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Full Length Article

Optimization of Physico-chemical Conditions for the Growth of *Pasteurella multocida* under *In Vitro*

ATTA HUSSAIN SHAH[1], A.A. KAMBOH†, N. RAJPUT‡ AND N.A. KOREJO¶
Department of Dairy Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan
†*Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan*
‡*Poultry Husbandry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan*
¶*Veterinary Medicine, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam Pakistan*
[1]Corresponding author's: e-mail: vetdr_atta@yahoo.com

ABSTRACT

A study on physico-chemical growth conditions of *Pasteurella multocida* was carried-out at Vaccine Production Unit, Tandojam, Sindh, Pakistan. The organism was grown in brain heart infusion medium containing different physical and chemical agents. A suitable temperature for its growth was recorded in between 35°C and 40°C, whereas poor activities were observed at below 30°C and the organism did not grew well at 50°C and above. The influence of shaking in terms of different rotation per minute (rpm) was also determined during investigation. Under shaking conditions (500 rpm for 24 h) good growth was achieved. Positive correlation of shaking and growth at 500 rpm for 24 h was observed. The influence of pH was recorded during the experiment. No growth occurred at pH 0.5 and 10.0 but best growth was obtained at pH ranged from 7.0-8.0. In addition to that, use of orange juice as supplement failed to support the growth, whereas apple juice (15%) showed positive influence on the growth of *P. multocida*. Moreover, different concentrations of succrose were used but only 1.2% supported the growth. As succrose was costly, it was replaced with table sugar at various concentrations, out of which only 0.6% well supported the growth.

Key Words: *Pasteurella multocida*; Growth; Physical factors; Chemical factors

INTRODUCTION

Microorganisms are omnipresent in this biosphere and cause fatal as well as non-fatal diseases in livestock. *Pasteurella multocida* remained one of them, which belongs to the family *Pasteurellacaeae*. *P. multocida* is a Gram-negative, multispecies pathogen that causes serious diseases in animals and humans (Frederiksen, 1993). The bacterium is the causative agent of fowl cholera in chickens and turkeys (Christensen & Bisgaard, 2000) and hemorrhagic septicemia in cattle and buffaloes (De Alwis, 1992). Moreover, in humans, it commonly causes cellulitis and localized superficial skin abscesses following an animal bite or scratch (Goldstein, 1992).

Haemorrhagic septicaemia (HS) is a major disease of cattle and buffaloes occurring as catastrophic epizootics in many Asian and African countries, resulting in high mortality and morbidity (Mustafa *et al*., 1978; Bain *et al*., 1982). The disease is per-acute, having a short clinical course involving severe depression, pyrexia, submandibular edema and dyspnea, followed by recumbency and death. In South Asia HS is caused by infection with *P. multocida* serotype B:2 in cattle and buffaloes with high mortality rates and economic significance predominates (De Alwis, 1995). Mostly, death results within 8 to 24 h after onset. But rarely, animal shows the signs like dullness, reluctant to move, hyperthermia, salivation, nasal discharge and swelling is frequently seen beginning in the throat region and spreading to the parotid region, neck and brisket (Shah *et al*., 1997).

Vaccine introduction is the best way to check 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 production are directly related to the growth of *P. multocida,* which is a major problem in vaccine production. Due to scare growth of the organism, only doses from culture have not been reduced but cost of vaccine per dose was also increased. Thus, current situation like disease frequency and high rise in cost of chemicals, reagents and media has made it necessary to work out on the growth conditions of the organism, thus can achieve better growth, which in turn increase the doses and reduce cost of vaccine. This study was performed to work out most favorable growth conditions for *P. multocida.*

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MATERIALS AND METHODS

Source and serotype of organism. *P. multocida* (Roberet type I; B:6) was obtained from Center of Advanced Studies for Vaccinology and Biotechnology (CASVAB), Quetta.

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 was kept in an incubator at 37^{0}C for two hours. Thereafter, introduction of the suspension into mice was carried-out for recovery of the organism. On death of mice after about 16 h., the organism was recovered from its heart blood. A smear was prepared from heart blood, stained with Gram's staininig method and examined under oil immersion objective for the morphological characteristics of the organism. Further that, heart blood was cultured aseptically in brain heart infusion agar (BHIA) and also on the MacConkey's agar plates. After 24 h incubation, the pure colonies were transferred on BHIA plates. No growth on MacConkey's medium was a typical identification marker of *P. multocida*. Later on, all primary growths were maintained on BHI broth and BHI agar.

The organism and basic growth media. *P. multocida* strain (Roberet Type-I, B:6) was grown in Brain heart infusion (BHI) under different physical and chemical environments in order to observe their influence on patterns of the growth. Pure culture about 0.25 mL (0.8 mg mL^{-1} biomass) was cultured into 10 mL of basic broth medium i.e., brain heart infusion.

Result interpretation. After 24 h incubation, results were interpreted in terms of dry weight (biomass).

RESULTS AND DISCUSSION

Influence of temperature on the growth of *P. multocida*. During present study the influence of different temperatures on survival and multiplication of *P. multocida* was investigated (Fig. 1). Favorable temperature for its growth was recorded at 35±5°C (1.15 mg mL^{-1}), whereas poor activities (<1.15 mg mL^{-1}) were observed at below 25±5°C and the organism did not grow at 50°C or more. A similar kind of study was also carried-out by Imtiaz (2001), who recorded the optimum temperature for growth of *P, multocida* as 35^{0}C, while poor growth was noted at 20^{0}C and 25^{0}C but at all no growth was recorded at 50^{0}C. However, in the present study, *P. multocida* showed good growth (1.15 mg mL^{-1}) at temperatures ranging from 30^{0}C to 40^{0}C but grew poorly at or above 40^{0}C and below 30^{0}C, whereas no growth was obtained at temperatures below 10^{0}C and above 50^{0}C. It is clear from the present results and those of above workers that temperature has a major role for the multiplication of bacterial organisms that can cause effect on its activities.

Influence of shaking on the growth of *P. multocida*. The influence of shaking on the growth of *P. multocida* was also observed. The experiments were conducted in shaking incubator at different rotation per minute (rpm) ranged from 50 to 500. When the culture was placed in shaking incubator

Fig. 1. Influence of temperature on the growth of *Pasteurella multocida*

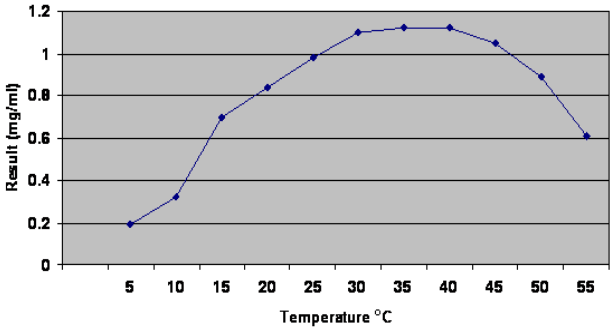


Fig. 2. Influence of Shaking on the growth of *Pasteurella multocida*

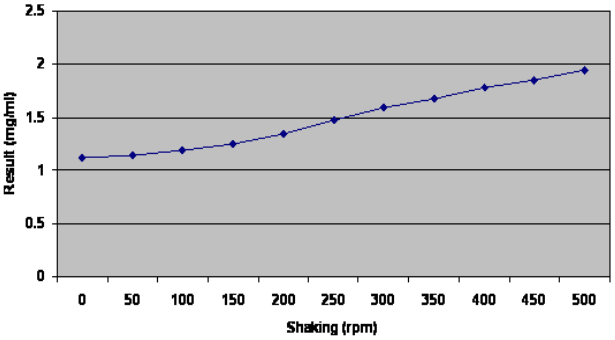
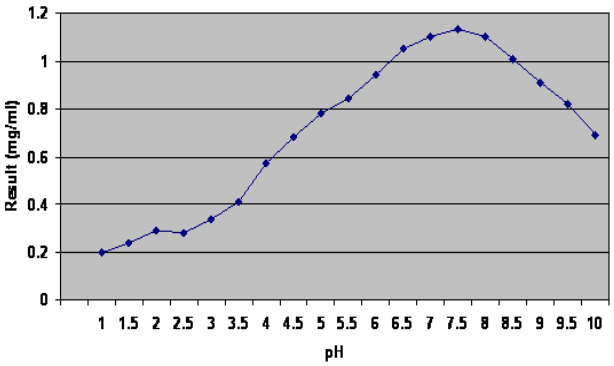


Fig. 3. Influence of pH on the growth of *Pasteurella multocida*



it was observed that the growth was directly proportional to the shaking rpm. At 500 rpm best growth (1.95 mg mL^{-1}) was observed (Fig. 2). Imtiaz (2001) conducted a study to observe the influence of agitation and stir on the growth of the cultures of *P. multocida*. He demonstrated in his investigation that when the agitation was increased at an increment of 100 rpm (rotation per minute) up to 700 rpm, a steady and slow increase in growth with the increase in agitation was recorded. At 600 rpm and with a vessel volume of one liter, maximum growth occurred, which dropped when agitation of culture rose to 600 rpm onward. These results agreed with those of the above author

Fig. 4. Influence of orange juice on the growth of *Pasteurella multocida*

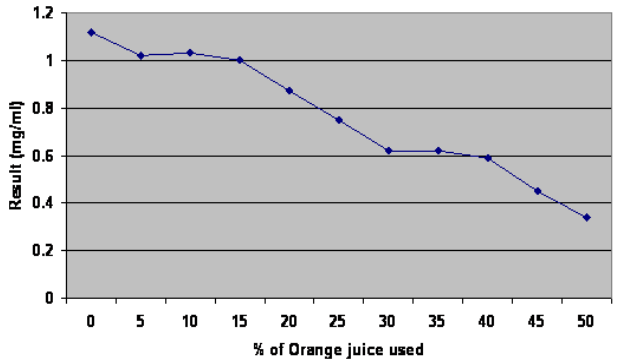
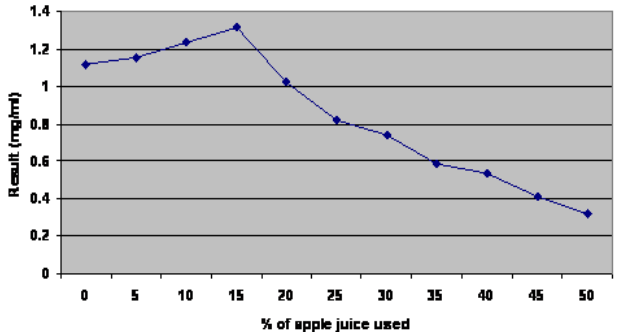


Fig. 5. Influence of apple juice on the growth of *Pasteurella multocida*



regarding the influence of shaking on growth of *P. multocida*.

Influence of pH on the growth of *P. multocida*. Different levels of pH were tested against the *P. multocida* and results were recorded. The organism did not grow at pH 2.0 and 10.0 but at pH ranged from 6.0-8.0, 1.16 mg mL^{-1} growth was obtained (Fig. 3). Imtiaz (2001) also studied the effect of pH on the *P.asteurella multocida* and found that pH 6.7 has supported the growth of organism well (1.20 mg mL^{-1}). It is clear that pH has a role, which affects the physiological activities of the organism. Further that while conducting such study, one should keep in his mind to provide appropriate pH that helps the organism to grow and multiply properly. Hence, the results of the present investigation regarding the influence of pH on the growth are in agreement with the above authors, who also recorded similar findings against the species.

Influence of citric acid on the growth of *P. multocida*. Study was conducted on the influence of different concentrations (5-50%) of commercially prepared orange juice (containing 30 mg citric acid 100-mL^{-1}) on the survival and multiplication of *P. multocida.* Out of different concentrations no one has supported the bacterial growth (Fig. 4). Otani (1963) studied the influence of vitamin C on *Bacillus pertussis*. Orange juice contains about 0.6-1.0% citric acid. Redoxon, L-ascorbic acid sodium (pH 6.4 to 6.6) was added to solid culture medium (pH 7.0) with different

Fig. 6. Influence of Succrose on the growth of *Pasteurella multocida*

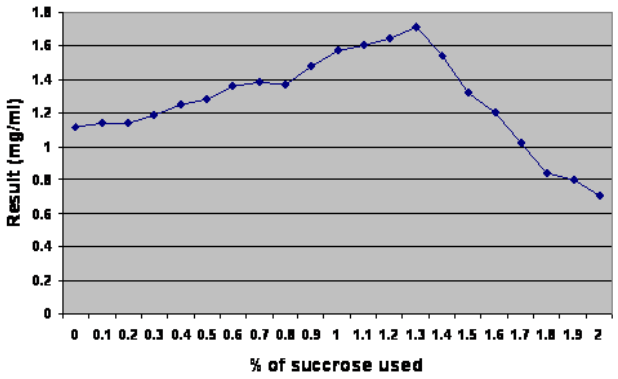
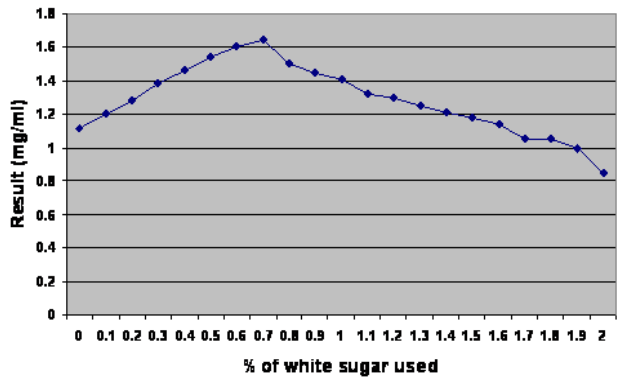


Fig. 7. Influence of home used sugar on the growth of *Pasteurella multocida*



pathogenic bacteria like *Pneumococcus, Influenza bacilli, Coliform bacilli, Dysentery bacilli, Typhus bacilli, Diphtheria bacilli, Staphylococcus, Streptococcus, Meningococcus, Bacillus subtilis, Bacillus prodigiosus* and *Bacillus pertussis*. It was concluded that the growth of only whooping cough bacillus was specifically retarded by vitamin C, while all other bacteria remained almost uninfluenced. The larger amount of vitamin C clearly disturbed the growh of *Bacillus pertussis*. Vitamin C has therefore a bactericidal effect on the *Bacillus pertussis*. The results regarding the influence of orange juice that contains major part-vitamin C did not show any positive influence against the growth of *P. multocida.*

Influence of apple juice on the growth of *P. multocida*. Commercially used apple juice with different concentrations ranging from 05 to 20% was used. Generally, positive influence of apple juice regarding growth of the species was observed. Good growth (1.32 mg mL^{-1}) was obtained at 15% in the medium (Fig. 5). It is concluded that the apple juice has positive influence on the growth of the species, which is due to the iron content of the juice. This is just hypothesis but it must need proper analysis of apple contents that helped the organism to grow.

Influence of succrose on the growth of *P. multocida*. During the study, succrose was used in different

concentrations (0.1 to 2.0%) to the basic medium. Addition of 0.5% succrose produced good results (1.30 mg mL^{-1}) but it yielded highest growth (1.70 mg mL^{-1}) at 1.2% (Fig. 6). Nandanlpeiris and De Alwis (1991) during their study added 0.6% succrose to the medium and found good growth. It is therefore, suggested that addition of 1.2% succrose to the medium should be used, which yielded higher biomass of *P. multocida*.

Influence of home used sugar (white sugar) on the growth of *P. multocida*. Due to high cost of succrose, it was replaced with home use sugar (white sugar). When white sugar was used in different concentrations (ranging from 0.1-2.0%), maximum growth (1.62 mg mL^{-1}) was obtained at 0.6% (Fig. 7). However, a steady decline in growth was observed when concentration of the home made sugar was increased.

CONCLUSION

P. multocida was found highly active at temperatures ranging from 35-40°C but failed to survive at 5 and 50°C. A positive correlation was observed between shaking and growth. An increased biomass was observed with increase in shaking up to 500 rpm. The species did not grow at pH 1 and 10; however, the best growth was observed at pH range 7 to 8. To some extent apple juice supported the growth of *P. multocida*. The organism's growth was supported by succrose at 1.2% concentration. However, white sugar also gave good results at a concentration of 0.6%.

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