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*Pseudomonas* sp. and *Bacillus* sp. Culture <sup>in</sup> on 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. which was grown together on skimmed milk media with the addition of liquid whey tofu. The results showed 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.  $23.54 \times 10^{10}$  CFU/ml and *Pseudomonas* sp. was  $67.8 \times 10^7$  CFU/ml.

**Key words:** *Pseudomonas* sp., *Bacillus* sp., Liquid Whey Tofu, Growth of Bacteria

*Pseudomonas* sp. commonly used in the aquaculture because of it produces protease, lipase, and amylase enzyme to help the process of digestion (Dalahi *et al.*, 2014, Armada and Rhoda 2016). While *Bacillus* sp. can also produce siderofor to inhibit pathogenic *Vibrio vulnificus* (Sugita *et al.* 1998). This study purpose was determining their growth by adding whey tofu in media. Whey tofu containing 0.05% carbohydrates, 9% proteins, 0.69% fat, and P<sub>2</sub>O<sub>5</sub> 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 <sup>was</sup> made by dissolving 30 grams in 1 L of aquadest water, then heated over hot plate until homogeneous. Sterilizing was conducted by using autoclave at 121°C for 15 minutes with 1 atm pressure. Dissolving 100 grams of skimmed milk powder in 1 L of distilled water, then heated over hot plate until homogeneous. Skim milk solution sterilized using autoclave at 121°C for 15 minutes with 1 atm pressure.

Liquid whey tofu <sup>was</sup> obtained from tofu factory at Pacar Keling, Surabaya, Indonesia. The liquid whey tofu used in this research <sup>was</sup> 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°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).

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*Pseudomonas* sp. and *Bacillus* sp. were calculated the density using spectrophotometer in 550 nm (Suminto, 2008). *Pseudomonas* sp. and *Bacillus* sp. taken as much 1 ml and inserted into each treatment. ~~The inoculation result was~~ incubated for  $1 \times 24$  hours at temperature of  $30-35^{\circ}\text{C}$ .

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 1.

Table 1. 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	$10^5$	$43.6 \times 10^{8a}$	$13.1 \times 10^{8a}$	$20.7 \times 10^{8b}$	$89.8 \times 10^{8a}$	$68.2 \times 10^{8b}$	$72.5 \times 10^{8b}$	$27.0 \times 10^{9c}$	$18.59 \times 10^{10a}$	$99.6 \times 10^{9a}$
P1	$10^5$	$25.0 \times 10^{7c}$	$10.4 \times 10^{8a}$	$31.2 \times 10^{8a}$	$71.8 \times 10^{8b}$	$71.7 \times 10^{8ab}$	$88.3 \times 10^{8ab}$	$34.6 \times 10^{9c}$	$23.54 \times 10^{10a}$	$80.0 \times 10^{9b}$
P2	$10^5$	$31.0 \times 10^{7d}$	$12.0 \times 10^{8a}$	$26.2 \times 10^{8b}$	$92.8 \times 10^{8a}$	$57.3 \times 10^{8b}$	$71.0 \times 10^{8b}$	$87.3 \times 10^{9a}$	$8.90 \times 10^{9b}$	$27.6 \times 10^{9c}$
P3	$10^5$	$51.0 \times 10^{7b}$	$87.0 \times 10^{7a}$	$28.3 \times 10^{8a}$	$14.0 \times 10^{8c}$	$97.6 \times 10^{8a}$	$10.38 \times 10^{9a}$	$58.3 \times 10^{9b}$	$11.9 \times 10^{9b}$	$64.8 \times 10^{9b}$

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 ( $p < 0.05$ ).

P1 reaches the optimum point of exponential phase at 42<sup>th</sup> hours with a density of  $23.54 \times 10^{10}$  CFU / ml. At the P2 treatment the optimum point of exponential phase occurs faster than P1, that is at 36 hours with  $87.3 \times 10^9$  CFU / ml. The optimum point of exponential phase in P3 occurs at 48<sup>th</sup> hour with  $64.8 \times 10^9$  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 12<sup>th</sup> hour showed different growth compared control treatment (P0). According to Le *et al.* (2003) that *Lactobacillus paracasei* cultured in liquid whey tofu is able to grow better than on media that contain only glucose.

*Bacillus* sp. which were cultured on the addition of 10% liquid whey tofu (P1) showed higher growth ( $23.54 \times 10^{10}$  CFU / ml) and were more stable compared 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 shows in Table 2.

Table 2. 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	$10^5$	$34 \times 10^{7a}$	$92 \times 10^{6d}$	$67 \times 10^{7d}$	$94 \times 10^{7c}$	$37.1 \times 10^{8b}$	$15.8 \times 10^{9b}$	$35.9 \times 10^{9a}$	$46.2 \times 10^{9a}$	$30.1 \times 10^{9a}$
P1	$10^5$	$10 \times 10^{6c}$	$35 \times 10^{7b}$	$20.0 \times 10^{8a}$	$15.4 \times 10^{8b}$	$59.7 \times 10^{8a}$	$45.8 \times 10^{8a}$	$3.3 \times 10^{9b}$	$67.8 \times 10^{9a}$	$57 \times 10^{9c}$
P2	$10^5$	$41 \times 10^{7a}$	$17 \times 10^{7c}$	$10.8 \times 10^{8b}$	$10.7 \times 10^{8c}$	$34.5 \times 10^{8b}$	$83 \times 10^{7c}$	$16.0 \times 10^{9a}$	$43.4 \times 10^{9a}$	$19.1 \times 10^{9b}$
P3	$10^5$	$60 \times 10^{6b}$	$71 \times 10^{7a}$	$13.3 \times 10^{8b}$	$35.3 \times 10^{8a}$	$67 \times 10^{7c}$	$91 \times 10^{7c}$	$23.7 \times 10^{9a}$	$20.7 \times 10^{9b}$	$32.0 \times 10^{9a}$

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 ( $p < 0.05$ ).

At treatment P1, *Pseudomonas* sp. growth faster than P0. Increasing growth in P1 occurred at 6<sup>th</sup> hour and the optimum point of exponential phase at 42<sup>th</sup> hour with cell count  $67.8 \times 10^9$  CFU / ml. *Pseudomonas* sp. which was cultured on P2 showed increased growth in the first hour, but decreased at 6<sup>th</sup> hour. The optimum point of exponential phase at P2 occurred at 42<sup>th</sup> hours with the number of cells as much as  $43.4 \times 10^9$  CFU / ml. The optimum point of the exponential phase at P3 was more slowly than P1, P2 and P3, which occurred at 42<sup>th</sup> hour with  $32.0 \times 10^9$  CFU / ml.

According Masangkay (2012) the present 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 42<sup>th</sup> 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. Culture media which was contain a lot of nutrients conduce increased of concentration and became hypertonic. According to Alawiyah (2015) that most hypertonic solutions (high nutrient levels) could inhibit bacterial growth because it caused plasmolysis in bacterial cells.

*Bacillus* sp. and *Pseudomonas* sp. which were grown together on skimmed milk media with the addition of liquid whey tofu show different growth. *Bacillus* sp. grew faster than *Pseudomonas* sp. According to Wulan *et al.* (2006), the differences amount of bacterial cells occurred because of the amount of Carbon (C), Nitrogen (N), Phosphor (P) and other elements contained in the culture medium different. Differences growth of bacteria between *Bacillus* sp. with *Pseudomonas* sp. which were cultured together were assumed 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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