The Potency of *Bacilus* sp as Particle-associating Bacteria in Laboratory Condition

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Abstract. The aim of this study was to know the potency of *Bacillus* sp in colonizing artificial particles in laboratory condition. Study of particle –associating capacity of *Bacillus* sp by using artificial-sphare agar as particle and transprant exopolimeric particles (TEP) produced by *Thalassiosira weisfloggii* as well as flocculating activity. The results of this study showed that *Bacillus* sp were able to associate both artificial agar spheres and transparent exopolimeric substances (TEP) as well as high flocculating activity produced by phytoplankton, *T. weissfloggii*. The mean number of *Bacillus* sp in colonizing artificial agar spheres increasing with incubation time was 3,50 x 10^5 cells/agar spheres and Transprant expolimeric particles, 2.9 x 10^8cells/mL; and flocculating activity, 92 % after four days incubation. In conclusion that *Bacillus* sp has a high potential as particle-associating bacteria used in intensive shrimp farming system.
Keywords: Particle–associating bacteria, *Thalassiosira weisfloggii*, *Bacillus* sp, Phytoplankton

INTRODUCTION

The FAO (2014) reported that the annual growth rate of aquaculture production during the last decade has reached 14.3 % which higher compared to the capture fishery which was 0.5 %. The increasing concerns related to environmental impact of shrimp farms led to development of production systems with little or zero water exchange. Aquaculture sector has high promising opportunity to be developed because production of captured fishery sector has been stagnant or decline in two last decade. The main goal of aquaculture expansion must be focused to produce aquaculture product without significantly increasing natural resources such as water and land as well as to develop sustainable aquaculture, efficient and friendly environment (Crab et al., 2012).

Aquaculture produces large quantities both organic and inorganic waste which can be detrimental to the environment without managed properly (Kuhn et al., 2010. Intensive aquaculture system achieved by increasing high density cultures, high amounts of feed and other equipment (Ekasari et al., 2010). The limiting factors of intensive aquaculture systems is water quality and economy because these system will take place rapid accumulation of feed residues, organic matter and toxic inorganic nitrogen (Avnimelech, 2006).

The control of aquaculture systems through the manipulation of microbial activity become an important technology in the development of intensive aquaculture. Biofloc technology make it possible to minimize water exchange and water usage in aquaculture systems through maintaining adequate water quality and biofloc within the culture unit rich in protein so it can be obtained a sustainable method to control water quality and producing protenaceous feed *in situ* (Crab et al., 2012, Crab et

al., 2019). The objective of this study was to know the potency of *Bacillus* sp in colonizing particles and flocculating activity for biofloc agent in intensive aquaculture systems.

MATERIALS AND METHODS

1. Test of Bacterial colonization on particles

Bacillus sp used in this experiment was originally isolated from brakish water particles. This bacteria was grown in batch culture enriched Marine Broth.. Bacterial colonization was determined according to Grossart et al., (2003) method by measuring the accumulation of bacteria on agar sphere as particle that was incubated in still seawater containing bacteria. Agar sphere was made by dripped warm agar solution 2 % with sterile pipette into seawater covered by paraffin oil. Diameter of agar sphere 1-2 mm. Every flask filled with sterile seawater and then inoculated with bacterial suspension and then filled 30 agar sphere per flask. Potency of *Bacillus* sp in colonizing particle by using agar sphere model as particles. Determination of bacterial colonization on particles by measured the number of bacteria accumulated on agar sphere particles which incubated Erlenmeyer 250 mL with sterile *Bacillus* sp culture. Agar sphere particles were made from Bacto agar.

Model agar particles were suspended on thin glass threads in suspensions of bacteria. Every 250 mL Erlenmeyer flask contained with sterile seawater with 25 ppt , then inoculated with *Bacillus* sp culture in exponential growth phase. Bacterial suspension with cell density 1,0 x 10^8 cells/mL. Every Erlenmeyer flask filled with 30 agar spheres. Sampling was conducted in 30 minutes interval. Every sampling of model agar sphere three times replication in counting chamber then stained with DAPI (4,6-diamidino-2 phenylindole). Free-living bacteria were quantified by filtering 1 mL solution of incubation water onto 0.2 µm pore size nucleopore filters and stained with DAPI and quantified by epifluorescence microscopy at magnifications of 1,000.

2. Test of Bacterial-flocculating Activity

Flocculating activity of *Bacillus* sp was determined according to the method of Gao et al (2006); Kaolin clay as test material, was suspended in distilled water with concentration 50 g/L at pH7,0. In every test tube filled with 9 ml Kaolin clay suspension, 0.25 ml of $CaCl_2$ solution and added 0,1 ml of bioflocculant solution. As reference used distilled water. All mixtures in final volume were made 10 ml with distilled water. After stiring with magnetic stirrer. All the test tubes were allowed to settle for 5 minutes. The absorbance of the upper phase was measured by using UV spectrophotometer, at 550 nm. Bioflocculating activity was calculated as follows: [(B-A)/B] x 100 %, which A and B were absorbances at 550 nm for sample and reference, respectively.

3. Interaction of Particle-associating Bacteria on Transparent polimer particle (TEP)

Test of Interaction B*acillus* sp with transparent polimer particle (TEP) produced by *Thalassiosira weisfloggii* by using Marine Broth and artificial sea water as medium. The number of bacteria accumulated in TEP measured according to Grossart et al., (2006). *Bacillus* sp was grown monoculture in rooling bottles size 400 mL and filled with artificial sea water, salinity 25 ppt. Bacterial inoculum with cell density 1.0 x 10^6 cells/mL then incubated 3.2 rpm, temperature 20 ± 1^0 C for 4 days. The abundance of free-living and attached bacteria from 1 ml subsamples after staining with DAPI (4'6'-diamidino-2-phenolindole) counted by epifluorescence microscopy at 1000 x magnification. A minimum of 10 replicates was counted for each samples.

RESULTS AND DISCUSSION

1. Bacterial Colonization on Particles

Colonization rate of *Bacillus* sp on the agar particles increased after 30 minutes incubation. Attached bacteria on agar spheres grew rapidly. The number of bacteria colonized particles average 3.0 x 10^3 cells/ agar sphere after 30 minutes incubation and then abundance of bacteria increased

rapidly because this bacteria grown in monoculture with batch system. The number of bacteria accumulated on particle after 24 hours incubation cell densities average 3.5 x 10^5 cells/ agar sphere. The result showed that *Bacillus* sp have capacity to colonize substrate . Based on bacterial behavior, there are three functional types of bacteria; (1) bacteria that specialize in colonizing particles; (2) free-living bacteria and (3) bacteria that can grow in suspension as well as on particles (Grossart et al., 2006). Particles play important role in aquatic ecosystem because they are important sites for biological processes such as production, decomposition and nutrient in the water column (Grossart wt al., 2003). The population dynamics of marine snow microbes are complex and dependent on several factors such as the rate of attachment, detachment, growth, and mortality of of the microbial populations (Kiorboe et al., 2003).

2. Bacterial- flocculating Activity

Flocculating activity of *Bacillus* sp was 2.5 % in the initial incubation phase and then increased rapidly after 24 hours incubation. Flocculating activity reached maximum hours incubation, 92 % then decreased after 72 incubation 83 % (Fig. 1). The growth exponential phase increased with gradual increase of EPS with cell weigh from 48 to 96 h the declined in the growth curve after 96 hours because of decreased production of EPS (Rao et al 2013).

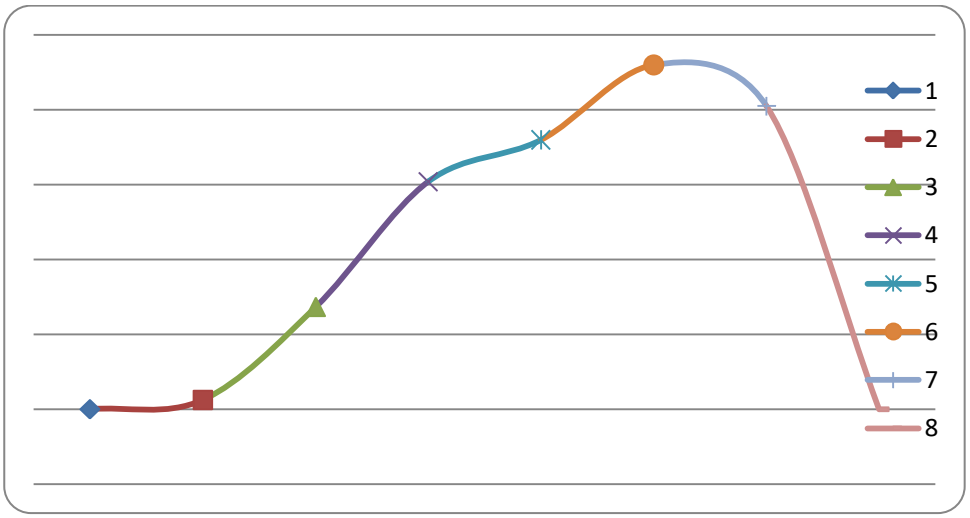

Figure 1. Flocculating activity of *Bacillus* sp after 4 days incubation

CONCLUSIONS

Bacillus sp has a high potency to be used as biofloc inoculum in intensive shrimp farming system. It has a good capacity in colonizing both model agar spheres , transparent expolimeric particle (TEP), and flocculating activity, 3.5 x 10^5 cells/agar spheres, 2.9 x 10^8 cells/ml and 92 %, respectively.

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