openaccess Journal
SCIENCE LETTERS
2014 | Volume 2 | Issue 1 | Pages 39-42

Effect of different media compositions and physicochemical conditions on the *in vitro* growth of *Pasteurella multocida*

Farzana Shahin*, Mudasser Habib, Muhammad Salahuddin Shah, Noubahar Hussain, Shahnaz Adeeb Khanum
Nuclear Institute for Agriculture and Biology (NIAB), Jhang Road, Faisalabad, Pakistan

Abstract
Hemorrhagic septicemia (HS) is an acute disease of cattle and water buffaloes. In Pakistan, it is frequently caused by B: 2 serotype of *Pasteurella multocida.* Vaccination is the principal mean of prevention. In vaccine manufacturing, higher yields of bacterial cell mass are of great value in terms of process economics. Present work extends the optimization of various physicochemical conditions of culture, e.g. effect of different media compositions, pH and inoculum size. Casein-sucrose-yeast media (CSY) was prepared with different concentrations of yeast extract, casein hydrolysate, and sucrose with different inoculums sizes. The maximum growth of *P. multocida* was achieved by using 0.6% sucrose and 8% inoculum size in CSY medium after 16 h incubation (OD 1.73) and at the pH range of 7.0-8.0. It was also found that different concentrations of media components and physical conditions have significant impact on the growth kinetics of *P. multocida.*
Key words: Hemorrhagic septicemia; *Pasteurella multocida;* physicochemical conditions**;** bacterial cell mass.

Received March 12, 2014; **Revised** May 02, 2014; **Accepted** May 12, 2014
Corresponding author: Farzana Shahin**;** Email: f.shaheen64@yahoo.com

To cite this manuscript: Shahin F, Habib M, Shah AS, Hussain N, Khanum SA. Effect of different media compositions and physicochemical conditions on the *in vitro* growth of *pasteurella multocida*. Sci Lett 2014; 2: 39-42.

Introduction

Hemorrhagic septicemia (HS) is an acute disease of cattle and water buffaloes [1]. The disease is caused *by Pasteurella multocida*, a gram-negative coccobacillus residing mostly as a commensal in the upper respiratory tract of animals. The HS in bovine is caused by specific serotypes of *P. multocida*. Serotypes B: 2 and E: 2 which are two common serotypes of *P. multocida* associated with disease in animals in Asia and Africa, respectively [2]. The HS occurs as catastrophic epizootics, resulting in high mortality and morbidity. The disease is per-acute, having a short clinical course involving severe depression, pyrexia, submandibular edema and dyspnea, followed by recumbency and death within 8 to 24 h of onset [3].

According to an estimate, Pakistan has a cattle population of 36.9 million and a buffalo population of 32.7 million heads [4]. In Pakistan, HS is considered as a disease of great economic importance. Importance of HS to buffalo rearing in Pakistan was highlighted by a recent survey which indicated its prevalence up to 49% [5]. The disease has a major impact on the livestock industry in countries of South and Southeast Asia [6, 7]. The young animals being are most at risk of successive outbreaks [8].

Vaccination is the best way to prevent the outbreak of the disease in bovine. For the economical production of vaccine, abundant growth of *P. multocida* along with well developed capsule around the cell is ensured. Sub-optimal growth leads to higher costs of production. Therefore, it is necessary to work out optimal requirements for the growth of organism. Current study was designed to optimize the growth conditions for *P. multocida.*

Material and methods

Source of micro-organism

An Isolate of *P. multocida* (B:2) was previously isolated and characterized [9]. Freeze dried seeds of *P. multocida* were procured from storage at ultra low temperature in Animal Sciences Division (ASD) at Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad, Pakistan.

Reactivation of the seed culture

Freeze-dried seed of *P. multocida* was re-activated in a test tube containing Brain heart infusion (BHI, Oxoid) broth and incubated at 37 ˚C for two hours. For the recovery of organism, the suspension was injected into rabbit intravenously (I/V). After the death of rabbit, heart and liver impressions were taken aseptically in brain heart infusion agar (BHIA) and also on the MacConkey's agar plates. After 24 h incubation, round, creamy, glistening colonies were observed on BHIA. On MacConkey agar no growth were observed (typical identification marker of *P. multocida)*. After Gram's staining and microscopic observation, the organism was appeared as Gram-negative, bipolar and short bacilli.

Media components optimization

The growth medium used for the growth of *P. multocida* was casein-sucrose-yeast (CSY) broth [10]. The media ingredients were as follows: yeast

extract (0.5 g), casein hydrolysate (0.3 g), sucrose (3 g), anhydrous potassium dihydrogen phosphate (0.3 g) and sodium chloride (0.5 g), by dissolving the ingredients in 100 ml distilled water. While to check the effect of different concentrations of yeast extract, casein hydrolysate and sucrose, one lower and one higher concentration were prepared along with the control CSY media.

After media preparation, the pH of the media was adjusted to 7.3-7.4 and autoclaved at 121°C and 15 bar pressure for fifteen minutes. After cooling of the media, a colony of the *P. multocida* was inoculated and all the flasks were incubated at 37˚C in incubator shaker. Dry cell mass were checked after 24 hours of the bacterial culture.

Table 1: Composition of different media ingredients

Ingredients	Lower conc.	Control (csy media)	Higher conc.
Yeast extract	0.25g/100ml	0.5g/100ml	1g/100ml
Casein hydrolysate	0.15g/100ml	0.3g/100ml	0.6g/100ml
Sucrose	0.15g/100ml	0.3g/100ml	0.6g/100ml
K_2HPO_4	0.3g/100ml	0.3g/100ml	0.3g/100ml
NaCl	0.3g/100ml	0.5g/100ml	0.3g/100ml

Effect of inoculum size

To study the effect of inoculum size on *P. multocida* growth, medium was prepared as described above. Ten flasks of CSY medium were prepared and each flask was inoculated with different percentages of starting culture (1-10%). After the inoculation, the flasks were incubated at 37˚C in incubator shaker at a shaking speed of 200 rpm. Dry cell mass in each flask was checked after 24 hours of culture.

Effect of pH

To study the effect of pH on *P. multocida* growth, media were prepared in 7 different flasks and pH was adjusted to 4, 5, 6, 7, 8, 9 and 10 and autoclaved. The flasks having media were inoculated and incubated at 37˚C in incubator shaker at 200 rpm. Dry cell mass was calculated after 24 hours.

Results

Effect of yeast extract

During present study, the effect of different yeast extract concentrations on the multiplication of *P. multocida* was investigated. Out of the different concentrations, the maximum growth was observed at 0.5% (2 mg mL^{-1}) and at 1% (1.9 mg mL^{-1}) while the lowest growth was determined at 0.25% (0.67 mg mL^{-1}) (Fig. 1).

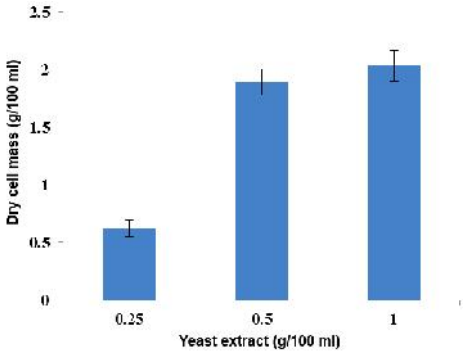

Fig. 1 Effect of yeast extract on dry cell mass of *P. multocida*.

Effect of casein hydrolysate

During the study, maximum growth of *P. multocida* was observed at 0.6% (1 mg mL^{-1}) and lowest growth was observed at 0.15% (0.33 mg mL^{-1}) concentration of casein hydrolysate (Fig. 2).

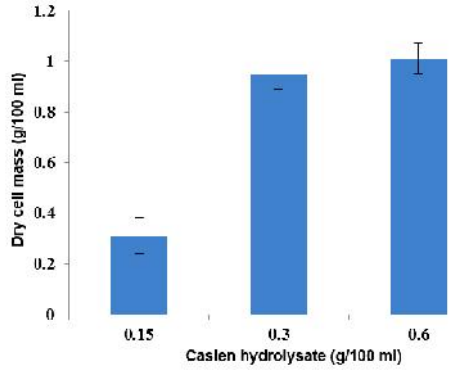

Fig. 2 Effect of casein hydrolysate on dry cell mass of *P. multocida*.

Effect of sucrose

Influence of different sucrose concentrations on the multiplication of *P. multocida* was investigated. Out of the different concentrations of sucrose, the maximum growth was observed at 0.6% (1.33 mg mL^{-1}) (Fig. 3). Bacterial growth diminished as the sucrose concentration reached as low as 0.2%.

Effect of inoculum size

To study the effect of inoculum size, different inoculum percentages were used in culture medium. Maximum growth (3.8 mg mL^{-1}) was observed at 8% inoculum volume, followed by 6% and 7%. Other inoculum sizes gave even lower cell masses. Minimum growth was observed at 1% inoculum size (0.4 mg mL^{-1}) (Fig. 4).

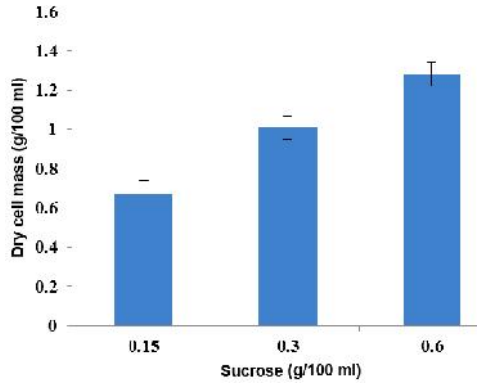

Fig. 3 Effect of sucrose on dry cell mass of *P. multocida*.

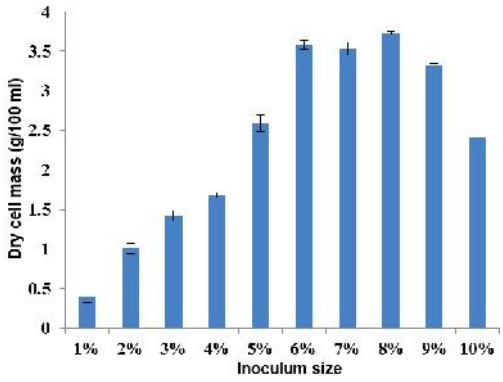

Fig. 4 Effect of inoculum size on dry cell mass yield of *P. multocida.*

Effect of pH

The *P. multocida* was cultured in media having different pH values. The organism did not grow at pH 10; however, at pH 7 maximum growth (1.98 mg mL^{-1}) was obtained (Fig. 5). Dry cell masses at pH 4, 5, 6, 8 and 9 were 0.33 mg mL^{-1}, 0.79 mg mL^{-1}, 1.66 mg mL^{-1}, 1.33 mg mL^{-1} and 0.66 mg mL^{-1}, respectively.

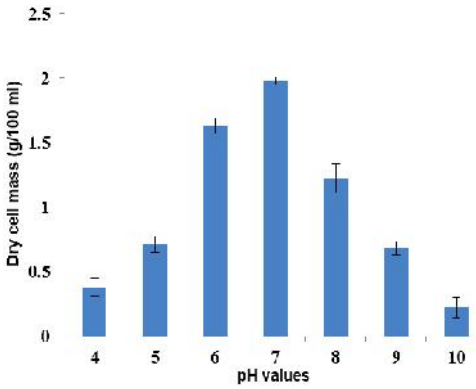

Fig. 5 Effect of pH on the growth of *P. multocida.*

Discussion

Hemorrhagic septicemia is one of the most important diseases of cattle and buffalo in Pakistan and causes heavy losses in livestock. The source of infection is infected or carriers animals. The causative agent does not survive for more than 2-3 weeks in soil or pastures. Vaccination is the best way to prevent the outbreak of the disease in bovine. For the economical production of vaccine, abundant growth of *P. multocida* along with well developed capsule around the cell is ensured. Doses of vaccine produced are directly related to the growth of *P. multocida*, good growth of *P. multocida* in culture media can reduce the cost of vaccine per dose. This study was conducted to optimize the growth conditions of the organism, to achieve better growth, which in turn increase the doses and reduce cost of vaccine.

Results showed that out of the different compositions of CSY media the maximum growth was observed with 1.3% (1.33 mg mL^{-1}) sucrose while Shah and De-Graaf [11] also observed the highest growth of 1.70 mg mL^{-1} at 1.2% sucrose. In contrast, OIE Terrestrial Manual [12] during their study added 0.6% sucrose to the medium and found good growth. Our results suggested that the addition of 1.3% sucrose to the medium should be used, which yielded higher biomass of *P. multocida*. We also observed that by increasing the concentration of casein and yeast extract in CSY media the growth of *P. multocida* was not increased. Our results were in line with Shah et al. [10].

Shah and De-Graaf [11] also studied the effect of pH on the growth of *P. multocida* and found that organism did not grow at pH 10.0, but pH ranging from 6.0-8.0 supported the growth (1.16 mg mL^{-1}). Munir et al. [13] also studied the effect of pH on the *P. multocida* and found that pH 6.7 supported the optimum growth of organism (1.20 mg mL^{-1}). Our results are in accordance with their findings. This study would help to optimize the growth conditions of *P. multocida* for vaccine production.

Conclusions

The results indicated that the different concentrations of media components and physical conditions have significant impact on the growth kinetics of *P. multocida.* It was concluded that CSY media with 0.6% sucrose should be used for better growth yield of *P. multocida.* Adaptation of optimized physicochemical conditions can make vaccine against *Hemorrhagic septicemia* more economical.

References

[1] Asadullah K, Saddique U, Ahmad R, Khan H, Mohammad Y, Zubair M. Sero-surveillance of hemorrhagic septicemia in cattle and buffaloes in district Malakand, NWFP. J Agri Biol Sci 2006; 1(4):11-14.

[2] Nandanlpeiris LKP, De Alwis MCL. Simplified techniques for haemorrhagic septicemia oil adjuvant vaccine production. In: Proceeding of the Forth-International Workshop on haemorrhagic septicemia. Kandy, Srilanka. FAO/APHCA Publication: 1991, p.13.

[3] Bain RVS, De Alwis MCL, Carter GR, Gupta BK. haemorrhagic septicaemia. FAO Animal Health and Production Paper No 33. Rome, Food and Agriculture Organization of the United Nations. 1982, p. 11-33

[4] Government of Pakistan, Economic Survey 2011-2012, Economic Advisory Wing, Finance Division, Islamabad.

[5] Farooq U, Hussain M, Irshad H, Badar N, Munir R, Ali Q.. Status of haemorrhagic septicemia based on epidemiology in Pakistan. Pak Vet J 2007; 27(2): 67-72.

[6] Ataei S, Burchmore R, Hodgson JC, Finucane A, Parton R, Coote JG. Identification of immunogenic proteins associated with protection against haemorrhagic septicemia after vaccination of calves with a live-attenuated aro A derivative of *Pasteurella multocida* B: 2. Res Vet Sci 2009; 87(2): 207-210.

[7] Imtiaz M. Evaluation of growth conditions for an improved biomass of *Pasteurella multocida* for vaccine production and immunogenicity, 2001. *MSc. Thesis*, Department of Veterinary Microbiology, Sindh Agriculture University, Tando Jam, Pakistan.

[8] De Alwis MCL, Sumanadasa MA. Naturally acquired immunity to hemorrhagic septicemia among cattle and buffalos in Sri-Lanka. Trop Anim Health Prod 1982; 14(1):27-8.

[9] Ashraf A, Tariq H, Shah MS, Nadeem S, Manzoor I, Ali S, Ijaz A, Gailani S, Mehboob S. Characterization of *Pasteurella multocida* strains isolated from cattle and buffaloes in Karachi, Pakistan. Afr J Microbiol Res 2011; 5(26):4673-4677.

[10] Shah AH, Kamboh AA, Rajpot N, Korejo NA. Optimization of Physico-chemical conditions for the growth of *Pasteurella multocida* under In Vitro. J Agri Soc Sci 2008; 4: 4.

[11] Shah NH, De-Graaf FK. Protection against haemorrhagic septicemia induced by vaccination of buffalo calves with an improved oil adjuvant vaccine. FEMS Microbiol Lett 1997; 55(2):203-7.

[12] OIE Terrestrial Manual. Haemorrhagic septicemia, Chapter 2.4.12, 2008, p.739-751.

[13] Munir R, Shahwar D, Farooq U, Nawaz I, Shahzad I, Khanum A. Outer membrane protein profiling of *Pasteurella multocida*. PakVet J 2007; 27(1): 1-4.