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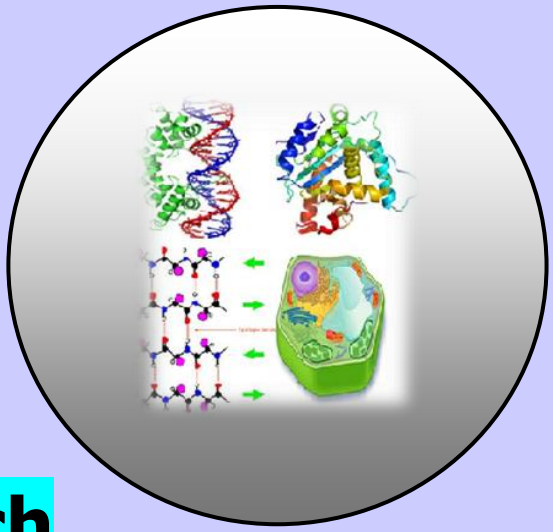

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Streptomyces sp. as a Biocontrol of Vibriosis on Larvae of *Macrobrachium rosenbergii* (de Man) Prawns

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ABSTRACT

Vibriosis is a limiting factor on production of M. rosenbergii (de Man) prawn hachlings. An alternative control of vibriosis can be undertaken through a biological pathways by using Streptomyces bacteria. Ten isolates of Streptomyces spp. were isolated in this research from rhizosphere of mangrove of Rhizophora spp. Three isolates namely Streptomyces sp.1, Streptomyces sp.3 and Streptomyces sp.4 could inhibit the growth of V. anguillarum (in vitro). Streptomyces sp.1 could inhibit the growth V. anguillarum best with diameter of inhibition of 21.03±1.42 mm. Filtrates of Streptomyces sp.1 could also inhibit V. anguillarum (in vitro) with MIC value of 10%. Aplication of Streptomyces sp.1 culture could give significant different impacts (p<0.05) on the percentage of survival (SR) of prawn larvae that had been infected by V. anguillarum compared to the control. Treatment of Streptomyces sp.1 culture could also significantly (p<0.05) reduce the total population of Vibrio on the maintenance media compared to the control.

Key words: Streptomyces sp., Vibrio anguillarum, Macrobrachium rosenbergii (de Man) Prawns, Vibriosis and Biocontrol Agent.

INTRODUCTION

The activity of nursery of shrimp larvae must be steril from patogenic infections. Infection on hatchling besides causing high financial lost, it may be also potential to be of an entry point of patogenic bacteria to the shrimp ponds (Atmomarsono *et al*., 2010; Patang, 2012).

Vibrio spp. act as the main pathogenic bacteria causing vibriosis on animals kept in aquaculture systems (Thiruvarangan *et al*., 2014). Increase in the population of Vibrio on aquaculture ponds can disturb physiological activities of shrimps that in turn may cause increase in mortality of captivated animals (Velmurugan *et al*., 2015). Some species that have been reported to cause vibriosis such as *V. harveyi*, *V. anguillarum*, *V. alginolyticus, V. parahaemolyticus*, *V. vulnificus*, *V. cholera*, *V. fischeri* and *V. splendidus* (Morris, 2003; Azizunnisa and Shreeramulu, 2013; Vaseeharan and Ramasamy, 2003).

Control of vibriosis by fishermen was generally by using antibiotics. Some antibiotics such as from the group of quinolones, flumequine and flouroquinolone are frequently used to supresse the vibriosis infections on aquaculture systems (Cabello, 2006; Balcazar *et al*., 2006). In practical, the use of the antibiotics may have negative impacts to the health as well as harmful to the environment. Applying antibiotics on aquaculture can increase the resistance of the pathogenic bacteria as well as causing bio-accummulation of their residue on the captivated animals (Isnansetyo, 2009).

The concept of *biological control agents* as what Aftabudin *et al*. (2013) and Isnansetyo (2009) stressed out was potential to be developed in controlling bacterial aquatic infections. As Selvakumar *et al*. (2010) postulated, Streptomyces bacterium is one of the biological controling agents which has a potential to be applied in aquaculture systems. Streptomyces is well known to have capability in antagonistic activities against other bacteria, including on the group of patogenic Vibrio (Selvakumar *et al*., 2010 ; Velmurugan *et al*., 2015).

The genus of Streptomyces is a group of Actinobacteria which are commonly exist in marine ecosystems (Velmurugan *et al*., 2015). Mohana and Radhakrishnan (2014) added that Streptomyces which is isolated from the beach area, especially from the mangrove areas, have high antagonistic activities on negative Gram bacteria. Hong *et al*. (2009) reported that mangrove ecosystems act as a potential habitat for Actinomycetes, the bacteria on which habitats have been limited explored. As the mangrove occupied intertidal zones, Wang *et al*. (2003) stated that this ecosystem act as an extremely productive areas, so they have a high potential to be used as a source of isolates for microorganisms producing bioactive compounds. Based on this assumption, the objective of this research was to reveal the potential of Streptomyces act as a biocontrol agent controlling vibriosis diseases on shrimp larvae.

MATERIAL AND METHODES

Bacterial Strain and Media

Strain of *V. anguillarum* causing vibriosis on prawn larvae of *M. rosenbergii* (*de Man*) was isolated from larvae ponds belong to Integrated Services Unit (UPT) of Nursery (Pembenihan), Office of Marine and Fisheries (Dinas Kelautan dan Perikanan), Bali Province (Bintari *et al*., 2016). Strains of *Streptomyces* spp. were isolated from the soil of mangrove rhizosphere at the Mangrove Information Centre, Suwung Kawuh Village, Kuta District, Badung Regency, Bali, and isolation was carried out at Microbiology Laboratory, Biology Department, Faculty of Natural Sciences and Mathematics of Udayana University, Bali. *Vibrio anguillarum* was cultured on *Trypic Soy Broth* (TSB) (Merk™), *Trypic Soy Agar* (TSA) (Merk™) and *Alcaline Peptone Water* (10 g of pepton, 10 g of NaCl, 1000 ml of water, final pH or acidity of 8.5) media.

Streptomyces spp. was cultured on *Yeast Extract Malt Agar*/ YEMA media (0.5 g of K_2HPO_4, 0.2 gof $MgSO_4.7H_2O$, 0.1 g of NaCl, 10 g of malt, 1 g of yeast extract, 15 gof agar, 600 ml of destiled water, 400 ml of sea water) and *Yeast Extract Malt Broth /* YEMB (media composition of 4 g (0.4%) of yeast extract, 10 g (1%) of malt, 0.4 g of glucose, 600 ml of distiled water, and 400 ml of sea water).

Isolating, identifying and characterisation of *Streptomyces* spp.

Isolation of *Streptomyces* spp. was undertaken through a *serial dilution method*. It was grown on YEMA media and incubated on 28^0C for 5 days. The colony was characterised macroscopically, microscopically, undergone Gram stained tests and acidic resistency. Identification of the genus was undertaken as stated on the *Guide to the Classification and Identification of the Actinomyces and Their Antibiotics* (Lechevalier and Waksman, 1973) as well as based on the *Bergey's Manual of Determinative Bacteriology* (Holt *et al*., 1994).

Antagonistic Activity of *Streptomyces* spp. against *Vibrio anguillarum*

Activity tests were carried out using dual culture methods (Bernal *et al*., 2015). In the test of activities, *Streptomyces* spp. was grown on YEMA culture for 5 days. *Vibrio anguillarum* was cultured on TSB for 24 hours (28 ± 30^0C) and standardized by Mc Farland 5%. The culture was grown on 200 µl TSA media through *pour plate*. When the media has already become solid, the culture of *Streptomyces* sp. (with the diameter of ± 5 cm) was placed in the centre of agar media. It was then incubated on 28^0C for 24 hours. Investigation was undertaken by observing the existence of inhibition zones emerging on the media. The isolate of Streptomyces that have the highest inhibition capability (in mm), was utilized in the following tests.

Isolation of *Streptomyces* sp. Filtrate and Determination of *Minimum Inhibitory Concentration* (MIC)

The colony of *Streptomyces* sp. was growned on YEMA media for 5 days. The colony grown on 100 ml YEMB media was by taking 5 rounded colonies using a cork borer (diameter ± 5 mm), and then incubated on 80 rpm speed of shaker (Retnowati 2010; Kawuri, 2012). The culture was incubated for 22 days and then isolation of the filtrate was undertaken periodically by centrifugating the culture on 11.000 rpm speed for 15 minutes. Supernatant was then shieved by 0.45 µm filter papers (Charoensopharat *et al*, 2008). The filtrate activity tests was undertaken by applying difusion well methods (Kawuri, 2012) for finding out the best incubation time of *Streptomyces* sp. isolates in producing metabolite compounds which was then followed by MIC tests. The filtrate for MIC tests were partitioned by N-butanol solution with the volume ratio of 1:1 (v/v). The water phase and N-butanol phase formed were evaporated by using evaporator on 40^0C until getting concentrated filtrates. The MIC tests were undertaken on several concentrations of filtrates (v/v): 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% and 10% as well as on 0% as control by applying difusion well methods (Kawuri, 2012 ; Laidi *et al*., 2013).

The Potential of *Streptomyces* sp. as a biocontrol for Vibriosis on Prawn Larvae

Prawns larvae which was used on the *M. rosenbergii* (de Man) larvae test was in the IV stadium. Larvae was placed in the plastic bag with 500 ml brakish water (salinity of 10 ppt), as many as 50 individuals each. The larvae was aclimatised and given fodder of *Artemia* sp. as many as 4 naupli/larvae in the morning and in the afternoon. The experimental design applied was the complete randomized design with 5 levels of treatments of Streptomyces sp. cultures.

The isolate of *Streptomyces* sp. was cultured on YEMB media and incubated on 80 rpm speed shaker for 5 days. The culture was harvested and standardised on 10^5 CFU/ml density. The suspension of the culture which was used in the treatment was 2.5 ml, 5 ml, 7.5 ml and 10 ml. Inoculation of the culture of the Streptomyces sp. was undertaken for 2 days respectively, each treatment undertaken once daily (Velmurugan *et al*., 2015), and on final treatment, change of 25% of maintenance water was undertaken. On the third days of treatments, *V. anguillarum* culture was inoculated as much as 10 ml. The isolate of *V. anguillarum* was cultured on APW media for 24 hours on ± 30^0C. The bacteria were harvested through centrifugation on 5000 rpm for 5 minutes. The pellets gained were then suspended again on 10 ml of NaCl 0.9% (w/v) and standardized by McFarland 5% (Chau *et al*., 2011). Observation was made after 24 hours inoculation of the patogenic by observing the percentage of survival rates of larvae (%SR) and the abundance or the total of Vibrio bacteria on the water where it was kept. The value of %SR was calculated by using the Goddard (1996) formula:

$SR = \frac{Nt}{No}\ x\ 100\%$, where SR = level of survival rate of larvae (%); Nt= number of larvae which is survive at the end of observation (individuals); No = Numbers of prawn larvae at the beginning of observation. Data were analyzed through *Analysis of Variance* (ANOVA), through F test on the level of 5 % and then followed by the Duncan (DMRT) test on the level of 5%.

RESULT

Isolation of *Streptomyces* spp.

Results of the isolation on rhizosphere of *Rhizophora* sp. showed that there were 10 isolates of Streptomyces with different macroscopis characteristics were successfully isolated. The general characteristic belongs to the isolates such as the colony were strongly attached to YEMA media, forming aerial hipha, Gram possitive stained, cannot stand to acid, possitive catalase, cained conidia with the size of 0.20-1.84 µm and having vegetative hifa with the diameter of 0.40-1.00 µm.

Antagonistic activity test

Antagonistic activity of the 10 isolates of Streptomyces was tested by utilizing dual culture methods. Results of the tests showed that 3 isolates namely *Streptomyces* sp.1, *Streptomyces* sp.3 and *Streptomyces* sp.4 (Figure 1) can inhibit the growth of *V. anguillarum* on the in vitro test (Table 1) which is indicated by the formation of inhibition zones around antagonistic bacteria (Figure 2). *Streptomyces* sp.1 has the best potential in inhibiting *V. anguillarum* by the diameter of inhibition of 21.03 ± 1.42 mm, which was then explorated for the next test.

Filtrate activity test and determination of MIC

Based on the result of the filtrate activity test, it was found that the *Streptomyces* sp.1 could produce metabolit compounds, the compound of which had antibacteria characteristics against *V. anguillarum*. *Streptomyces* sp.1 was active, starting producing metabolit compounds which had antibacteria characteristics on day 12^{th} and the production was optimum on the day of 18^{th} (Figure 3). The filtrate on the 18 day incubation periods was then undergone a purification process by extracting the filtrates by n-butanol solution for 24 hours. Results of purification processes showed that the filtrates on the n-butanol fase was capable in inhibiting the *V. anguillarum* in vitro by inhibition diameter of 20.4±0.31 mm.

Results of MIC tests showed that in this research, the concentration of the filtrates of 10% was the minimum concentration that was capable to inhibit the *V. anguillarum* by the inhibition capability of 12.5±0.13 (Table 2). Nevetheless the acquired value of MIC was still high, so, the reduction of filtrate concentrationunder 10% needed in order to get the maximum MIC value.

The potential of *Streptomyces* sp1. as a biocontrol of vibriosis on prawn larvae

Results of application tests showed that treatment of *Streptomyces* sp.1 culture brought impacts significantly ($p<0.05$) on increasing the percentage of the survival rate (%SR) of prawn larvae (Table 3.). Treatment of culture of *Streptomyces* sp.1 by volume of 5 ml (A2B1), 7.5 ml (A3B1) and 10 ml (A4B1) gave significantly different impacts ($p<0.05$) compared to the control (A0B1). Adding 10 ml culture of *Streptomyces* sp.1 on the media found to be the most effective on improving the percentage of survival rates of larvae. Application of *Streptomyces* sp.1 was also found to gave impacts on the decrease in the total population of Vibrio on the liquid component of the media (Table 3). Treatment of A4B1, A3B1 and A2B1 known to give significant different impacts ($P<0.05$) on the total Vibrio bacteria on the media compared to the control.

DISCUSSION

As many as 10 isolates of *Streptomyces* spp. were successfully isolated and characterized from rhizosphere of mangrove *Rhizophora* sp. Three isolates (Figure 1) namely *Streptomyces* sp.1, *Streptomyces* sp.3 and *Streptomyces* sp.4 had an antagonistic activity against *V. anguillarum* that caused vibriosis on the *M. rosenbergii* (de Man) larvae (Table 1, Figure 2). The rhizosphere of mangrove according to Usha *et al*. (2010) and Rao and Rao (2013) acted as a potential source of exploration of Actinomyces. As it is located in intertidal areas, Wang *et al*. (2003) claimed that the mangrove areas were productive so they have a potential to be used as a source of microorganism isolates producing bioactive compounds.

The antagonistic ability of Streptomyces on pathogenic bacteria in aquaculture has been frequently reported. Some species of Streptomyces such as *Streptomyces* strains VM-8, VM-15, VM21 (Velmurugan *et al*., 2015) and the *Streptomyces* strain A1 (Chau *et al*., 2011) which were isolated from the sediment of ponds was revealed to be having potentials as the biocontrol against vibriosis of shrimps. Some spesies of Streptomyces such as *S. flocculus, S. pancagri* (Bernal *et al*., 2015), *S. xantholiticus*, *S. aureofasciclus*, *S. vastus*, *S. galbus*, *S. rimosus* (Sahu *et al*., 2007) and *S. fradieae* (Aftabuddin *et al*., 2013) were also known to have antagonistic activity agaist the patogenic Vibrio on the aquaculture.

The genus of Streptomyces has been known broadly to be able to produce metabolite compounds such as antibiotics (Remya and Vijayakumar, 2008), enzymes and melanines (Manivasagan *et al*., 2013) as well as siderophores (You *et al*., 2005) which were having function in antagonistic activities. Results of exploration on *Streptomyces* sp.1 on this research showed that this isolate can actively producing secondary metabolite compounds on water culture. This was shown by the capability of inhibition of the filtrate produced by the *Streptomyces* sp.1 (on the in vitro test*)* against *V. anguillarum*. On this research it was revealed that the optimum production of metabolite compound by *Streptomyces* sp.1 occured on the day 18^{th} of the incubation periods (Figure 3), with MIC value of 10% (Table 2). The filtrate of Streptomyces has a high potential in producing antibacterial compounds.

The filtrate of the *S. violaceusniger* strain HAL64 was reported to contain high quantities of casinostatines, the compounds of which was known to be effective in inhibiting Gram positive and negative bacteria (El-Naggar, 2007). The filtrates of *S. longwoodensis* and *S. viridiviolaceus* were also known to contain vast spectrum of antibiotics against patogenic bacteria and fungy (Remya and Vijayakumar, 2008).

Table 1. Inhibition capability of the isolateof Streptomyces on the *V. anguillarum.*

No.	Isolates of the Streptomyces	Diameter of inhibition zone (mm)
1.	*Streptomyces* sp.1	21.03±1.42[b]
2.	*Streptomyces* sp.2	0±0.00[d]
3.	*Streptomyces* sp.3	9.57±0.75[c]
4.	*Streptomyces* sp.4	8±0.00[c]
5.	*Streptomyces* sp.5	0±0.00[d]
6.	*Streptomyces* sp.6	0±0.00[d]
7.	*Streptomyces* sp.7	0±0.00[d]
8.	*Streptomyces* sp.8	0±0.00[d]
9.	*Streptomyces* sp.9	0±0.00[d]
10.	*Streptomyces* sp.10	0±0.00[d]
11.	Chloramfenicol positive control	31.43±0.51[a]

Notes :

1). Values on the table ± standard deviation (average of 3 replications).

2). Different letters on the same columns indicated significantly different results (P<0.05) based on Duncan tests.

Table 2. Results of MIC test of filtrates of *Streptomyces* sp.1 against the *V. anguillarum*.

No.	Filtrate consentration	Diameter of inhibition power (mm)
1.	100%	20.4±0.31a
2.	90%	17.8±0.05ab
3.	80%	17.6±0.05ab
4.	70%	17.4±0.12ab
5.	60%	16.4±0.15abc
6.	50%	15.8±0.14bc
7.	40%	15.8±0.15bc
8.	30%	14.9±0.07bc
9.	20%	12.5±0.13c
10.	10%	12.5±0.13c
11.	Control (n-butanol)	0.00±0.00d

Notes:

1). Values on the table ± standard deviation (average of 3 replications).

2). Different letters on the same columns indicated significantly different results (P<0.05) based on Duncan tests.

The culture of *Streptomyces* sp.1 which was applied on maintenance containers was known to be capable of increasing the percentage of survival rates of larvae and decreasing the total of Vibrio on the maintenance water (Table 3). Adding 10 ml of the culture of *Streptomyces* sp.1 on the maintenance media was known to be the most effective on increasing the percentage of survival rates of larvae as much as 26.8 ± 6.09 %, and decreasing the total of Vibrio from the origin population, from 1 x 10^7 CFU/ml into 1.7x10^3 CFU/ml. Most of the species of Vibrio were opportunistic patogenic bacteria on the aquaculture. According to Kimura *et al*. (1998), when the total Vibrio on the culture system increased, it then would be correlated with the increament of infections. Based on this, the application of Streptomyces on the culture system will be excellent because it will be able to reduce the total population of the Vibrio.

Table 3. The percentageof Survival Rates (SR) of larvae of *Macrobrachium rosenbergii* (*in vivo* tests).

Treatment	Bacteria Culture of*Streptomyces*sp. 1 (A)	Bacteria Culture of*V. anguillarum* (B)	% SR	Total of Vibrio at the Beginning (CFU/ml)	Total of Vibrio at the End (CFU/ml)
A0B1	0 ml	10 ml	9.6 ± 1.67^{b}	10^7	5.28 x 10^{3a}
A1B1	2.5 ml	10 ml	18 ± 6.78^{ab}	10^7	4.62 x 10^{3ab}
A2B1	5 ml	10 ml	23.2 ± 11.09^{a}	10^7	2.12 x 10^{3bc}
A3B1	7.5 ml	10 ml	25.2 ± 4.14^{a}	10^7	2.1 x 10^{3bc}
A4B1	10 ml	10 ml	26.8 ± 6.09^{a}	10^7	1.7 x 10^{3c}

Notes :
1). Values on table ± standard deviation(average of 5 replications).
2). Different letters on the same columns indicated significantly different results (P<0.05) based on Duncan tests.

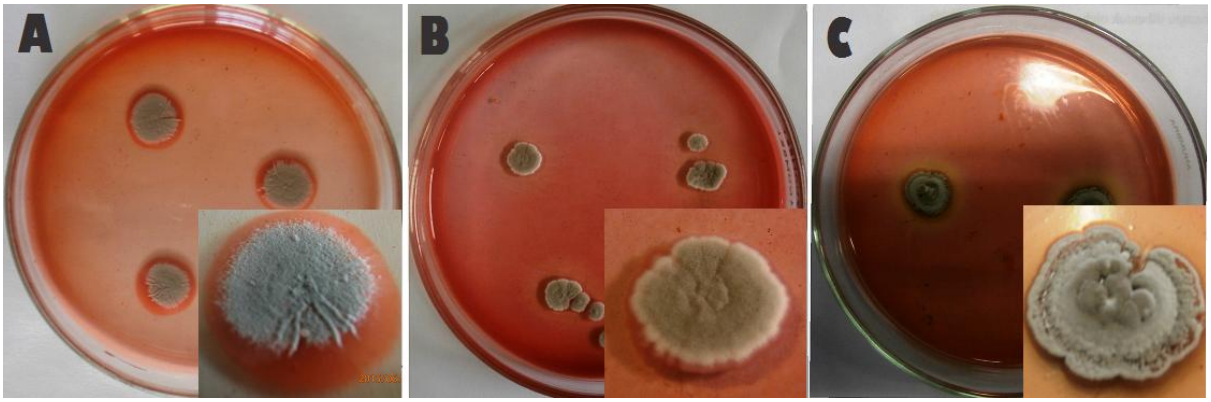

Figure 1. The colony of *Streptomyces* sp.1 (A), *Streptomyces* sp.3 (B) and*Streptomyces* sp.4 (C) on the YEMA media.

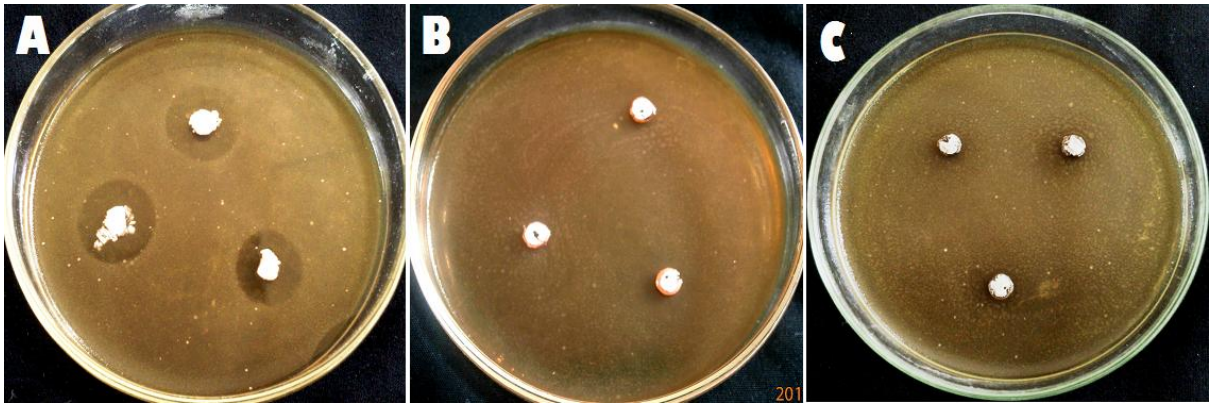

Figure 2. The antagonistic activitiy tests of isolates of *Streptomyces* sp.1 (A), *Streptomyces* sp.3 (B) and *Streptomyces* sp.4 (C) against patogenic*V. anguillarum* (*in vitro).*

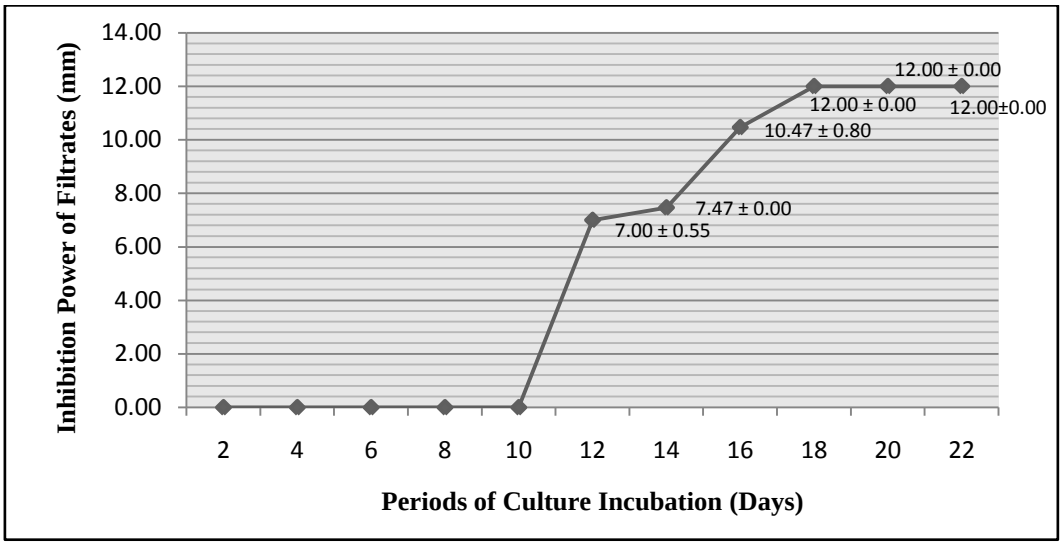

Figure 3. The activity of inhibition power of the filtrates of *Streptomyces* sp. 1 on different incubation periods against *V. anguillarum*.

This result also supported a research by Aftabuddin *et al*. (2013), the research of which found that the application of *Streptomyces* sp. on the larvae ponds would be effective in reducing the population of the Vibrio. On that research it was known that the *Streptomyces fradiae* which was applied on the maintenance ponds of larvae of *P. monodon* can reduce the population of the Vibrio significantly compared to the control. *Streptomyces fradiae* was known to be able to secretan antibiotic as a mechanism of inhibiting the patogenic Vibrio. The ability of the antagonistic microbes in reducing the population of the *Vibrio* spp. on the maintenance media according to Banaerjee *et al*. (2007) can also caused by the production of vibriostatic compounds and because of niche competitions between the Vibrio bacteria and the antagonistic bacteria. The ability of the Streptomyces in controlling the vibriosis infections on shrimps has also been reported by Velmurugan *et al*. (2015). On his research, the culture of *Streptomyces VM-15*, *Streptomyces VM-21*, and *Streptomyces VM-8* were found to be effective in reducing vibriosis infections on post larvae of *P. monodon*.

Das *et al*. (2010) were also reported that the application of the culture of *Streptomyces* sp. on the consentration of 1% (v/v) was known can reduce vibriosis infection by *V. harveyi* and *V. proteolyticus* on adult *Artemia* sp. (age 15 days).

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REFFERENCES

Aftabuddin, S., M.A. Kashem., M.A. Kader., M.N.A. Sikder and M.A. Hakim (2013). Use of*Streptomyces fradiae* and *Bacillus megaterium* as Probiotics in the Experimental Culture of Tiger Shrimp *Penaeus monodon* (Crustacea, Penaeidae). *AACL Bioflux*. 6 (3): 253-267.

Atmomarsono, M., Muliani. and B.R. Tampangallo (2010). Aplikasi Bakteri Probiotik Untuk Peningkatan Sintasan dan Produksi Udang Windu di Tambak. *Prosiding Forum Inovasi Teknologi Akuakultur*. 269-278.

Atta, H.M., M.H. El-Sehrawi., N.M. Awny. and N.I. El-Mesady (2012). Cirramycin –B antibiotic production by *Streptomyces cyaneus*-AZ-13Zc: Fermentation, purification and biological activity. *N.Y.Sci.J*. 4: 35-42.

Azizunnisa and K. Sheeramulu (2013). A Study on Luminiscent Bacteria in Shrimp Post Larvae in Hatcheries and Rearing Tanks in East Godavari District of Andhra Pradesh, India. *International Journal of Advantacements in Research and Technology*. 4(2): 109-116.

Balcazar, J.L., I.D. Blas., I. Ruiz-Zarzuela., D. Cuningham., D. Vendrell and J.L. Muzquiz (2006). The role of probiotics in aquaculture. *Vet. Microbiol*. 144: 173-186.

Banarjee, S., T.N. Devaraja., M. Syariff and F.M. Yusoff (2007). Comparion of four antibiotics with indigenous marine *Bacillus* spp. in controlling pathogenic bacteria from shrimp and artemia. *J. Fish Dis.* 30(7): 383-389.

Bernal, M.G., A.I.Campa-Sordova., P.E. Saucedo., M. C. Gonzalez., R. M. Marrero., J.M. and Mazon-Suastegui (2015). Isolation and in Vitro Selection of Actinomycetes Strains as Potential Probiotics for Aquaculture. *Veterinary World*. 8: 170-176.

Bintari, N.W.D., R. Kawuri and A.A.G.R. Dalem (2016). Identifikasi Bakteri Vibrio Penyebab Vibriosis Pada Larva Udang Galah (*Macrobrachium rosenbergii* (de Man)). *Jurnal Biologi*. 20(2): 53-58.

Cabello, F. C. (2006). Heavy Use of Prophylactic Antibiotics in Aquaculture: a Growing Problem For Human and Animal Health and for the Environment. *Environ. Microbiol*. 8(7): 1137–1144.

Charoensopharat, K., P. Thummabenjapone., P. Sirithorn and S. Thammasirirak (2008). Antibacterial Substance Produced by *Streptomyces* sp. No. 87. *African Journal of Biotechnology*. 7(9) 1362-1368.

Chau, N.T.T., N.X. Hieu., L.T.N. Thuan., M. Matsumoto and I. Miyajima (2011). Identification and Characterization of Actinomyces Antagonistic to Pathogenic *Vibrio* Spp. Isolated From Shrimp Culture Pond Sediments in Thua Thien Hue-Viet Nam. *J. Fac. Agr. Kyushu Univ.* 56(1): 15-22.

Das, S., L.R. Ward and C. Burke (2010). Screening of marine *Streptomyces* spp. for potential use as probiotics in aquaculture. *Aquaculture*. 305: 32-41.

El-Naggar, M.Y. (2007). Kosinostatin, a moajor secondary metabolite isolated from the culture filtrate of *Streptomyces violaceusniger* strain HAL64. *J. Microbiol*. 45(3): 262-267.

Holt, J.G, N.R Krieg, P.H.A Sneath, J.T Staley and S.T Williams (1994). *Bergey's Manual of Determinative Bacteriology*. Ninth Edition. Williams & Wilkins. Baltimore.

Hong, K., A-H. Gao., Q-Y. Xie. H. Gao., L. Zhuang. H-P. Ln. H-P. Yu., J. Li. X-S. Yao., M. Goodfellow and J.S. Ruan (2009). Actinomycetes for marine drug discovery isolated from mangrove soils and plants in China. *Mar. Drugs*. 7: 24-44.

Isnansetyo, A., I. Istiqomah., Muhtadi., S. Sinansari., R.K. Hernawan., Triyanto. J. Widada (2009). A Potential Bacterial Biocontrol Agent, Stain S2V2 Against Pathogenic Marine *Vibrio* In Aquaculture. *World J. Microbiol Biotechnol*. 25(1): 1103-1113.

Kawuri, R. (2012). "Pemanfaatan Streptomyces Thermocarboxydus Untuk Mengendalikan Penyebab Penyakit Busuk Daun Pada Lidah Buaya (Aloe Barbadensis Mill.) Di Bali" (disertasi). Denpasar: Universitas Udayana.

Kimura, I., K. Yamano., H. Nakano., K. Momoyama., M. Hiraoka and Inouye (1996). Detection of penaeid rod –shaped DNA virus (PRDV) by PCR. *Fish Pathol*. 31: 93-98.

Laidi, R.F., A. Abderrahmane and A.A.H. Norya (2013). Identification and antibiosis of a novel Actinomycete strain RAF-11 isolated from Iraqi soil. *International Journal of Sciences : Basic and Applied Research*. 12(1): 141-159.

Lechevalier, H.A. and S.A. Walkman (1973). *Guide to the Classification and Identification of the Actinomycetes and Their Antibiotics*. Baltimore: Williams and Wilkins.

Manivasagan, P., J. Vankatesan., K. Sivakumar and S.K. Kim (2013). Marine actinobacterial metabolites: Current status and future perspectives. *Microbiological Research*. 168: 311-322.

Mohana, S. and M. Radhakrishnan (2014). *Streptomyces* sp. MA7 Isolated from Mangrove Rhizophere Sediment Effective Against Gram Negative Bacterial Pathogens. *International Journal of PharmTech Research*. 6(4): 1259-1264.

Morris, J.G. (2003). Cholera and Other Types of Vibriosis: A Story of Human Pandemics and Oysters on the Half Shell. *Clinical Infectious Diseases* 37**:** 272-280.

Patang (2012). Pengaruh Penggunaan Berbagai Antibiotik dan Probiotik dengan Dosis Berbeda Terhadap Pertumbuhan dan Kualitas Air Pada Larva Udang Windu (*Penaeus monodon* Fabricus). *Jurnal Agrisistem*. 8(2): 77-86.

Remya, M. and R. Vijayakumar (2008). Isolation and characterization of marine atagonistic Actinomycetes from west coast of India. *Facta universitatis: Series Medicine and Biology*. 15(1): 13-19.

Sahu, M.K., M. Murugan., K. Sivakumar., T. Thangaradjou and L. Kannan (2007). Occurrence and distribution of Actinomycetes in marine environs and their antagonistic activity against bacteria that is pathogenic to shrimps. *The Israeli Journal of Aquaculture*. 59(3): 155-161.

Selvakumar, D., K. Arun., S. Suguna., D. Kumar and K. Dhecendaran (2010). Bioactive potential of Streptomyces against fish and shellfish pathogen. *Iranian Journal of Microbiology*. 2(3): 157-164.

Thiruvarangan, M., T. Kumar and A.P. Rajan (2014). Prognosis of biofilm formation of Vibrio bacteria on shrimp and diagnosis of vibriosis. *World Journal of Pharmaceutical Research*. 3(2): 3295-3314.

Velmurugan, S., S.T. John., D.S. Nagaraj., T.A. Ashine., S. Kumaran and S.R. Pugazhvendan (2015). Isolation of Actinomycetes From Shrimp Culture Pond and Antagonistic to Pathogenic *Vibrio* Spp. and WSSV. *International Journal of Current Microbiology and Applied Sciences*. 4(7): 82-92.

Veseeharan, B. and P. Ramasamy (2003). Abudance of potentially pathogenic micro-organism in *Penaeus monodon* larvae rearing systems in India. *Microbiol. Res.* 158: 299-308.

Wang, B. S. Liang. W. Zhang and Q. Zan. (2003). Mangrove flora of the world. *Acta Botanica Sinica*. 45. 644-653.

You, J.L., L.X. Cao., G.F. Liu., S.N. Zhou., H.M. Tan and Y.G. Lin (2005). Isolation and characterization of Actinomycetes antagonistic to pathogenic *Vibrio* Spp. from nearshore marine sediments. *World Journal of Microbiology and Biotechnology*. 21. 679-682.

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