Available online at BIOMA: Jurnal Ilmiah Biologi
Websites:http://journal.upgris.ac.id/index.php/bioma/index
BIOMA: Jurnal Ilmiah Biologi, 12 (1), April 2023, 31-43
Doi: https://doi.org/10.26877/bioma.v11i2.14387

GROWTH PATTERN OF *Bacillus* spp. ISOLATED FROM GASTROINTESTINAL OF NYPA PALM WORM (*Namalycastis rhodochorde*) WITH DIFFERENT COMBINATION OF PH AND SALINITY

Rikhsan Kurniatuhadi[1*], Ari Hepi Yanti[1], Tri Rima Setyawati[1], Eka Karunia[1]
[1]Biology Department, Mathematics and Natural Sciences Faculty, Tanjungpura University
Jl. Prof. Dr. Hadari Nawawi, Pontianak City, West Kalimantan, Indonesia, 78241)
**Corresponding author:rikhsan.kurniatuhadi@fmipa.untan.ac.id*

ARTICLE INFO

Article history

Submission	2023-01-08
Revision	2023-03-14
Accepted	2023-04-09

Keywords:
Bacillus spp.
Nypa palm worm
Growth pattern
pH and salinity

ABSTRACT

Bacillus spp. (NrLtF1, NrLtF5, and NrLtG2) isolated from the digestive tract of the Nypa palm worm (Namalycastis rhodochorde) were assumed to have growth characteristics according to the habitat conditions of the nypa worm. Salinity and pH are two environmental factors affecting gastrointestinal isolates' growth from nypa palm worms. This study aimed to determine the effect of pH and salinity on the media on growth patterns and determine the optimum combination of pH and salinity for Bacillus spp. The study was conducted using a spectrophotometry method using a microplate reader with Glucose Yeast Peptone (GYP) as a medium for bacterial growth. The medium's pH and salinity values were adjusted by adding 5M NaOH and 5M HCl to obtain pH values of 4, 5, 6, and 7, respectively. Addition of NaCl to the growing medium to get salinity values of 5%, 10%, and 15%. Optimization was determined by incubation at a density of 595 nm at a temperature of 31°C for 24 hours. The results showed that Bacillus spp. could grow well at pH 6 with an interval of salinity value from 5%-15%. The growth optimum of three isolates showed that Bacillus NrLtF1, NrLtF5, and NrLtG2 occurred at combination P6S5, P6S10, and P6S15 based on contour plot design, respectively. The Optimum growth of Bacillus spp. with pH and salinity expects to be a reference for developing feed products based on indigenous nypa palm worms.

INTRODUCTION

Bacterial growth characteristics are related to their habitat and relationship with other living things. The bacteria found in associated animals will have optimal growth

characteristics following the adaptation character of these animals to environmental factors in their habitat. Several types of associations of bacteria with sponges, groups of worms, and insects have been identified, and their growth patterns have been studied based on the habitat of the host animal. Research conducted on the growth of indigenous isolates associated with animals showed a growth pattern with optimal growth characteristics following the physicochemical factor values of the host animal such as mollusks (Lyudmila et al., 2008), polychaetes (Rizzo et al., 2013), sponges (Yi et al., 2014), and shrimp (Subagiyo et al., 2015). Subagiyo et al. (2016) stated that bacteria can survive in media or media according to the nutritional components needed for growth and optimal growth characteristics according to their habitat. According to Bayane et al. (2007) and Ikhwani et al. (2017), bacteria can carry out physiological processes and adapt well to the environment if the growth medium conditions are favorable.

Nypa palm worms (*N. rhodochorde*) are worms from the *Polychaeta* group that live in estuary areas exposed to seawater intrusion. This worm is a new and endemic species in West Kalimantan. Nypa palm worms have strategic economic importance because they are used as feed components for aquaculture cultivation. Junardi (2015) has developed a method of fertilization and cultivation of nypa palm worms in a laboratory. However, the growth of worms in the laboratory is not as progressive as in their habitat.

Isolation and characterization of the cellulolytic and proteolytic ability of *Bacillus* spp. isolated from the intestine of nypa palm worms which was carried out in a previous study to find local and indigenous isolates with economic potential (Yanti et al., 2017; Yanti et al., 2020; Setyawati et al., 2021). Local isolates were used as a degrading agent for nypa midribs natively. This agent is related to providing feed products for nypa palm worm cultivation and developing beneficial indigenous bacteria-based probiotic products. Our previous research investigated the potential of probiotic bacteria isolated from the gastrointestinal tract of nypa palm worms to develop feed for nypa palm worms in the laboratory (Yanti et al., 2018). Karunia et al. (2021) showed that the growth of *Bacillus* bacteria isolated from nypa palm worms tended to follow the environment of the nypa palm worms. The results of the study before showed that three isolates had similar characteristics to *Bacillus* and had cellulolytic, proteolytic, and inhibitory abilities to pathogens, namely *Bacillus* NrLtF1, *Bacillus* NrLtF5, and *Bacillus* NrLtF2. These three bacteria are probiotic candidates for making aquaculture feed formulations. However, the

viability and optimum growth of the three bacteria have yet to be known with certainty against fluctuations in the pH value and salinity of the medium.

The pH and salinity of the substrate play a role in the growth of nypa palm worm association bacteria. *Bacillus* spp., isolated from the intestine of the nypa palm worm (*N. rhodochorde*), is thought to have growth characteristics adapted to the character of the growing environment of its host. Junardi and Setyawati (2008) stated that nypa palm worms (*N. rhodochorde*) live in rivers and estuaries, which are still influenced by salinity from the seawater. Each bacterium lives in media with optimum salinity and different pH values. Several studies have been carried out by researchers related to the optimum growth factors of bacteria isolated from primary mangrove areas, such as *Bacillus* , *Staphylococcus, Vibrio, Micrococcus, Alteromonas, Escherichia,* and *Listeria* from several locations in Indonesia (Ambeng et al., 2019; Sumardi et al., 2021; Fani et al., 2022). However, research and scientific discussion regarding the optimum growth of bacterial isolates isolated from the intestines of nypa palm worms with an estuary or secondary mangrove habitats still need to be included in the information. It is necessary to explain basic information for applying bacteria as feed probiotics for nypa palm worms.

This study aims to determine the effect of salinity and pH of the media on the growth of *Bacillus* spp. (NrLtF1, NrLtF5, and NrLtG2) were isolated from the intestine of the Nypa palm worm (*N. rhodochorde*). Therefore, it is necessary to study the optimum growth of indigenous bacteria based on the habitat or living conditions of the host specially for pH and salinity factors. Determination of optimal growth for *Bacillus* spp., isolated from the intestinal of nypa palm worms, is expected to be a scientific basis for developing fermented feed for cultivating nypa palm worms.

MATERIALS AND METHODS

Bacterial strains and medium

The strains of *Bacillus* spp. (NrLtF1, NrLtF5, and NrLtG2) used in this study were isolated from the intestines and fecal pellets of nypa palm worms (*N. rhodochorde*) (Setyawati et al., 2021). The NrLtF and NrLtG codes describe the origin of the isolate samples, where F was isolated from fecal pellets, while G was isolated from the nypa

palm worm intestine. The optimum growth temperature of the strains studied was 30^{o}C (Yanti et al., 2020).

The growth medium used was the initial isolation medium, namely de Man Rogosa Sharp (MRS) Agar (Merck), while the liquid medium for the growth experiment used was Peptone Yeast Glucose Broth media (peptone 20 g; glucose 10 g; yeast extract 10 g; $MgSO_4$ 8 mg; K2HPO4 40 mg; aquades 1 L). The media was then sterilized using an autoclave at 121oC for 15 minutes and a pressure of 2 atm (Irianto, 2006).

Experimental design

The experimental design used a factorial completely randomized design (CRD) consisting of two treatment factors, namely the medium's salinity and pH treatment consisted of 10 combinations for each bacterial isolate. The first factor was the pH treatment consisting of three levels, namely pH (code: P) 4, 5 and 6. The second factor was the salinity treatment (code: S) with five treatment levels namely 5%, 10% and 15% based on the salinity of fresh water, brackish water, and salt water. pH 7 and 0% salinity as controls (**Table 1**). Each treatment was repeated three times to obtain 30 experimental units.

Table 1. Experimental design of treatments in the medium's salinity and pH combination against three isolates

Treatments	pH (**code: P**)		
Salinity (**code: S**)	4	5	6
5%	P4S5	P5S5	P6S5
10%	P4S10	P5S10	P6S10
15%	P4S15	P5S15	P6S15

Rejuvenation of Bacillus* spp. *Culture

One loop of *Bacillus* spp. (NrLtF1, NrLtF5, and NrLtG2) inoculated in MRS agar medium which had solidified aseptically. Inoculation is carried out by scraping the loop needle containing the culture on the surface of the agar continuously (zig-zag). Furthermore, the culture was incubated at 30oC for 24-48 hours to obtain a stock bacterial culture (Subagiyo et al., 2016).

Growth Measurement of Bacillus spp. with a microplate reader

Measurement of *Bacillus* spp. (NrLtF1, NrLtF5, and NrLtG2) Cells were carried out using the spectrophotometric method. Spectrophotometry was carried out on the bacterial suspension on a microplate reader with a wavelength of 595 nm with a shaking

speed of 10 S. Measurement was made by taking 200 microliths of the suspension, which had been inoculated with *Bacillus* . Then the suspension of *Bacillus* spp. incubated for 24 hours. Optical density measurement of *Bacillus* spp. performed every two hours within 24 hours. Optical density values were then recorded and interpreted using the counterplot method to determine the effect of pH and medium salinity values on the growth of *Bacillus* spp..

Data Analysis

The optical density (OD) measurement data is presented in tables and graphs. The treatment's growth response and cell density data were then analyzed using the Minitab 16 Response Methodology Surface (RSM) software: The contour plot and surface 2D optimize the method. The response surface and contour plots were drawn to illustrate the main and interactive effects of the independent variables on the pH and salinity values of the medium. The optimum values of the selected variables were obtained by analyzing the response surface contour plot.

RESULTS AND DISCUSSION

The growth curves of *Bacillus* spp. (NrLtF1, NrLtF5, and NrLtG2) showed that the three bacteria could grow in all treatment compositions of pH and salinity of the medium. However, the graphic pattern showed that the decrease of the pH medium value causes the growth of the three bacterial isolates also decreases. In contrast to the pH factor of the medium, the increase in the salinity value of the medium affected the optimum growth character for all bacterial isolates (**Figure 1**). This result is correlated with the optimum growth value based on the bacteria coefficient, where the medium's pH value is 6-7, and the salinity is between 5-15 percent.

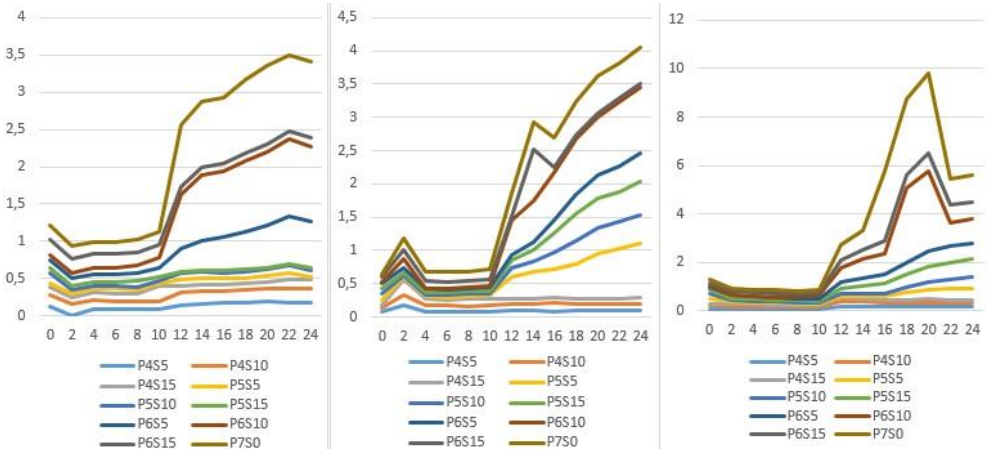

Figure 1. Growth pattern of *Bacillus* spp. NrLtF1, NrLtF5, and NrLtG2 with different salinity and pH values of the broth medium (abscis: OD value; ordinate: incubation time)

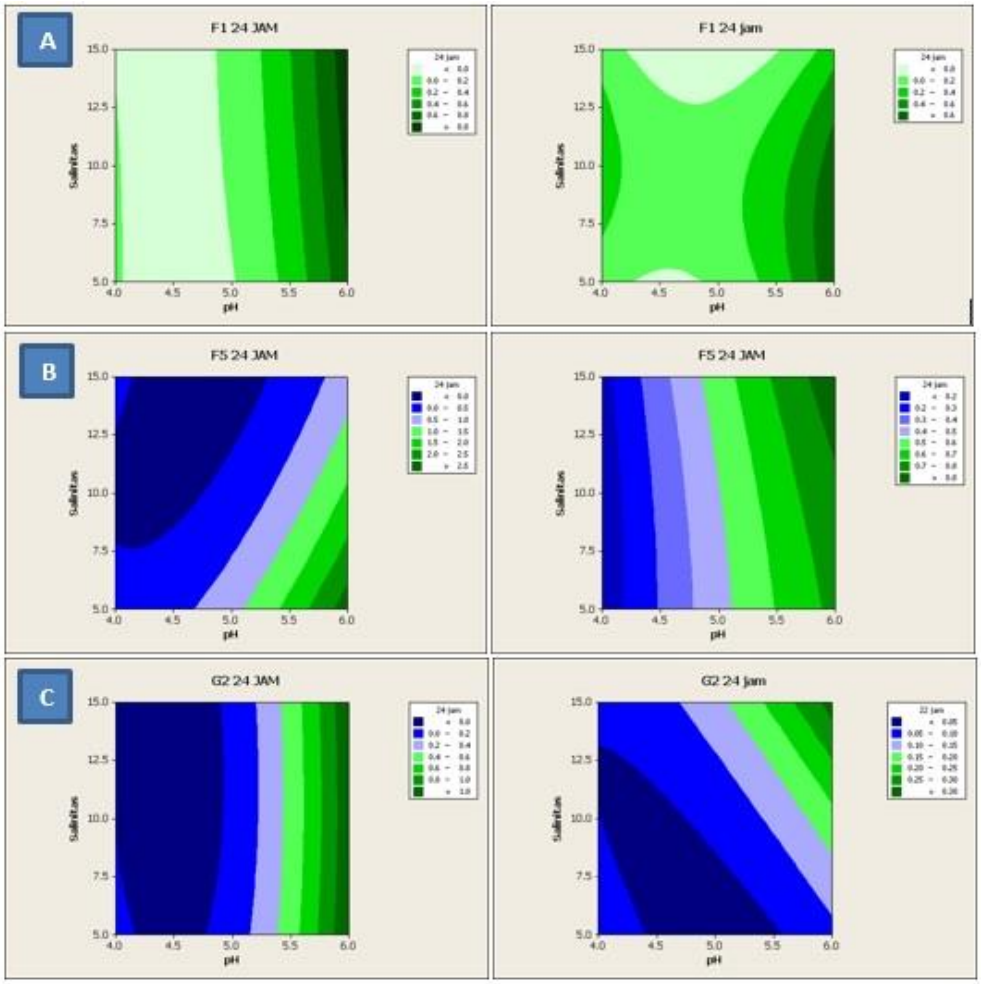

Figure 2. Response surface curves for *Bacillus* spp. (NrLtF1 fig. A, NrLtF5 fig. B, and NrLtG2 fig.C) biomass as a function of temperature and pH. The surface graph and the contour plot on the right show bacterial growth with agitation during incubation, while the chart on the left shows no agitation

The optimum growth optimization patterns and interval of the pH and salinity values of the medium showed that *Bacillus* sp. NrLtF1 was the excellent-growing bacteria from the color zone of the surface contour plot graph. In contrast, NrLtF5 and NrLtG2 bacteria have different color zones than the previous isolate (**Figure 2**). The two bacteria showed a pattern of slowing growth which was indicated by the color of the blue part of the graph. However, the growth pattern of *Bacillus* in the incubation model with agitation (left side) and without agitation (right side) showed almost the same pattern for each type of isolate. The dark blue color on the graph shows the concentration of NrLtF5 and NrLtG2 bacterial cell density less than 0.2 x 10^5 cells/mL, light blue color indicates a cell density of 0.2-0.3 x 10^5 cells/mL, lavender purple color indicates a cell density of 0.3 -0.5 x 10^5 cells/mL, light green color indicates cell density of 0.5-0.8 x 10^5 cells/mL, and dark green color indicates cell density >0.8 x 10^5 cells/mL.

Table 2. The experimental design used in response surface methodology studies showing observed pH and salinity of medium affecting the biomass of *Bacillus* spp.

Incubation time (hour)	Growth Optimization Value		Cell Prediction Value (OD)
	pH	Salinity (%)	
NrLtF1			
2	6,0	15,0	0,14
4	4,0	5,0	0,11
6	4,0	5,0	0,10
8	6,0	15,0	0,16
10	4,0	15,0	0,17
12	6,0	8,43	0,49
14	6,0	8,13	0,60
16	6,0	7,72	0,62
18	6,0	7,62	0,67
20	6,0	5,0	0,60
22	**6,0**	**6,91**	**0,77***
24	6,0	7,02	0,76
NrLtF5			
2	4,0	15,0	0,11
4	4,0	15,0	0,10
6	4,0	15,0	0,09
8	5,25	10,35	0,14
10	6,0	11,56	0,12
12	6,0	11,26	0,42
14	6,0	13,58	0,56
16	6,0	15,0	0,66
18	6,0	10,45	1,70
20	**6,0**	**10,35**	**1,90***
22	6,0	15,0	0,86
24	6,0	15,0	0,86
NrLtG2			
2	6,0	15,0	0,13

Incubation time (hour)	Growth Optimization Value		Cell Prediction Value (OD)
	pH	Salinity (%)	
4	4,0	15,0	0,12
6	4,0	15,0	0,10
8	4,0	15,0	0,09
10	4,0	15,0	0,10
12	6,0	15,0	0,17
14	6,0	15,0	0,22
16	6,0	15,0	0,25
18	6,0	15,0	0,29
20	6,0	15,0	0,31
22	**6,0**	**15,0**	**0,33***
24	6,0	15,0	0,32

Note: Values in bold and with an asterisk are the optimum growth values at the pH and salinity of the medium during the study.

The bacterial isolate NrLtF1 was detected as being able to grow in all salinity and pH treatments, which were marked with green areas on the graph. The other isolates showed a decreased growth pattern marked in blue areas. The results of statistical analysis using the Minitab 16 Response Methodology Surface (RSM) showed that each isolate had a combination point with optimum growth for the salinity and pH of the medium when viewed from the incubation time. *Bacillus* sp. NrLtF1 achieved good growth after 22 hours of incubation with a combination of pH 6 and salinity of 7%. The best growth of *Bacillus* sp. NrLtF5 occurred at a combination of pH 6 and 10% salinity at an incubation time of 20 hours during *Bacillus* sp. NrLtG2 reached optimum growth at 22 hours with a combination of pH 6 and 15% salinity. NrLtG2 is the only isolate that can grow optimally at any incubation time at a salinity value of 15% (**Figure 2**).

Discussion

Bacillus is a bacterium that has a cosmopolitan character and extensive habitat adaptation. Beleneva (2008) stated that *Bacillus* is an indigenous component of marine bacterial communities and is often isolated from such marine environments as water, sediments, and animals. This group of bacteria's adaptability is correlated with their physiological activities, including enzymatic activities that support their life on various substrates. The results of previous studies showed that the bacteria isolated from the gastrointestinal of the nypa palm worm (*N. rhodochorde*) had an excellent cellulolytic ability. Enzymatic activity is highly correlated with the optimum growth character of each bacterium. The proteolytic and cellulolytic activity and performance studied previously

from *Bacillus* , isolated from the nypa palm worm intestine, will be influenced by environmental factors or the appropriate medium.

Bacillus is one of the genera that have the potential to be developed into probiotics (Dewi, 2014). This is because *Bacillus* can produce antibiotic compounds against pathogens (Baraga et al., 2022). Olmos and Michel (2014) stated that *Bacillus* could be used as a probiotic for aquaculture cultivation after knowing the character of its enzymatic activity and optimum growth. This study tested how the optimum growth of bacteria that were later identified from the *Bacillus* group could grow well based on their natural association with nypa palm worm. The study is significant and related to the application of these bacteria when the nypa palm midrib fermentation is carried out to manufacture nypa palm worm feed. Subagiyo et al. (2015) state that each species or strain of bacteria has a different pH and medium salinity adaptation value to carry out specific growth and biological activities.

The results showed that the four isolates of *Bacillus* spp. have different characters for the pH range and salinity of the tested medium. *Bacillus* sp. NrLtF1 was very well adapted to all tested pH and salinity values (**Figure 1**). The source of isolates is essential in determining the character of bacterial growth. *Bacillus* sp. NrLtF1 has a suitable growth interval with a more comprehensive pH and salinity during *Bacillus* sp. NrLtF5 and NrLtG2 have a green zone at pH 6 on the surface and contour plot graphs. The results of the research by Gorlach-Lira et al. (2010) on *Bacillus* sp. isolated from the semi-arid pasture soil of Northeast Brazil have similar character optimization of pH medium with *Bacillus* spp., which was isolated from the intestine of the nypa palm worm. Both have a similar excellent bacterial growth zone (green zone on the surface contour plot) at the pH interval of the medium 5-7. Research Emoyoma et al. (2020) also described the pH of the nypa palm tree substrate in the range of 5-7. *Bacillus* spp. Isolated from nypa palm worms, it will adapt well to substrate and host conditions.

The growth curve of the three isolates of *Bacillus* spp. isolated from the intestine of nypa palm worms showed low growth at pH four compared to other pH values. This condition occurred in all combinations of medium salinity treatments (**Figure 1**). pH 7 is a medium pH value with a good growth chart of nypa palm worm isolates. Karunia et al. (2021) measured the pH of palm worms' intestinal and coelomic cavities. The results of these measurements indicate that the pH of the intestine and coelom cavity is 7. Growth

conditions of *Bacillus* spp. showed similarities with the results of research by Sumardi et al. (2019), which showed that the optimum growth of *Bacillus* isolated from the estuary of Hanura Beach, Lampung, occurred at an interval of 7-10 pH values of the medium. However, from the optimization of the surface contour plot graph (**Figure 2**), *Bacillus* spp., which was isolated from nypa palm worms, could still grow well at pH 5 and 6. This result indicates that the ability of *Bacillus* spp., isolated from the nypa palm worms' intestine, was better adapted to the estuary's physical and chemical conditions and the worms, especially for the range of pH and salinity values of the medium.

Optimum growth of *Bacillus* sp. NrLtF1 was the most variable in medium with different salinity values. The growth optimization value data shows that the optimum value occurs in at least five different salinity values with an interval of 5-15%. *Bacillus* sp. NrLtF5 is intermediate with optimal growth at 10-15% salinity intervals, while NrLtG2 only grows optimally at 15% salinity. The results showed that the OD values of the three bacterial isolates fluctuated from the 20th to the 24th hour, where the NrLtF5 isolates reached the highest value on turbidity observations (**Table 2**). It shows that the significant increase in growth was better for this isolate when compared to the other two isolates. Although the optimum growth characteristics of salinity are different from each other, the overall character shows the character of the estuary. According to Remane's 1934 drawing of estuary species diversity patterns, species show estuary salinity intervals between 5 and 15% (Smith & Elliot, 2016). This percentage of salinity can change based on tidal, daily, fortnightly, monthly (lunar), seasonal, and equinoctial cycles (Wolanksi & Elliott, 2015). It is the scientific basis for describing the optimum growth character of *Bacillus* spp. isolated from nypa palm worms based on the salinity values of their habitat.

CONCLUSION

The graph of the number of bacterial growth cells and the contour plot test showed that the growth of *Bacillus* spp. has different incubation times. The higher the pH and salinity value, the increased bacteria growth. However, some isolates experienced decreased growth at low pH and salinity values. *Bacillus* spp. can grow at all tested pH and salinity. The media's optimal salinity and pH values for bacterial growth at 24 hours of incubation occurred at a combination of 15% salinity and pH 6 in the media. Bacterial

growth in this combination showed a higher number of cells. The optimal salinity and pH of the medium gives an overview of the growth of *Bacillus* spp when it is developed into a probiotic product for nypa palm worm feed.

ACKNOWLEDGMENT

The authors want to thank the Faculty of Mathematics and Natural Sciences, Tanjungpura University, for assisting this research through DIPA funds from Tanjungpura University, nypa palm worm research team (Eka Karunia), and Mr. Novik Nurhidayat from the Health Microbiology Laboratory of the Indonesian Institute of Sciences (LIPI/BRIN) for assisting the research process.

REFERENCES

Ambeng, H., Zubair, N.P., Oka, and Tonggiroh, A. (2019). Isolation and characterization of bacteria from mangrove sediment at the coastal area in Pangkep South Sulawesi, *Journal of Physics: Conference Series IOP Publishing* 1341 (2019) 022016 doi:10.1088/1742-6596/1341/2/022016.

Apri, A., Badami, K., Araninda, D.G., and Wardani, M.K. (2017). Dampak Perbedaan Salinitas Terhadap Viabilitas Bakteri *Vibrio fluvialis*. *Jurnal Ilmiah Perikanan dan Kelautan*, 9(2). https://doi.org/10.20473/jipk.v9i2.7636

Baraga, P.V., Mahyarudin, and Rialita, A. (2022). Aktivitas antibakteri metabolit sekunder isolat bakteri endofit kunyit (*Curcuma longa* L.) terhadap *Propionibacterium acnes*, *BIOMA: Jurnal Ilmiah Biologi*, 11(1), 103-120. https://doi.org/10.26877/bioma.v11i1.10558

Bayane, A. (2007). Isolation and characterization of new spore-forming lactic acid bacteria with prospects of use in food fermentations and probiotic preparations, *African journal of microbiology research*, vol. 4(11), 1016-1025.

Beleneva, I.A. (2008). Distribution and Characteristics of *Bacillus* Bacteria Associated with Hydrobionts and the Waters of the Peter the Great Bay, Sea of Japan, *MICROBIOLOGY*, 77(4), 497–503.

Dewi, E.R.S. (2014). Pertumbuhan kultur probiotik hasil isolat bakteri non patogen dalam berbagai jenis media, *BIOMA: Jurnal Ilmiah Biologi*, 3(1), 53-65. https://doi.org/10.26877/bioma.v3i1,%20April.655

Elliot, M. and Smyth, K. (2016). *Chapter 9: Effects of changing salinity on the ecology of the marine environment. Stressor in the marine environment*. Oxford University Press.

Junardi. (2008), Karakteristik morfologi dan habitat cacing nipah (*N. rhodochorde*) (*Polychaeta*: Neredidae: Namanereididae) Di Kawasan Hutan Mangrove Estuaria Sungai Kakap Kalimantan Barat, *Jurnal Sains MIPA*, 14(2), 85-89.

Emoyoma, U.O., Numbere, A.O., and Woke, G.N. (2020). Impact of nypa palm (*Nypa fruticans* Wurmb) and mangroves forest on benthic macro invertebrate community in Andoni River, Nigeria. *International Letters of Natural Sciences*, 77, 51-62. doi:10.56431/p-0eqs18.

Fani, E.F., Rahmawati, and Kurniatuhadi, R., (2022). Identification and detection of proteolytic activity of endophyte bacterial isolated from *Avicennia Marina* leave in Mempawah Mangrove Center, *LenteraBio*, 11(2), 293-299. https://doi.org/10.26740/lenterabio.v11n2.p293-299

Gorlach-Lira, K., Pedrosa, M.L.V., Pokojska, A.B., Rozycki H., and Dahm, H. (2010). Response surface analysis on the effect of temperature and pH on growth and proteolytic activity of thermophilic *Bacillus* sp. *Brazilian Atchives of Biology and Technology*, 53(5), 1067-1072. https://doi.org/10.1590/S1516-89132010000500009

Ikhwani, A.Z.N., Nurlaila, H.S., Ferdinand, F.D.K., Fachria, R., Hasan, A.E.Z., Yani, M., Setyawati, I., and Suryani. (2017). Preliminary study: optimization of pH and salinity for biosurfactant production from *Pseudomonas aeruginosa* in diesel fuel and crude oil medium. IOP Conf. Series: Earth and Environmental Science 58: doi:10.1088/1755-1315/58/1/012056.

Karunia, E., Yanti, A.H., Kurniatuhadi, R. (2022). Karakterisasi *Bacillus* sp. (kode NrLtF5) yang diisolasi dari usus cacing nipah (*Namalycastis rhodochorde*). *Jurnal Protobiont*, 10(3), 69-73. http://dx.doi.org/10.26418/protobiont.v10i3.55574

Lyudmila, A., Romanenko, Masataka, U., Natalia, I.K., Valery, V., and Mikhailov. (2008). Isolation, phylogenetic analysis and screening of marine mollusk-associated bacteria for antimicrobial, hemolytic and surface activities, *Microbiological Research*, 163(6), 633-644. https://doi.org/10.1016/j.micres.2006.10.001.

Olmos, J., and Michel, J.P. (2014). *Bacillus subtilis* A Potential Probiotic Bacterium to Formulate Functional Feeds for Aquaculture, *Journal of Microbiology and Biochemical Technology*, 6(7), 361-365. DOI: 10.4172/1948-5948.1000169.

Ricco, C., Michaud, L., Syldatk, C., Hausmann, R., Emilio, D.D., and Giudice A.L. (2013). Influence of salinity and temperature on the activity of biosurfactants by polychaete-associated isolates. *Environmental Science and Pollution Research*, 21(4). DOI: 10.1007/s11356-013-2259-8.

Subagiyo, Margino, S., Hadisaputro, T., and Ari W. (2015). Effects of pH, temperature and salinity in growth and organic acid production of lactic acid bacteria isolated from penaeid shrimp intestine. *ILMU KELAUTAN Indonesian Journal of Marine Sciences*, 20(4). DOI:10.14710/ik.ijms.20.4.187-194.

Subagiyo, Ria, A.T.N., Willis, A.S., and Adi, S. (2016). Optimasi suhu dan pH pertumbuhan *LactoBacillus lactic* isolat ikan kerapu, *Jurnal Kelautan Tropis*, 19(2), 166-170. https://doi.org/10.14710/jkt.v19i2.844

Sumardi, Rima, K., Farisi, S., and Widiastuti, E.L. (2021). Probiotic candidate proteolytic *Bacillus* sp. collected from Mangrove of Margasari, Lampung. *Indonesian Journal of Forestry Research*, 8(1), 73-84. http://repository.lppm.unila.ac.id/id/eprint/32476

Setyawati, T.R., Yanti, A.H., and Kurniatuhadi, R. (2021). identification and probiotic potency screening of cellulolytic bacilli isolated from fecal pellets and gastrointestinal tract of nypa palm worm (*Namalycastis rhodochorde*), West Kalimantan, Indonesia. *Jurnal Biologi Indonesia*, 17(2), 189-195, DOI: 10.47349/jbi/17022021/189.

Yanti, A.H., Setyawati, T.R, and Kurniatuhadi, R. (2017). Potential probiotic microflora isolated from coelom fluid, gastrointestinal fluid, and fecal pellets of nipah worm (*Namalycastis rhodochorde*) Research Report on Novice Educators Pontianak Tanjungpura University.

Yanti, A.H., Setyawati, T.R., Kurniatuhadi, R., (2018), Mikroflora probiotik potensial yang diisolasi dari cairan coelom, gastrointestinal dan feses cacing nipah (*N. rhodochorde)*, Laporan Hasil Penelitian. Universitas Tanjungpura.

Yanti, A.H., Setyawati, T.R, and Kurniatuhadi, R. (2020). Isolation and characterizationof lactid acid bacteria from fecal pellets, coelomic fluid, and gastrointestinal tract of nypa worm (Namalycastis rhodochorde) from West Kalimantan, Indonesia. Biodiversitas, 21(10), 4726-4731. **DOI** https://doi.org/10.13057/biodiv/d211036

Yi, K.N., Hodson, M., Hewavitharana, A., Bose, U., Shaw, P.N., and Fuerst, J. (2014). Effects of salinity on antibiotic production in sponge-derived salinispora Actinobacteria. *Journal of Applied Microbiology*, 117(1). DOI:10.1111/jam.12507.

Wolanksi, E. and Elliott, M. (2015). *Estuarine Ecohydrology: An Introduction, 2nd edition*. Elsevier Science, Amsterdam.