Pseudomonas sp. and Bacillus sp. Culture in Whey Tofu: A Way to Increase Aquaculture Production

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py have been used to treat the TVT but chemotherapy has been shown to be the most effective and easily available practical therapy Otter *et al.* (loc cit.). The chemotherapeutic agents such as vincristine, vinblastine, doxorubicin and cylophosphamide have been used. However, vincristine sulphate intravenous injection (0.025 mg / kg body weight) is considered to be the most effective therapy for canine TVT (Feldman and Nelson, 1996: Murugan *et al., loc cit.*).

Summary

Proper examination of animals prior to mating in order to eliminate animals with patent infection from the breeding population and strict measures against mingling with stray animals will control the disease. TVT is curable in almost all cases with chemotherapy using vincristine injection. Sustained animal birth control programme of stray dogs shall decrease the incidence of TVT cases to a great extent.

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Pseudomonas sp. and *Bacillus* sp. Culture in Whey Tofu: A Way to Increase Aquaculture Production

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Abstract

The purpose of this study was to determine the growth of *Pseudomonas* sp. and *Bacillus* sp. grown together on skimmed milk media with the addition of liquid whey of tofu. The results showed that the optimum growth of *Pseudomonas* sp. and *Bacillus* sp. was found at addition 10% of liquid whey tofu and the increased growth of *Bacillus* sp. faster than *Pseudomonas* sp. The optimum of exponential phase was done at 42 hours with the average number of *Bacillus* sp. 23.54×10^{10} CFU/ml and *Pseudomonas* sp. was 67.8×10^{9} CFU/ml.

Key words: *Pseudomonas* sp., *Bacillus* sp., Growing on Liquid Whey Tofu

Pseudomonas sp. is commonly used in the aquaculture because of it produces protease, lipase, and amylase enzyme to help the

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process of digestion (Dalahi *et al*., 2014, Armada and Rhoda, 2016). While *Bacillus* sp. can also produce siderofor to inhibite pathogenic *Vibrio vulnificus* (Sugita *et al*., 1998). This study purpose was to determining their growth by adding whey tofu in media. Whey tofu contains 0.05% carbohydrates, 9% proteins, 0.69% fat, and P_2O_5 about 228.85ppm (Karina *et al*., 2016, Asmoro 2008).

Materials and Methods

Materials used in this research were *Pseudomonas* sp. and *Bacillus* sp. bacteria derived from intensive pond sediments, liquid whey tofu obtained from tofu factory at Pacar Keling, Surabaya, Indonesia. This study was conducted using Completely Randomized Design (CRD) with four treatments and five replications. The treatments used in this research were:

P0 : 10% TSB + 10% skimmed milk + 6% glucose (control)

P1 : 10% TSB + 10% skimmed milk + 6% glucose + 10% liquid whey tofu

P2 : 10% TSB + 10% skimmed milk + 6% glucose + 20% liquid whey tofu

P3 : 10% TSB + 10% skimmed milk + 6% glucose + 30% liquid whey tofu

TSB media was made by dissolving 30 grams in 1 L of aquadest water, then heated over hot plate until homogeneous. Liquid whey tofu was obtained from tofu factory at Pacar Keling, Surabaya, Indonesia. The liquid whey tofu used in this research were from mixture of a variety of other liquid wastes that flows from the process of making the tofu to the sewer / tub, for further discharge into the river or sewer. The liquid whey tofu was filtered using filter paper to separate the tofu waste with water, then sterilized using an autoclave at 121^{0}C for 15 minutes at 1 atm pressure. *Pseudomonas* sp. and *Bacillus* sp. was done on 20 ml volume test tube with media filling only half of the total volume (10 ml).

Pseudomonas sp. and *Bacillus* sp. were calculated for the density using spectrophotometer in 550 nm (Suminto, 2008). *Pseudomonas* sp. and *Bacillus* sp. taken as much 1 ml and inserted into each treatment and incubated for 1 × 24 hours at temperature of 30-35^{0}C.

Calculation of bacterial growth in this study was done by calculating bacterial colonies used Total Plate Count (TPC) method (Waluyo, 2007). Data of *Pseudomonas* sp. and *Bacillus* sp. calculated using TPC method and then analyzed using ANOVA (Analysis of Variance) and Duncan test.

Results and Discussion

Data of *Bacillus* sp. which were cultured on skimmed milk media by the addition of liquid whey tofu are presented in Table I.

P1 reaches the optimum point of exponential phase at 42th hours with a density of 23.54×10^{10} CFU / ml. At the P2 treatment the optimum point of exponential phase occurs faster than P1, that is at 36 hours with 87.3×10^9 CFU / ml. The optimum point of exponential phase in P3 occurs at 48th hour with 64.8×10^9 CFU / ml.

Bacillus sp. which were cultured in skimmed milk media with 10% (P1), 20% (P2) and 30% (P3) with additions of liquid whey tofu at the 12th hour showed different growth compared control treatment (P0). *Lactobacillus*

Table I. Average number of *Bacillus* sp. bacteria from 1 to 48 hour.

Treatment	Time of Calculation (CFU/ml)									
	0	1	6	12	18	24	30	36	42	48
P0	10^5	$43.6\times10^{8\,a}$	$13.1\times10^{8\,a}$	$20.7\times10^{8\,b}$	89.8×10^{8a}	$68.2\times10^{8\,b}$	$72.5\times10^{8\,b}$	$27.0\times10^{9\,c}$	$18.59\times10^{10\,a}$	99.6×10^{9a}
P1	10^5	$25.0\times10^{7\,c}$	$10.4\times10^{8\,a}$	$31.2\times10^{8\,a}$	71.8×10^{8b}	71.7×10^{8ab}	$88.3\times10^{8\,ab}$	$34.6\times10^{9\,c}$	$23.54\times10^{10\,a}$	80.0×10^{9b}
P2	10^5	$31.0\times10^{7\,d}$	$12.0\times10^{8\,a}$	$26.2\times10^{8\,b}$	92.8×10^{8a}	$57.3\times10^{8\,b}$	$71.0\times10^{8\,b}$	$87.3\times10^{9\,a}$	8.90×10^{9b}	$27.6\times10^{9\,c}$
P3	10^5	$51.0\times10^{7\,b}$	$87.0\times10^{7\,a}$	$28.3\times10^{8\,a}$	14.0×10^{8c}	$97.6\times10^{8\,a}$	10.38×10^{9a}	$58.3\times10^{9\,b}$	$11.9\times10^{9\,b}$	64.8×10^{9b}

Description: P0: 0% liquid whey tofu, P1: 10% liquid whey tofu, P2: 20% liquid whey tofu, P3: 30% liquid whey tofu. The notation shown with different superscript letters in the same column shows the comparison between treatments having significant differences ($p < 0.05$).

Table II. Average number of *Pseudomonas* sp. bacteria from hour 1 to 48 hour.

Treatment	Time of Calculation (CFU/ml)									
	0	1	6	12	18	24	30	36	42	48
P0	10^5	34×10^{7a}	92×10^{6d}	67×10^{7d}	94×10^{7c}	37.1×10^{8b}	15.8×10^{8b}	35.9×10^{9a}	46.2×10^{9a}	30.1×10^{9a}
P1	10^5	10×10^{6c}	35×10^{7b}	20.0×10^{8a}	15.4×10^{8b}	59.7×10^{8a}	45.8×10^{8a}	3.3×10^{9b}	67.8×10^{9a}	57×10^{8c}
P2	10^5	41×10^{7a}	17×10^{7c}	10.8×10^{8b}	10.7×10^{8c}	34.5×10^{8b}	83×10^{7c}	16.0×10^{9a}	43.4×10^{9a}	19.1×10^{9b}
P3	10^5	60×10^{6b}	71×10^{7a}	13.3×10^{8b}	35.3×10^{8a}	67×10^{7c}	91×10^{7c}	23.7×10^{9a}	20.7×10^{9b}	32.0×10^{9a}

Description: P0: 0% liquid whey tofu, P1: 10% liquid whey tofu, P2: 20% liquid whey tofu, P3: 30% liquid whey tofu. The notation shown with different superscript letters in the same column shows the comparison between treatments having significant differences ($p<0.05$).

paracasei cultured in liquid whey tofu is able to grow better than on media that contain only glucose (Le *et al.*, 2003).

Bacillus sp. which were cultured on the addition of 10% liquid whey tofu (P1) showed higher growth (23.54×10^{10} CFU / ml) and were more stable compared to others. This is assumed because the content of N and P at the addition of liquid whey tofu 20% and 30% is too excessive, so *Bacillus* sp. unable to absorb N and P. and decrease the growth of bacteria (Zouari *et al.*, 2000).

Growth of *Pseudomonas* sp. was less than *Bacillus* sp. The growth of *Pseudomonas* sp. which were cultured on skimmed milk media with the addition of liquid whey tofu shown in Table II.

At treatment P1, *Pseudomonas* sp. growth faster than P0. Increasing growth in P1 occured at 6th hour and the optimum point of exponential phase at 42th hour with cell count $67,8\times10^9$ CFU / ml. *Pseudomonas* sp. which was cultured on P2 showed increased growth in the first hour, but decreased at 6th hour. The optimum point of exponential phase at P2 occured at 42th hours with the number of cells as much as 43.4×10^9 CFU / ml. The optimum point of the exponential phase at P3 was more slowly than P1, P2 and P3, which occurred at 42th hour with 32.0×10^9 CFU / ml.

According Masangkay (2012) the presence of whey tofu in the culture medium could increase the growth of *Mycobacterium tuberculosis*. The addition of 10% (P1), 20% (P2) and 30% (P3) liquid whey tofu in this study also had significant effect on the growth of *Pseudomonas* sp. At 42th hour *Pseudomonas* sp. which was cultured on the addition of 10% whey tofu (P1) showed the best treatment.

It was assumed that the nutrient concentration was too much or excessive, so that *Pseudomonas* sp. was unable to use the nutrients present in the media. Most hypertonic solutions (high nutrient levels) could inhibit bacterial growth because it caused plasmolysis of bacterial cells (Alawiyah, 2015).

Bacillus sp. grew faster than *Pseudomonas* sp. since the differences amount of bacterial cells occurred because of the amount of Carbon (C), Nitrogen (N), Phosphorous (P) and other elements contained in the culture medium different (Wulan *et al.*, 2006). Differences growth of bacteria between *Bacillus* sp. with *Pseudomonas* sp. which were cultured together assessed were due to the character of *Pseudomonas* sp. was non-fermentative bacteria (Suyono and Salahudin, 2011), while *Bacillus* sp. was fermentative (Whitman, 2009).

Summary

The results of this study indicated that whey tofu could increase the *Pseudomonas* sp. and *Bacillus* sp growth. This was good finding since the culture of the two bacteria would increase the aquaculture production in the future.

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Prevalence of Lentivirus Antibodies in Small Ruminants of South Gujarat

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Abstract

The study was undertaken to evaluate the presence of lentivirus antibodies in small ruminants of South Gujarat. Out of 416 goats tested 35 (8.41%) and from 127 sheep, 34 (26.77%) animals had antibodies against lentivirus by competitive ELISA. Seropositivity was higher in sheep as compared to goats and it was significant ($p<0.05$) statistically. Sex-wise analysis revealed, prevalence in male and female goats as 1.65% and 11.19%, respectively and the difference was significant ($p=0.0015$). Whereas in male sheep 29.27% and in female 25.58% animals were found to be positive and the difference was non-significant ($p=0.661$) at 95% level of confidence. While combining sheep and goats, prevalence in males was 8.64% and in females 14.44 % with an overall prevalence of 12.71 per cent. Overall, higher prevalence was recorded in females as compared to males.

Key words: Competitive ELISA; Goats; Lentivirus; Sheep

Small ruminant lentiviruses are slow growing retroviruses which infect a wide range of species including ruminants. They cause progressive degenerative diseases viz; Caprine-Arthritis Encephalitis (CAE) in goats and Maedi-Visna (MV) in sheep. CAE and MV are characterized by lifelong persistence of the causal agent in host monocyte and macrophages. Most infected sheep and goats do not exhibit clinical disease but remain persistently infected and are capable of transmitting the virus via

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