P-ISSN: 2355-729X
E-ISSN: 2614-5014

EFFECT OF TYPE OF DILUENT ON THE GROWTH OF BACTERIAL COLONIES *Bacillus sp.* ON THE ISOLATION PROCESS IN BACTERIAL CULTURE

Riko Darmawan*, Indra Wirawan, Maria Agustini

Aquaculture Study Program, Faculty of Agriculture, Dr. Soetomo University

*Corresponding author: Rikod1212@gmail.com

Manuscript Recived: 16 February 2025
Revision Accapted: 22 April 2025

ABSTRACT

Bacillus sp. are bacteria that play an important role in shrimp farming, especially in maintaining the balance of the pond ecosystem and as probiotics. This study aims to determine the effect of the type of diluent on the number of *Bacillus sp.* colonies formed in the isolation process and determine the most effective diluent. Three types of diluents were used, namely distilled water, NaCl solution, and infusion water (NaCl 0.9%). This study used a completely randomized design (CRD) with 9 replicates in each treatment. The data obtained were analyzed using one-way ANOVA to determine the significance of differences between treatments, followed by Tukey test and LSD 5%. The results showed that distilled water produced the highest number of colonies (1711.56), followed by infusion water (1222) and NaCl solution (771.44). The ANOVA test showed highly significant differences ($p<0.05$) between treatments, with Tukey and LSD 5% test results supporting these findings. 0.9% infused water was proven to be an effective diluent for the isolation of *Bacillus sp.* which provides an accurate picture of the bacterial population, because growth is optimal and remains controlled. This study recommends the use of infusion solution in the process of bacterial isolation to be applied in pond culture, in order to improve water quality through the utilization of probiotic bacteria.

Keywords: *Bacillus sp.*, Colonies, Diluent, Isolation

INTRODUCTION

Bacillus bacteria belong to a genus that is essential in various fields, including the food industry, agriculture, pharmaceuticals, and scientific research. Additionally, *Bacillus* is commonly used in shrimp aquaculture due to its role in maintaining the balance of pond ecosystems and serving as a probiotic. This bacterium has the ability to inhibit the growth of pathogenic bacteria and improve water quality. As previously discussed by Kuebutornye *et al.* (2019), *Bacillus* as a probiotic exhibits superior characteristics compared to other probiotics, including its ability to produce spores and metabolites that effectively combat various pathogenic microbes. The growth of *Bacillus* bacterial colonies is significantly influenced by various environmental factors, one of which is the type of diluent used during the bacterial isolation process.

The diluent plays a crucial role in reducing microbial concentration, facilitating the dispersion of bacterial cells on the medium, and preventing contamination during inoculation. Microorganisms can grow and develop under specific conditions, which is why different

P-ISSN: 2355-729X
E-ISSN: 2614-5014

diluents are used (Widiastiti *et al.*, 2019). However, the type of diluent used can significantly affect bacterial cell viability and colony formation. Some commonly used diluents in the isolation process include sterile distilled water, aquades, saline (physiological salt solution), and phosphate buffer. The appropriate diluent helps maintain the osmotic stability of bacterial cells and supports optimal conditions for colony growth. According to a study by Jones and Patel (2021), sterile saline supports bacterial cell osmotic balance, thereby enhancing the viability and colony growth of *Bacillus sp.* during the isolation process.

Additionally, the cleanliness and sterility of the diluent are crucial factors. Contamination in the diluent can lead to inaccurate isolation results. The selection of an appropriate diluent not only affects the quantity and quality of the formed colonies but also plays a vital role in determining the success of *Bacillus sp.* isolation. Therefore, it is essential to understand the effects of different types of diluents on bacterial colony growth, particularly in the isolation process of *Bacillus sp.* in pure culture.

MATERIAL AND METHOD

Method

This study was conducted at CV Sukses Indah Prima from November 10 to 24, 2024. Three types of diluents were used: NaCl solution, infusion water (0.9% NaCl), and distilled water. The research method employed a Completely Randomized Design (CRD) with nine replications for each treatment. The number of treatments and replications was determined using the following formula:

$$(t-1)(n-1) \geq 15 \qquad (1)$$

Where:

t = Number of treatments

n = Number of replications

Each treatment was diluted 11 times. The treatments were as follows:

1. Treatment A: Dilution using NaCl solution, 900 µl = 0.9 ml
2. Treatment B: Dilution using infusion water (0.9% NaCl), 900 µl = 0.9 ml

Treatment C: Dilution using distilled water (aquades), 900 µl = 0.9 ml

Materials and Equipment

This study used three types of diluents: NaCl solution, infusion water (0.9% NaCl), and distilled water. The media used were TSA and TCBS. *Tryptic Soy Agar* (TSA) is a commonly used growth medium for isolating and counting bacterial colonies from various microbial species, including *Bacillus sp.* Meanwhile, *Thiosulfate Citrate Bile Salts Sucrose* (TCBS) agar is a selective medium specifically designed to detect and isolate *Vibrio sp.* The *Bacillus sp.* bacterial samples were obtained from a pre-prepared culture. The equipment used included sterile test

P-ISSN: 2355-729X
E-ISSN: 2614-5014

tubes, micropipettes, microchips, Petri dishes, a spreader, a Bunsen burner, and an incubator.

Dilution Procedure

A total of 900 µl of each diluent was added to a sterile test tube. Then, 100 µl of the *Bacillus sp.* bacterial sample was aseptically inoculated into the first tube (10^{-1} dilution) at a 1:9 ratio. The suspension was mixed until homogeneous, then 100 µl was taken using a micropipette and aseptically transferred to the next tube (10^{-2} dilution). This process was repeated until the 10^{-11} dilution, ensuring thorough mixing at each step and using a different microtip for each dilution level. Aseptic principles were strictly maintained throughout the procedure.

Incubation and Observation

The diluted suspension was inoculated into the appropriate growth media and incubated at 30°C for 24 hours. The resulting *Bacillus sp.* bacterial colonies were observed and counted for further analysis.

The bacterial isolation process was carried out using the serial dilution method, and colony counting was performed using the colony-forming unit (CFU) method. Data were analyzed using one-way ANOVA, followed by Tukey HSD and LSD 5% tests to determine significant differences between treatments.

RESULTS AND DISCUSSION

Growth of *Bacillus sp.* Colonies

Based on the research results on the effect of different diluents on the growth of *Bacillus sp.* colonies during the bacterial isolation process, the total bacterial count and average colony growth varied among the three treatments: NaCl solution, 0.9% infusion water, and distilled water (aquades)

Table 1. Growth of *Bacillus sp.* bacterial colonies.

Treatment	Bacteria Total	Average	Standard Deviation
NaCl Solution	6,943	771.44	225.17
0.9% Infusion Water	10,998	1,222	284.58
Aquades	15,404	1,711.56	483.19

The research results indicate that distilled water (aquades) produced the highest average colony count (1,711.56), followed by 0.9% infusion water (1,222) and NaCl solution (771.44). The highest standard deviation was observed in distilled water (483.19), indicating greater variability in colony counts compared to the other diluents.

P-ISSN: 2355-729X
E-ISSN: 2614-5014

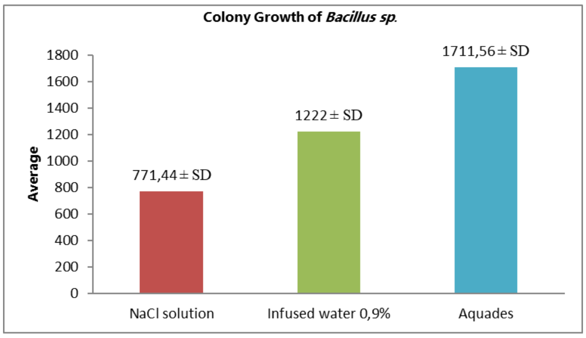

Figure 1. Graph of *Bacillus sp. colonies growth*

The graph of Bacillus sp. colony growth (Figure 1) shows that distilled water (aquades) supports higher colony growth compared to infusion water and NaCl. However, the excessive number of colonies in aquades makes enumeration difficult, reducing the accuracy of the count.

Observations after 24 hours on TSA media (Figure 2) revealed differences in colony characteristics as follows:.

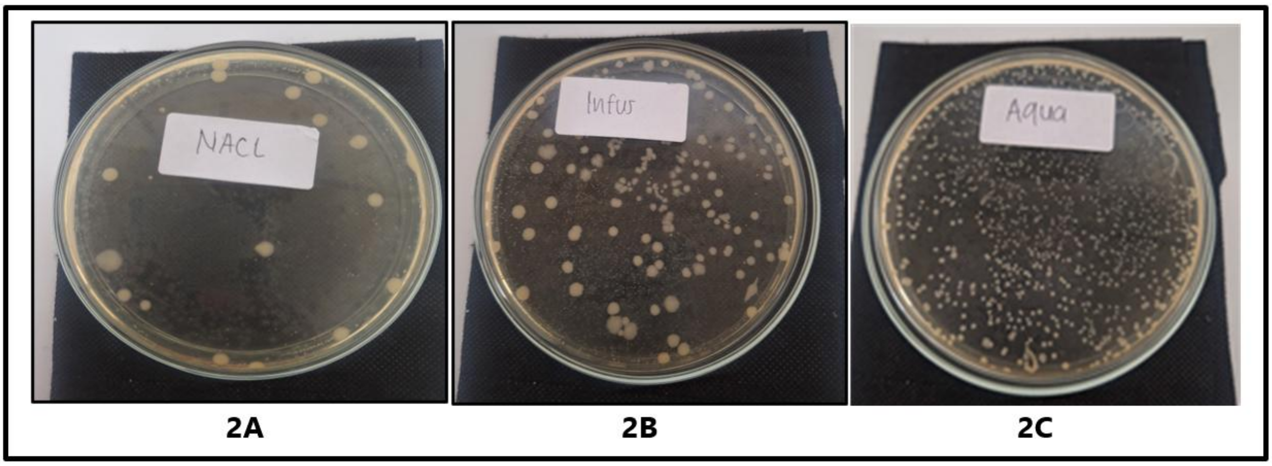

Figure 2. *Bacillus sp.* colonies on TSA Media. (2A) *Bacillus sp.* colonies in NaCl solution; (2B) *Bacillus sp.* colonies in infusion water; (2C) *Bacillus sp.* colonies in aquades.

Based on the image above, characteristics of *Bacillus sp.* colonies for each diluent.

2A. NaCl Solution: Round-shaped colonies, larger in size, scattered, and the least in number.

2B. 0.9% Infusion Water: Smaller, denser colonies, and more abundant than in NaCl.

2C. Aquades: Small colonies, widespread, and extremely numerous, often overlapping.

To ensure that only *Bacillus sp.* colonies grew on TSA media, a *Vibrio sp.* screening was conducted using TCBS media. The presence of *Vibrio sp.* was not allowed, as it could affect the research results. If *Vibrio sp.* was detected, the *Bacillus sp.* colonies on TSA would be considered invalid. However, throughout this study, no *Vibrio sp.* growth was observed on TCBS media, confirming that the colonies growing on TSA were exclusively *Bacillus sp.* without contamination.

Based on the ANOVA variance analysis, the results showed an F-value of 16.347 with $p < 0.001$, indicating a highly significant difference ($p < 0.05$) among treatments. This suggests that the type of diluent (NaCl, infusion water, and distilled water) has a significant effect on the growth of *Bacillus sp.* colonies.

P-ISSN: 2355-729X
E-ISSN: 2614-5014

After ANOVA confirmed a significant difference, a 5% LSD test and Tukey HSD test were conducted to determine the extent of differences between treatments in influencing the growth of *Bacillus sp.* colonies.

Table 2. Tukey HSD test for *Bacillus sp.* colony growth

Treatment	N	Subset for alpha = 0,05		
		1	2	3
P1	9	771,44[a]		
P1	9		1222[b]	
P3	9			1711,56[c]
Sig.		1000	1000	1000

Based on the table above, the post-hoc test results indicate that:

a. P1 (NaCl) has an average colony count of 771.44 and falls into Subset 1.
b. P2 (Infusion water) has an average colony count of 1222 and falls into Subset 2.
c. P3 (Distilled water) has an average colony count of 1711.56 and falls into Subset 3.

The three treatments showed significant differences as each fell into a different subset ($p < 0.05$). P1 was significantly different from P2 and P3, and P2 was also significantly different from P3. Distilled water (P3) resulted in the highest colony count, while NaCl (P1) had the lowest. The significance value (Sig = 1.000) within each subset indicates that the differences between treatments are significant at a 95% confidence level.

Effect of Diluent Type on *Bacillus sp.* Colony Growth.

Effect of NaCl on Bacillus sp. Colony Growth.

NaCl solution is commonly used in microbiological applications. However, in this study, 0.95% NaCl resulted in the lowest *Bacillus sp.* colony count compared to infusion water and distilled water. This occurs due to the osmotic pressure effect, which causes bacterial cell dehydration and inhibits *Bacillus sp.* growth. The concentration of NaCl in the diluent significantly affects bacterial growth, as an increase beyond physiological levels can induce osmotic stress, limiting cell proliferation.

A study by Budiharjo *et al.* (2017) demonstrated that increasing NaCl concentrations can affect enzymatic activity and the growth of halophilic bacteria. Similarly, research conducted by Amalia *et al.* (2016) found that NaCl concentrations of 15%-30% inhibited the growth of *Staphylococcus aureus*,

P-ISSN: 2355-729X
E-ISSN: 2614-5014

aligning with findings that higher NaCl levels also negatively impact *Bacillus sp.* growth.

The Effect of 0.9% Infusion Water on Bacillus sp. Colony Growth.

Although the concentration difference between 0.95% NaCl and 0.9% NaCl may seem small, its impact on the growth of *Bacillus sp.* is quite significant. Infusion water with 0.9% NaCl supports moderate colony growth, making it ideal for isolation and enumeration as it is neither too low nor excessive. This concentration provides a balanced environment, promoting the growth of *Bacillus sp.* without causing overpopulation.

This is consistent with the study conducted by Astriani and Feladita (2022), which found that a 0.9% physiological saline solution is the best medium for maintaining bacterial isolate viability. Similarly, the findings of Li *et al.* (2021) identify 0.9% NaCl as another standard physiological solution used for various purposes in handling cells or animal tissues to maintain cellular metabolic activity.

Effect of Aquades on the Growth of Bacillus sp. Colonies.

According to Khotimah *et al.* (2018), distilled water (aquades) is the primary solvent used in laboratory practices and is characterized as pure, clear, odorless, and tasteless. The absence of NaCl or other ions in distilled water leads to significantly high growth of *Bacillus sp.* colonies. The hypotonic environment created by distilled water causes bacterial cells to absorb water excessively. However, *Bacillus sp.* can adapt and reproduce rapidly due to minimal osmotic stress.

Nevertheless, excessive growth can cause colonies to overlap, making isolation and colony enumeration challenging. This is consistent with the findings of Azizah and Soesetyaningsih (2020), who reported that BPW diluent and 0.85% physiological saline resulted in more consistent colony counts compared to distilled water, which tended to produce highly variable colony counts.

Effect of NaCl Concentration on Bacillus sp. Colony Growth.

The growth of *Bacillus sp.* colonies is influenced by environmental factors, including osmotic conditions resulting from different NaCl concentrations in various diluents. These concentration differences play a role in the water balance within bacterial cells, which can either support or inhibit metabolic activity and bacterial cell division.

A 0.95% NaCl solution creates a hypertonic environment with a higher solute concentration outside the bacterial cells than inside, leading to osmosis that draws water out and increases the risk of plasmolysis. This condition can inhibit growth or even cause bacterial cell death if the osmotic pressure becomes too high. High NaCl concentrations

P-ISSN: 2355-729X
E-ISSN: 2614-5014

are often used to suppress bacterial growth by reducing intracellular water availability. This aligns with the study by Fatmariza *et al.* (2017), which states that when bacteria are exposed to a hypertonic solution with a higher concentration than inside the bacterial cells, fluid exits through the cytoplasmic membrane in a process called plasmolysis. Similarly, Ramadhann and Asmarani (2024) agree that hypertonic solutions cause fluid to be drawn out of bacterial cells, gradually leading to cytoplasmic shrinkage and eventual cell death. This phenomenon was observed in the 0.95% NaCl treatment, where colony growth significantly decreased compared to the infusion solution.

Isotonic solutions, such as infusion water containing NaCl at a concentration balanced with bacterial cell fluids, do not cause changes in cell size since there is no significant movement of water into or out of the cells. This condition provides optimal osmotic stability for the growth of *Bacillus sp.* without interference from external osmotic pressure. This is also supported by Rumpoko (2022), who stated that, in general, bacteria require an isotonic medium for their growth.

Aquades is classified as a hypotonic solution because it has a lower solute concentration than the fluid inside bacterial cells, causing water to enter the cells to balance osmotic pressure. This condition increases turgor pressure and, without protective mechanisms, can lead to cell lysis due to excessive water absorption. Fatmariza *et al.* (2017) stated that bacteria in a hypotonic solution might undergo plasmolysis, where the cells burst due to excessive water influx. However, *Bacillus sp.*, which is more resistant to osmotic pressure, can take advantage of this condition for faster growth in liquid media. Kusumaningtias and Susanto (2018) also noted that certain *Bacillus sp.,* which are aerobic, can survive by forming endospores—structures with minimal water content that are more resistant to unfavorable environmental conditions compared to active bacterial cells.

In this study, the 0.9% infusion water diluent produced more *Bacillus sp.* colonies than NaCl but fewer than aquades, which led to excessive colony density on TSA. Therefore, 0.9% infusion water was selected as the optimal option for more accurate colony enumeration.

Effectiveness of Diluents in Bacillus sp. Colony Isolation.

Effectiveness of NaCl Solution

0.95% NaCl solution is hypertonic and can influence the osmotic pressure within *Bacillus sp.* cells, thereby inhibiting their growth. This finding aligns with research by Li *et al.* (2021), which demonstrated that high NaCl concentrations suppress the growth of *Escherichia coli* and virulence-related

P-ISSN: 2355-729X
E-ISSN: 2614-5014

phenotypes. In this study, NaCl 0.95% significantly inhibited *Bacillus sp.* growth compared to other diluents, as indicated by the lower colony count. While effective in reducing unwanted bacterial contamination, high osmotic pressure slows down or limits *Bacillus sp.* cell division.

The use of this material presents several advantages and disadvantages that are crucial for its application. On the advantageous side, it is efficient in material usage, cost-effective in production, and particularly suitable for single-use applications, which helps to minimize the risk associated with reuse. However, there are notable disadvantages as well; the preparation time required can be a drawback, and if the material is not handled in a sterile manner, it poses a significant risk of contamination. Furthermore, this material may inhibit the growth of *Bacillus sp.*, potentially making its isolation less effective when compared to other diluents.

Effectiveness of 0.9% Infusion Water

The 0.9% Infusion Water has a NaCl composition close to isotonic conditions, creating an optimal environment for the growth of *Bacillus sp.* compared to NaCl solution and distilled water. This diluent allows bacterial growth in moderate amounts, neither restricting nor excessively promoting it, resulting in well-separated colonies without excessive density as seen with distilled water. Several studies have used 0.9% NaCl solution as a suspension medium for *Bacillus sp.*. Zaini *et al.* (2024) stated that *Bacillus subtilis* can survive in this solution with a certain turbidity level before experimentation. Abdurrasyid *et al.* (2024) also used 0.9% NaCl solution for endophytic bacteria before applying them to agar media.

In this study, 0.9% infusion water proved effective in supporting the growth of *Bacillus sp.* colonies as it creates a balance between bacterial growth inhibition and stimulation. Although its growth was not as dense as in distilled water, this condition allowed for more efficient colony isolation and counting. However, the use of 0.9% infusion water still has advantages and disadvantages that need to be considered in this research.

The 0.9% infusion water presents several advantages and disadvantages in its application. On the positive side, it is ready-to-use, practical, and produced under sterile conditions, which significantly minimizes the risk of contamination. Additionally, the appropriate sodium chloride (NaCl) concentration of 0.9% supports optimal growth of *Bacillus sp.* without inducing excessive osmotic pressure. However, the large-scale use of this infusion water can lead to increased production costs and may pose a contamination risk if reused without adhering to strict sterile procedures. Therefore, while the benefits of using 0.9% infusion water are evident, careful consideration must be given to its potential drawbacks in practical applications.

Effectiveness of Aquades

Aquades is a commonly used diluent in microbiological research due to its purity, minimizing the risk of contamination from other compounds. Khotimah *et al.* (2018) stated that aquades is distilled water free from impurities, making it a pure substance in laboratory settings.

As a neutral diluent with little to no dissolved substances, aquades supports the growth of *Bacillus sp.* colonies more effectively than NaCl solution, though not as optimally as infusion water. This aligns with the findings of Saraha *et al.* (2019), which showed that sterile aquades does not create an inhibition zone for Bacillus subtilis or Escherichia coli, indicating that it does not have an inhibitory effect on the growth of these bacteria.

However, without significant osmotic pressure as in NaCl solutions, aquades can lead to excessive colony growth, making the counting process challenging due to overlapping colonies. Therefore, although aquades is effective in supporting bacterial growth, its use in research still has advantages and disadvantages that need to be considered.

Aquades offers several advantages and disadvantages that are important to consider in its application. One of the primary advantages is that it is ready to use, practical, and cost-effective, particularly due to its availability in large volumes, which enhances efficiency in large-scale testing. However, there are notable disadvantages associated with its use. The risk of contamination is higher when compared to infusion water, which can lead to excessive growth of *Bacillus sp.* colonies, complicating the counting process. Additionally, the frequent need for repeated dilutions can consume significant time and effort.

Infusion water is the most effective diluent for isolating *Bacillus sp.* as it supports optimal growth without excessive colony density, though there is a risk of contamination if not used properly. NaCl solution can inhibit other bacteria, but a concentration of 0.95% is too high for *Bacillus sp.*, so a range of 0.85%–0.9% is recommended to maintain optimal growth. Meanwhile, distilled water produces a large number of colonies, but excessive density makes counting difficult, making it less ideal for analyses requiring accurate data. The choice of diluent depends on the research objectives and isolation need

CONCLUSION

The type of diluent significantly affects the colony growth of *Bacillus sp.* during the isolation process. While aquades produced the highest number of colonies, it often led to overgrowth

P-ISSN: 2355-729X
E-ISSN: 2614-5014

and difficulty in quantification. NaCl solution showed limited support for bacterial growth. In contrast, 0.9% infusion water provided optimal and controlled colony development, making it the most suitable diluent for accurate *Bacillus sp.* isolation. These findings suggest that 0.9% infusion water can serve as a suitable alternative diluent for *Bacillus sp.* isolation, particularly for applications related to improving water quality in aquaculture systems.

REFERENCES

Abdurrasyid, H., Mubarok, I., Mustikaningtyas, D., & Dewi, P. (2024). **Variasi Metode Ekstraksi Metabolit Sekunder Bakteri Endofit Daun Rambutan (Nephelium lappaceum L.) terhadap Pertumbuhan Escherichia coli dan Bacillus subtilis.** *Life Science*, *13*(2).

Amalia, A., Dwiyanti, R. D., & Haitami, H. (2016). **Daya Hambat NaCl terhadap Pertumbuhan Staphylococcus aureus.** *Medical Laboratory Technology Journal*, *2*(2), 42.

Astriani, R., & Feladita, N. (2022). **Perhitungan Angka Lempeng Total (Alt) Bakteri Pada Jamu Gendong Beras Kencur Yang Beredar Di Pasar Tradisional Way Kandis Dan Pasar Tempel Way Halim.** *Jurnal Analisis Farmasi*, *7*(2), 175–184.

Azizah, A., & Soesetyaningsih, E. (2020). **Akurasi Perhitungan Bakteri pada Daging Sapi Menggunakan Metode Hitung Cawan**. *Berkala Sainstek*, *8*(3), 75.

Budiharjo, R., Sarjono, P. R., & Asy'ari, M. (2017). **Pengaruh Konsentrasi NaCl Terhadap Aktivitas Spesifik Protease Ekstraseluler dan Pertumbuhan Bakteri Halofilik Isolat Bittern Tambak Garam Madura**. *Jurnal Kimia Sains Dan Aplikasi*, *20*(3), 142–145.

Fatmariza, M., Inayati, N., Analis Kesehatan, J., & Kemenkes Mataram, P. (2017). **Tingkat Kepadatan Media Nutrient Agar Terhadap Pertumbuhan Bakteri Staphylococcus Aureus**. *Jurnal Analis Medika Bio Sains*, *4*(2), 69–73.

Jones, P., & Patel, R. (2021). **The role of dilution media in bacterial colony isolation: A comparative study**. *Microbial Biotechnology*, *12*(7), 155–163.

Khotimah, H., Anggraeni, E. W., & Setianingsih, A. (2018). **Karakterisasi Hasil Pengolahan Air Menggunakan Alat Destilasi**. *Jurnal Chemurgy*, *1*(2), 34.

Kuebutornye, F. K. ., Abarike, E. ., & Lu, Y. (2019). **A review on the application of Bacillus as probiotics in aquaculture**. *Fish Shellfish Immunol*, *87*, 820–828.

Kusumaningtias, A., & Susanto, A. H. (2018). **E-Modul Direktorat Pembinaan SMA**. *E-Modul Biologi Kelas X*, 1–52.

Li, F., Xiong, X. S., Yang, Y. Y., Wang, J. J., Wang, M. M., Tang, J. W., Liu, Q. H., Wang, L., & Gu, B. (2021). **Effects of NaCl Concentrations on Growth Patterns, Phenotypes**

P-ISSN: 2355-729X
E-ISSN: 2614-5014

Associated With Virulence, and Energy Metabolism in Escherichia coli BW25113. *Frontiers in Microbiology*, *12*(August), 1–19.

Ramadhann, B. H., & Asmarani, Y. K. (2024). **Pengaruh Berkumur Larutan Air Garam terhadap Jumlah Koloni Streptococcus sobrinus pada Siswa SDN Keniten 2 Kabupaten Kediri.** *Bhakta Dental Journal*, *2*(1), 1–7.

Rumpoko, S. W. (2022). ***Fraksinasi Ekstrak Etanol Daun Jeruk Purut (Citrus hystrix D.C) Dengan Metode Kromatografi Cair Vakum (KCV) DAN Uji Aktivitas Bakteri Terhadap Bakteri Staphylococcus Epidermidis.*** Skripsi. Fakultas Sains, Teknologi, Dan Kesehatan. Universitas Sahid Surakarta: Surakarta

Saraha, N., Hastuti, U. S., & Lukiati, B. (2019). **Uji Daya Antibakteri Ekstrak Biji Pala Myristica fragans houtt Varietas Tidore 1 terhadap Bakteri Bacillus Subtilis dan Escherichia coli secara In Vitro serta Analisis Kandungan beberapa Senyawa Aktif Antibakteri**. *Jurnal Ilmu Hayat*, *3*(1), 13–21.

Widiastiti, I. G. A. A. M., Putra, I. W. W. P., Duniaji, A. S., & Darmayanti, L. P. (2019). **Analisis Potensi Beberapa Larutan Pengencer Pada Uji Antibakteri Teh Temu Putih (Curcuma zedoaria (Berg.) Roscoe) Terhadap Escherichia coli**. *Media Ilmiah Teknologi Pangan*, *6*(2), 117–125.

Zaini, N., Mayasari, U., & Amelia Nasution Rizki. (2024). **Uji Aktivitas Minyak Atsiri Daun Ekor Kucing (Acalypha hispida) Sebagai Disenfektan Alami Terhadap Bakteri Bacillus subtilis Dan Klebsiella pneumoniae Secara In-Vitro.** *Jurnal Biologi Makassar*, *9*(1), 87–96.

Yulianto, A. (2006). **Keanekaragaman Kepiting di Hutan Mangrove Desa Tungkal, Tanjung Jabung Barat, Jambi.** [Skripsi] Fakultas Perikanan. Bogor.

Zala, M., Sipai, S., Bharpoda, T., Patel, B. (2018). **Molluscan pests and their management : a review.** Agres. 7(2):126–132.