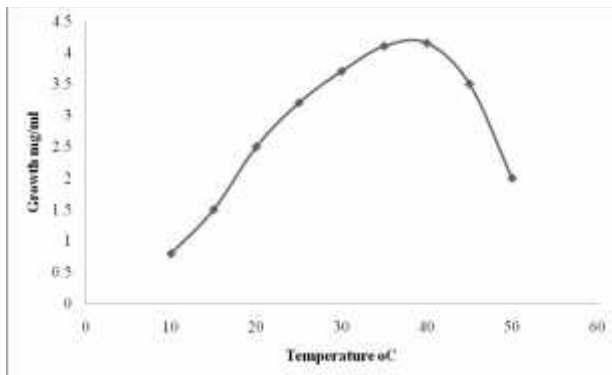


Fig.2. Effect of Temperature on *P. multocida* Growth

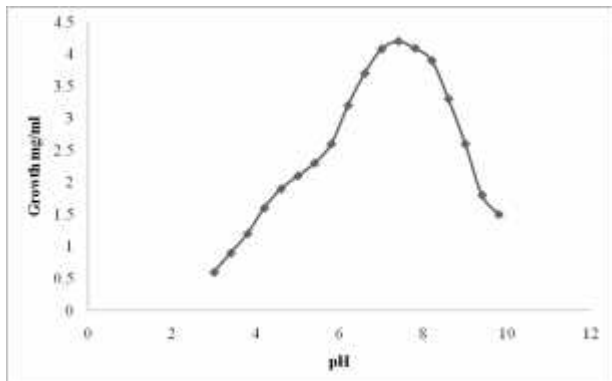


Fig.3. Effect of pH on *P. multocida* Growth

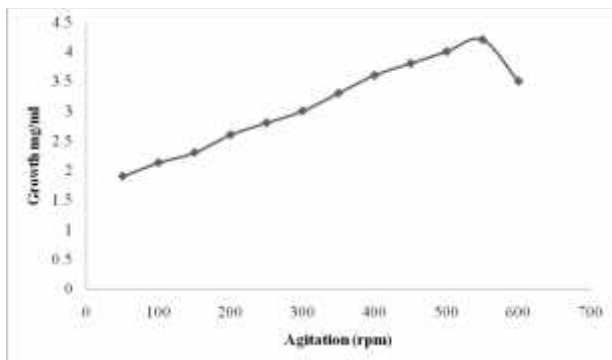


Fig.4. Effect of agitation on *P. multocida* growth

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