HAYATI
Journal of Biosciences

Vol. 32 No. 4, July 2025 1039-1052
DOI:10.4308/hjb.32.4.1039-1052
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

Check for updates

OPEN ACCESS

# The Gene Analysis and Probiotic Potential Characterization of *Pseudomonas alcaligenes* SG03

**Baso Manguntungi[1], Iman Rusmana[1], Apon Zaenal Mustopa[2*], Anja Meryandini[1,3]**

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680, Indonesia*
[2]*Research Center for Genetic Engineering, National Research and Innovation Agency (BRIN), Jakarta Pusat 10340, Inonesia*
[3]*Biotechnology Research Centre, Institut Pertanian Bogor. Jl. Kamper, Bogor 16680, West Java, Indonesia*

ARTICLE INFO

*Article history:*
Received January 21, 2025
Received in revised form March 18, 2025
Accepted March 21, 2025

*KEYWORDS:*
Characterization,
Evaluation,
Gene,
Probiotic,
*Pseudomonas alcaligenes* SG03

## ABSTRACT

AHPND causes up to 80% of shrimp mortality, severely impacting Indonesia's aquaculture. Rising antibiotic resistance necessitates sustainable alternatives. This study investigates the probiotic potential of *Pseudomonas alcaligenes* SG03, a bacterium with unique genomic traits, to address antibiotic resistance and promote animal health. Genome analysis revealed a 6.17 Mb genome with 4,446 protein clusters, including genes for carbohydrate metabolism (26.63%), amino acid metabolism (19.93%), and energy metabolism (10.88%). Key probiotic-related genes, such as acid resistance (rpoS, actP), salinity tolerance (gshA, cysK), antibiotic resistance (vanB, gyrA), and metal resistance (copZ, zwf), were identified. *In vitro*, assays evaluated probiotic properties under conditions mimicking the shrimp gastrointestinal tract. Osmo-tolerance tests showed optimal growth at 10% glucose, with a significant drop in viability at higher concentrations. Autoaggregation increased progressively, reaching 0.78 at 18 hours and 0.13 at 48 hours. Optimal growth occurred at 30°C, with a 2.5-fold increase in optical density compared to 25°C. NaCl tolerance peaked at 2% ($20.33\times10^{12}$ CFU at 48 hours), while bile salt tolerance was highest at 0.1% ($27.00\times10^{12}$ CFU at 48 hours). pH tolerance was optimal at pH 5 ($20.00\times10^{12}$ CFU at 48 hours). Antioxidant (53.00%) and anti-inflammatory (60.33%) activities peaked after 48 hours. Phenotypic antibiotic resistance was observed against Tetracycline, Amoxicillin, Cefixime, Streptomycin, and Chloramphenicol, with proteolytic activity but no lipolytic or hemolytic properties. These findings highlight *P. alcaligenes* SG03's potential as a probiotic in aquaculture to support shrimp health and reduce antibiotic dependency. Future studies should focus on *in vivo* validation and formulation development.



## 1. Introduction

Acute Hepatopancreatic Necrosis Disease (AHPND), caused by *Vibrio parahaemolyticus*, poses a severe threat to Indonesia's shrimp farming industry, which is among the largest in the world, contributing approximately 1.5 million tons annually. AHPND has resulted in significant economic losses, with mortality rates reaching up to 80% in shrimp ponds, particularly in regions such as Sulawesi and Java (Saputra *et al.* 2023). The pathogenicity of *V. parahaemolyticus* is closely linked to quorum sensing (QS). This mechanism enables bacterial communication and collective regulation of virulence gene expression, enhancing its ability to infect the hepatopancreas of shrimp. Current

* Corresponding Author
E-mail Address: azae.mustopa@gmail.com

control strategies rely heavily on antibiotics, with surveys indicating that up to 70% of shrimp farmers in Indonesia use antibiotics without proper oversight (Mizan *et al*. 2020; Lin *et al*. 2022). This practice has led to the detection of antibiotic residues in shrimp products and the environment, raising concerns about antimicrobial resistance and regulatory restrictions in international markets, such as those imposed by the EU and US (Mizan *et al*. 2020; Gupta *et al*. 2022).

Given these challenges, there is an urgent need for sustainable and environmentally friendly alternatives to antibiotics. One promising approach is the use of biocontrol agents with probiotic properties to combat pathogens by inhibiting quorum sensing (QS). Probiotics isolated from Indonesian aquatic environments can compete with *V. parahaemolyticus* for resources and produce anti-QS compounds, reducing the pathogen's virulence. Research has shown that applying probiotics in shrimp ponds can reduce infection rates by up to 60% (Gupta *et al*. 2022; Lubis *et al*. 2024). Among these probiotics, *Pseudomonas alcaligenes* has emerged as a promising biocontrol agent due to its unique ability to inhibit QS and its probiotic properties. Whole-genome sequencing (WGS) analysis has revealed that *P. alcaligenes* harbors two key genes encoding the enzymes AHL lactonase and AHL acylase, which degrades acyl-homoserine lactones (AHL), the chemical signaling molecules used by *V. parahaemolyticus* to regulate virulence gene expression (Fang *et al*. 2020; Zhang *et al*. 2023). By inactivating AHL, *P. alcaligenes* disrupt bacterial communication, reducing virulence factor expression and inhibiting biofilm formation. This prevents *V. parahaemolyticus* from establishing persistent infections, thereby reducing shrimp mortality (Reina *et al*. 2019; Rusmana *et al*. 2022).

In addition to its QS-inhibitory capabilities, *P. alcaligenes* acts as a probiotic by colonizing the shrimp gut, promoting beneficial microbiota, and inhibiting pathogenic growth. The bacterium produces antimicrobial compounds and secondary metabolites that enhance shrimp health. Studies have demonstrated that incorporating *P. alcaligenes* in shrimp farming ponds can increase shrimp growth rates by up to 30% and improve water quality, creating a more sustainable aquaculture environment (Fernandes *et al*. 2019; Phan *et al*. 2022; González-Palacios *et al*. 2020). Using *P. alcaligenes* as a probiotic offers a significant innovation in controlling AHPND caused by *V. parahaemolyticus* and reducing reliance on antibiotics. This dual approach not only supports shrimp health by enhancing immune responses and promoting beneficial microbiota but also suppresses pathogenic bacterial growth through QS inhibition. The implementation of *P. alcaligenes* in shrimp farming will reduce antibiotic use, lowering the risk of residues in shrimp products and the environment, and addressing antimicrobial resistance concerns (Gupta *et al*. 2022; Lubis *et al*. 2024). This innovation aligns with international market standards, such as EU and US regulations on antibiotic residues, enhancing the competitiveness of Indonesian shrimp products globally.

## 2. Materials and Methods

### 2.1. Genome Analysis

Isolation and sequencing genome preparation of *Pseudomonas alcaligenes* SG03 were conducted as described in previous studies. The raw data in the form of FastQ files obtained from base calling were processed using program tools on the Galaxy Europe10 server. The FastQ data underwent quality control (QC) using the Nanoplot program, followed by assembly processing using Flye. The assembly results were then polished to obtain higher-quality sequences using MEDAKA. Genome completeness was evaluated using Quast and BUSCO. Overall, draft assembly annotation was performed using PROKKA, RAST webserver, and eggNOG mapper. To resolve discrepancies between these tools, annotations were cross-validated, and the final annotation was determined based on consensus among the tools, prioritizing PROKKA for gene prediction and eggNOG for functional annotation. Specific annotation related to probiotic candidates was conducted following the method of Sylvere *et al*. (2023), predicting antibiotic resistance gene sequences using CARD and ResFinder 4.4.2. The ability to harbor virulence was determined through predictions from VirulenceFinder 2.0 (Kaktcham *et al*. 2025).

### 2.2. Osmo-Tolerances Assay

Zobell broth media was prepared by adding glucose at 4%, 6%, 8%, and 10% (with three repetitions) and then sterilized using an autoclave at 121°C for 15 minutes. A 100 µl bacterial isolate was taken using a sterile pipette and inoculated into 50 ml of the prepared media for each glucose concentration, with each treatment performed in separate test tubes. The inoculated cultures were then placed in an incubator set at 30°C and 37°C, with

shaking at 180 rpm for 48 hours. During the incubation period, 1 ml of sample was taken at 18 hours, 24 hours, and 48 hours using a sterile pipette to prevent cross-contamination. The optical density of the samples was measured using a UV-Vis spectrophotometer at a wavelength of 600 nm, and the absorbance values were recorded for each sample at the time of collection. As a positive control, bacterial cultures without added glucose were also prepared and measured simultaneously (Muche *et al*. 2023).

### 2.3. Uji Auto-Aggregation Assay

A 5 µL bacterial isolate was inoculated into 5 ml of Zobell broth media, then incubated at 30°C for 24 hours with shaking at 180 rpm to support bacterial growth. After the incubation period, the bacterial culture was inoculated into 1× PBS media at a concentration of 2% bacterial density (OD600 ≈ 0.8). The culture in PBS was then incubated at 30°C for 48 hours to facilitate further growth. Observations were made at 18, 24, and 48 hours. For growth analysis, samples were taken from the culture at each time point, and the optical density was measured using a UV-Vis spectrophotometer at a wavelength of 600 nm (Ekawati *et al*. 2024). The absorbance results were calculated using the following equation:

$$(\%)\ \text{Auto-aggregation} = \left[\frac{(A0 - A1)}{A0}\right] \times 100$$

Explanation: A1 = Absorbance of the sample at 18, 24, and 48 hours, and A0 = Absorbance at 0 hours.

### 2.4. Temperature Resistance Assay

A liquid culture with a volume of 500 µL was inoculated into the medium and incubated at four different temperatures, namely 25°C, 30°C, 37°C, and 42°C, for 48 hours. These temperatures were chosen to represent the range of environmental conditions in shrimp farming habitats, with 30°C being the optimal growth temperature for shrimp and 37°C and 42°C representing potential stress conditions. During the incubation, the culture was stirred at 180 rpm to ensure homogeneity and even nutrient distribution in the medium. Sampling was conducted at time intervals of 18 hours, 24 hours, and 48 hours. At each interval, samples were taken from each temperature treatment using a sterile pipette to prevent contamination. The optical density of each sample was measured using a UV-Vis spectrophotometer at a wavelength of 600 nm (Anita *et al*. 2018).

### 2.5. NaCl Resistance Assay

A 24-hour-old bacterial isolate grown in Zobell broth was inoculated into Zobell medium supplemented with NaCl at concentrations of 2%, 4%, and 6%. To perform serial dilution, 100 µL of the isolate culture was mixed with 900 µL of 0.85% NaCl to achieve a 10-1 dilution. Further serial dilutions were performed to reach a 10-10 dilution. Subsequently, 100 µL from each dilution was evenly spread onto the surface of the prepared Zobell agar medium. The cultured plates were incubated for 24, 48, and 72 hours. At each time interval, the colonies that grew were counted to determine the colony-forming units (CFU) per ml at each NaCl concentration. As a positive control, the isolate grown in Zobell broth without NaCl treatment was also analyzed. (Muche *et al*. 2023).

### 2.6. Anti-Inflammatory Assay

The assay was conducted by adding 500 µL of Bovine Serum Albumin (BSA) into a microtube, followed by the addition of 100 µL of bacterial supernatant. The mixture was then incubated at room temperature for 10 minutes to allow initial interaction between the BSA and components in the supernatant. After incubation at room temperature, the microtube was transferred to an incubator at 51°C for 20 minutes to continue the anti-inflammatory reaction. As a control, growth media without the bacterial isolate was used to ensure no reaction occurred due to external factors. A positive control was prepared using diclofenac in the same reaction mixture. After the incubation phase, the absorbance of each reaction was measured at a wavelength of 660 nm using a Spectrophotometer. Measurements were performed in triplicate for each sample to ensure consistency and accuracy of the data obtained (Garbi *et al*. 2017). The absorbance value was calculated using the equation:

$$(\%)\ \text{Inhibition} = \left[\frac{(A1 - A2)}{A0}\right] \times 100$$

Explanation: A1 = Sample Absorbance, A2 = Sample Control Absorbance, and A0 = Positive Control Absorbance.

### 2.7. Antioxidant Assay with DPPH (*1,1-Diphenyl-2-picrylhydrazyl*)

The antioxidant assay was conducted by adding 100 µL of DPPH solution (0.2 mM) to a microplate, followed by adding 100 µL of bacterial supernatant. The

microplate was then incubated for 30 minutes in the dark to prevent light from affecting the stability of DPPH. A blank control (DPPH solution without supernatant) was used to normalize the absorbance values. As a positive control, vitamin C at a concentration of 100 ppm was also added with the same treatment. After the incubation period, the absorbance of each reaction was measured at a wavelength of 517 nm using the Multiskan Go Microplate Spectrophotometer. This measurement was performed to determine the antioxidant activity of the bacterial supernatant by comparing the absorbance values between the sample and the positive control (Fidien *et al*. 2021; Hertati *et al*. 2023; Nurjannah *et al*. 2024). The absorbance values were calculated using the following equation:

$$(\%)\ \text{Inhibition} = \left[\frac{(A0 - A1)}{A0}\right] \times 100$$

Explanation: A1 = Sample Absorbance, A2 = Sample Control Absorbance, and A0 = Positive Control Absorbance.

## 2.8. pH Resistance Assay

The bacteria cultured in Zobell media for 24 hours were inoculated into Zobell media with pH variations of 2, 3, 4, and 5. Each pH variation was prepared by adding the appropriate buffer solution to achieve the desired pH. After inoculation, the culture was incubated at 30 °C for 24 hours with shaking at 180 rpm to ensure even growth. The Total Plate Count (TPC) method counted the number of colonies formed. A 100 µL sample of the bacterial culture was taken and mixed with 900 µL of 0.85% NaCl to obtain a 10-1 dilution. Further serial dilutions were performed to reach 10-10. Then, 100 µL from each dilution was spread evenly on the surface of Zobell agar media. After spreading, the culture was incubated for 24, 48, and 72 hours. The growing colonies were observed and counted to determine the colony-forming units per ml at each pH variation. A positive control was conducted using an isolate cultured in Zobell broth without any treatment (Sylvere *et al*. 2023).

## 2.9. Bile Salt Resistance Assay

The 24-hour-old bacterial isolate grown in Zobell media was inoculated into Zobell media containing varying concentrations of bile salt, specifically 0.1%, 0.3%, and 0.5%. Each media variation was prepared by adding bile salt to previously sterilized Zobell Marine Agar media. After inoculation, the culture was incubated at 30°C for 24 hours with shaking at 180 rpm to ensure even bacterial growth. For observation, the Total Plate Count (TPC) method was used to count the number of colonies formed. A 100 µL sample of the bacterial culture was taken and mixed with 900 µL of 0.85% NaCl to obtain a 10-1 dilution. Further serial dilutions were performed to reach a 10-10 dilution. Each dilution was then spread evenly on the surface of prepared Zobell Marine Agar agar media, with 100 µL applied to each. After spreading, the culture was incubated at the same temperature for 24, 48, and 72 hours. The colonies were counted at intervals to determine the colony-forming units per ml at each bile salt concentration. A positive control was performed using an isolate grown in Zobell Marine broth without bile salt treatment to compare the growth results (Sylvere *et al*. 2023).

## 2.10. Antibiotic Resistance Assay

A 5 ml bacterial isolate, aged for 24 hours, was taken and resuspended in Zobell agar media until homogeneous. The bacterial suspension was then poured into pre-prepared Petri dishes and gently mixed to ensure even distribution. Once the media solidified, antibiotic discs were placed on the surface of the agar. The discs used included Tetracycline (30 µg/disc), Penicillin (10 µg/disc), Amoxicillin (30 µg/disc), Cefixime (5 µg/disc), Streptomycin (10 µg/disc), and Chloramphenicol (30 µg/disc), with sufficient spacing to prevent overlapping inhibition zones. The Petri dishes were then incubated for 48 hours at 37°C. After the incubation period, the Petri dishes were examined to observe the presence of clear zones around the antibiotic discs. These clear zones indicate areas where bacterial growth was inhibited, demonstrating the sensitivity of the bacteria to the antibiotics (Akinyemi *et al*. 2024).

## 2.11. Proteolytic, Lipolytic, and Hemolytic Activity Assay

The bacterial isolate was tested using Zobell Marine Agar media enriched with 1% skim milk to evaluate protease enzyme activity. After preparing the media, the bacterial isolate was evenly inoculated onto the agar surface. The Petri dishes were then incubated for 48 hours at 30°C to allow bacterial growth and enzymatic activity. During the incubation period, bacteria that produce protease enzymes degrade the protein in the skim milk, creating a clear zone around the colonies that indicates proteolytic activity. After incubation, the Petri dishes were inspected for clear or transparent

zones around the colonies (Ekawati *et al*. 2024). For lipolytic activity testing, the bacterial isolate was tested on Zobell Marine Agar media with the addition of Tween 80. The isolate was spot-inoculated at the base of the media surface and then incubated at 30°C for 48 hours. The presence of clear zones indicates that the isolate can degrade lipids. For hemolysis assay, the bacterial isolate was streaked onto blood agar media and incubated at 30°C for 48 hours. Positive controls were *E. coli* and *Staphylococcus aureus* ATCC, while the negative control was a lactic acid bacteria strain from BRIN that had been tested. Observations were made to detect the formation of zones, including clear zones (β-hemolysis), green zones (α-hemolysis), or no zones around the bacterial colonies (γ-hemolysis) (Tran *et al*. 2023).

## 3. Results

Genome analysis data of *P. alcaligenes* SG03 began with analyzing eggNOG annotation results, Clusters of Orthologous Groups of proteins (COGs), and Kyoto Encyclopedia of Genes and Genomes (KEGG). The results of the eggNOG analysis are displayed in Table 1, COG in Figure 1, and KEGG in Figure 2.

From a total genome size of 6.17 Mb, 4,446 clusters of orthologous groups of proteins were identified. The largest cluster consisted of genes with unknown functions, comprising 1,108 genes (24.92% of the total). The second-largest cluster was associated with transcription, containing 426 genes (9.58%), followed by a cluster related to cell motility, with 396 genes (8.91%). The high proportion of transcription-related genes suggests a robust regulatory capacity, which may enable the strain to adapt to varying environmental conditions. Additionally, the significant number of genes associated with cell motility could enhance its ability to colonize host environments, a critical trait for probiotic functionality.

The results of the KEGG analysis showed that there were 597 genes in total across various pathway clusters, with the carbohydrate pathway containing the highest number of genes, specifically 159, which accounts for approximately 26.63%. Other pathway clusters that were quite numerous were related to amino acid metabolism, comprising 119 genes (around 19.93%), and those related to energy metabolism, comprising 65 genes (around 10.88%) of the total gene count. The dominance of carbohydrate metabolism genes aligns with the strain's potential to thrive in environments where carbohydrates are abundant, such as the gastrointestinal tract of hosts. The COG and KEGG analyses collectively highlight the presence of genes involved in key probiotic functions, such as carbohydrate metabolism (G), amino acid metabolism (E), and cellular defense (V). For example, the mapping of genes to pathways like lactate fermentation and fatty acid biosynthesis suggests mechanisms for energy production and membrane stability, which are crucial for survival under stress conditions. These pathways also play a role in producing metabolites that can benefit the host, such as short-chain fatty acids (SCFAs), which are known to modulate host immune responses and gut health.

Table 1. Results of eggNOG annotation related to probiotic characteristic genes of *P. alcaligenes* SG03

| Gene cluster and function | Name and gene product | Locus/size |
|---|---|---|
| Acid resistance (low pH) | rpoS (RNA polymerase, sigma S (sigma 38) factor | 934.600-935.601 (1.001 bp) |
| | actP (acetate/glycolate/cation symporter) | 1212217-1213884 (1.667 bp) |
| | Ndh (NADH:quinone oxidoreductase) | 4.514.096-4.515.397 (1.301 bp) |
| | gltS (Sodium/glutamate symporter) | 2.813.569-2.814.783 (1.214 bp) |
| | arcA (Arginine deiminase) | 5.983.238-5.984.488 (1.250 bp) |
| | trpE (Anthranilate synthase component 1) | 5.019.688-5.021.175 (1.487 bp) |
| | glnA (Glutamine synthetase) | 6.038.168-6.039.604 (1.436bp) |
| | ompR (DNA-binding transcriptional dual regulator OmpR) | 5.955.222-5.955.953 (731 bp) |
| Salinity resistance | gshA (Glutamate--cysteine ligase) | 5.958.975-5.960.561(1.586 bp) |
| | cysK (Cysteine synthase) | 1.495.129-1.496.043 (914 bp) |
| | mutS (DNA mismatch repair protein MutS | 936.525-939.104 (2.579 bp) |
| | vanB (Vanillate O-demethylase oxidoreductase) | 2.379.118-2.380.062 (944 bp) |
| Resistance antibiotic gene | gyrA (DNA gyrase subunit A) | 1.375.508-1.378.270 (2.762 bp) |
| | gyrB (DNA gyrase subunit B) | 5.693.063-5.695.483 (2.420 bp) |
| Metal resistance | copZ (Copper chaperone CopZ) | 207.482-207.679 (197 bp) |
| | Zwf (Glucose-6-phosphate 1-dehydrogenase) | 2964184-2965290 (1.106 bp) |
| Active removal of stress | spG (="(E)-4-hydroxy-3-methylbut-2-enyl-diphosphate synthase (flavodoxin) | 692.736-693.848 (1.112 bp) |
| | fpr (Ferredoxin/flavodoxin---NADP+ reductase) | 949.282-950.019 (737 bp) |

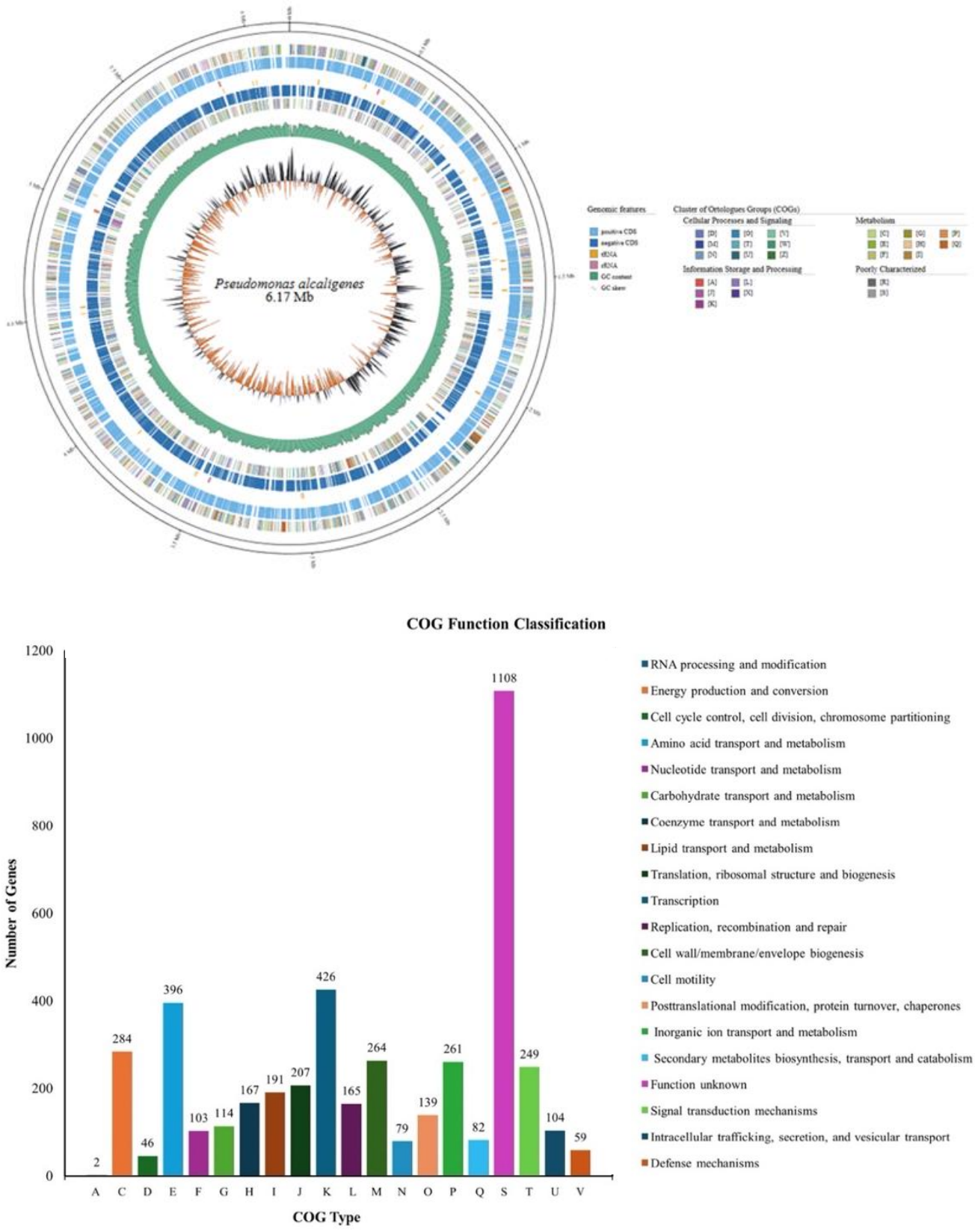


Figure 1. Results of analysis of clusters of orthologous groups of proteins (COGs) *P. alcaligenes* SG03

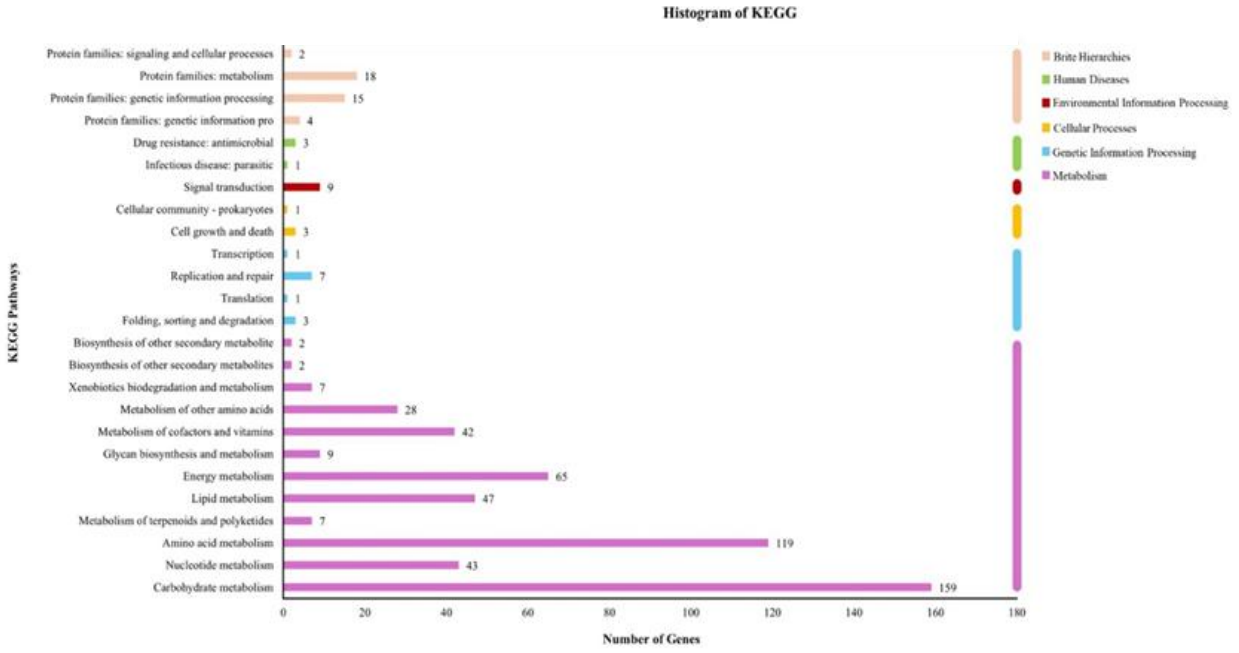


Figure 2. Analysis results of the kyoto encyclopedia of genes and genomes (KEGG) *P. alcaligenes* SG03

Table 1 lists several genes in *P. alcaligenes* SG03 associated with probiotic traits, including acid resistance, salinity tolerance, antibiotic resistance, metal resistance, and stress removal. Acid Resistance Genes: Genes such as rpoS (RNA polymerase sigma factor, 1,001 bp) and arcA (arginine deiminase, 1,250 bp) play critical roles in acid tolerance. For instance, arcA is involved in arginine metabolism, which not only aids in acid resistance but also contributes to energy production through the arginine deiminase pathway. This pathway converts arginine to ornithine, ammonia, and $CO_2$, generating ATP and helping the cell maintain pH homeostasis under acidic conditions. Salinity Tolerance Genes: Genes like gshA (glutamate-cysteine ligase, 1,586 bp) and cysK(cysteine synthase, 914 bp) are involved in the synthesis of glutathione, a key molecule in oxidative stress defense and osmoprotection. Glutathione helps mitigate the effects of high salinity by stabilizing cellular proteins and membranes, which is essential for survival in saline environments. Antibiotic Resistance Genes: The presence of gyrA (DNA gyrase subunit A, 2,762 bp) and gyrB (DNA gyrase subunit B, 2,420 bp) suggests mechanisms for DNA repair and replication under stress. However, the strain's sensitivity to tetracycline, amoxicillin, cefixime, streptomycin, and chloramphenicol (Table 2) indicates a low risk of transferring antibiotic-resistance genes, making it a safer probiotic candidate. Metal Resistance Genes: Genes such as copZ (copper chaperone, 197 bp) and zwf (glucose-6-phosphate dehydrogenase, 1,106 bp) suggest mechanisms for detoxifying heavy metals, which could enhance survival in polluted environments. For example, copZ helps sequester excess copper ions, preventing cellular damage. Stress Removal Genes: Genes like spG (flavodoxin synthase, 1,112 bp) and fpr (ferredoxin-NADP+reductase, 737 bp) are involved in oxidative stress responses, further supporting the strain's resilience. These genes play a role in neutralizing reactive oxygen species (ROS), which are byproducts of metabolic processes and environmental stressors.

The osmotolerance test, conducted using glucose concentrations of 4%, 6%, 8%, and 10%, revealed that the highest cell density was achieved at 20% glucose, except at the 18-hour mark (density = 0.99). As glucose concentration increased, cell density decreased (Figure 3). This suggests that *P. alcaligenes* SG03 is better adapted to moderate osmolarity, which is consistent with the conditions found in many host environments. Compared to other probiotic strains, such as *Lactobacillus* spp., which typically thrive at lower osmolarity, *P. alcaligenes* SG03 demonstrates a broader tolerance range, making it suitable for diverse applications.

The autoaggregation test on *P. alcaligenes* SG03 isolate showed aggregation ability along with the incubation time. After 18 hours of incubation, the density reached 0.78, which increased to 0.82 at 24 hours, with a significant rise at 48 hours, reaching 0.13. The autoaggregation data for *P. alcaligenes* SG03 isolate can be seen in Figure 4.

Table 2. Antibiotic resistance testing, proteolytic, lipolytic, and hemolytic activities of *P. alcaligenes* SG03

| Antibiotic | Clear zone (mm) / hours | | | Antibiotic activity | Resistance classification |
|---|---|---|---|---|---|
| | Ke-18 | Ke-24 | Ke-48 | | |
| Tetracyclin | 18,55 | 20,55 | 25,33 | Very strong | Sensitive |
| Penicillin | 0,00 | 0,00 | 0,00 | No activity | Resistance |
| Amoxicillin | 19.24 | 22,32 | 25,54 | Very strong | Sensitive |
| Cefixime | 18.62 | 20,65 | 22,33 | Very strong | Sensitive |
| Streptomycin | 20,43 | 22,62 | 25,62 | Very strong | Sensitive |
| Chloramphenicol | 18,66 | 22,66 | 25,62 | Very strong | Sensitive |

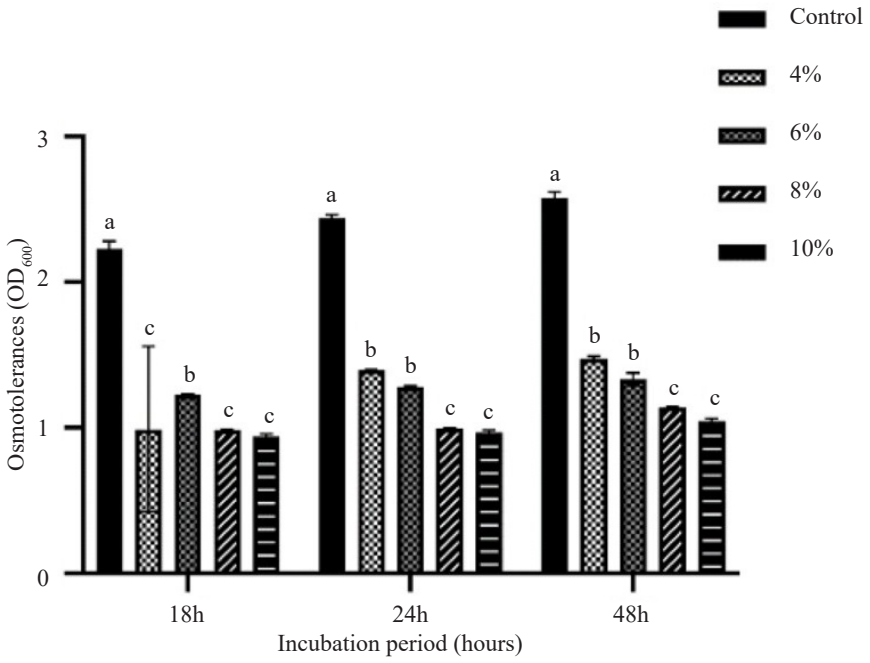


Figure 3. Results of osmotolerance test of *P. alcaligenes* SG03 isolates with various glucose concentrations. The average values with different letters indicate significant differences ($p<0.05$) based on the DMRT test

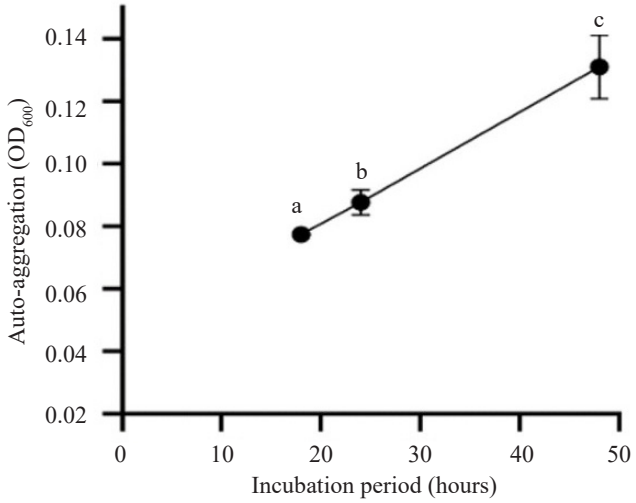


Figure 4. Autoaggregation test of *P. alcaligenes* SG03 isolates with incubation time. The average values with different letters indicate significant differences ($p<0.05$) based on the DMRT test

The temperature tolerance test, conducted at 25°C, 30°C, 37°C, and 42°C, identified 30°C as the optimal temperature, with cell densities of 1.27 (18 hours), 1.47 (24 hours), and 1.74 (48 hours) (Figure 5). This range is suitable for probiotics used in shrimp farming, where water temperatures typically range from 25°C to 35°C. The strain's ability to thrive at 30°C aligns with the environmental conditions of aquaculture systems, suggesting its potential as a probiotic in this field.

The resistance test to NaCl concentration was conducted with various concentrations of 2%, 4%, and 6%, along with different incubation times. Based on the results, it was observed that as the NaCl concentration increased, the CFU of *P. alcaligenes* SG03 decreased. The 2% NaCl concentration resulted in the highest CFU: $15.33\times10^{12}$ (18 hours), $18.33\times10^{12}$ (24 hours), and $20.33\times10^{12}$ (48 hours). The resistance data for the *P. alcaligenes* SG03 isolate can be seen in Figure 6.

The NaCl resistance test showed that *P. alcaligenes* SG03 exhibited the highest colony-forming units (CFU) at 2% NaCl, with values of $15.33\times10^{12}$ (18 hours), $18.33\times10^{12}$ (24 hours), and $20.33\times10^{12}$ (48 hours). As NaCl concentration increased, CFU decreased (Figure 6). Similarly, the bile salt resistance test revealed that 0.1% bile salt yielded the highest CFU, with values of $15.67\times10^{12}$ (18 hours), $21.33\times10^{12}$ (24 hours), and $27.00\times10^{12}$ (48

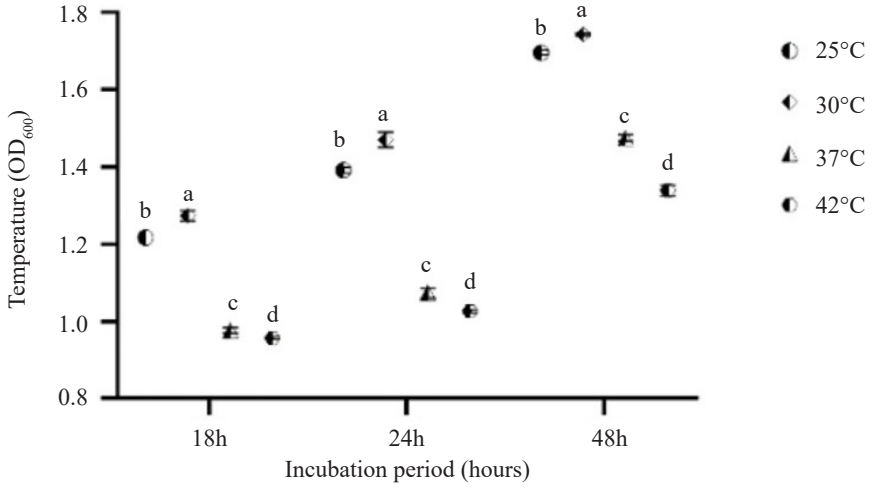


Figure 5. Temperature resistance test of *P. alcaligenes* SG03 isolates with various temperatures and incubation times. The average values with different letters indicate significant differences ($p<0.05$) based on the DMRT test

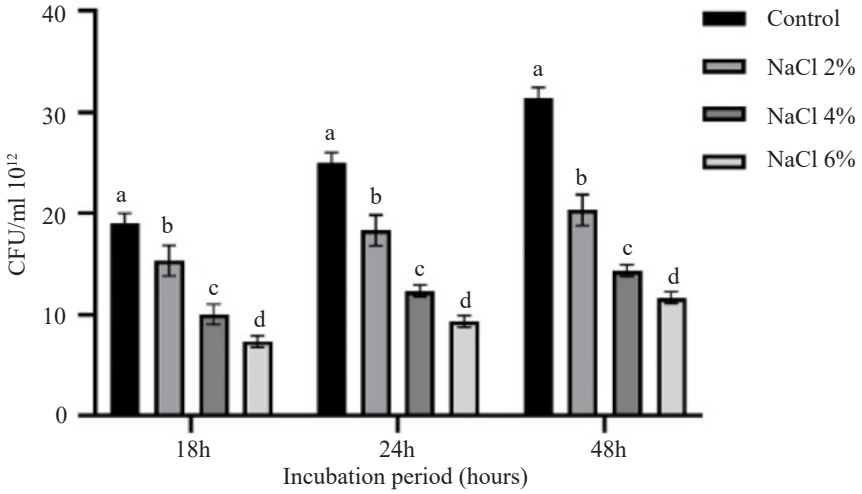


Figure 6. NaCl resistance test of *P. alcaligenes* SG03 isolates with various concentrations and incubation times. The average values with different letters indicate significant differences ($p<0.05$) based on the DMRT test

hours) (Figure 7). These results indicate that *P. alcaligenes* SG03 can tolerate moderate levels of NaCl and bile salts, which are critical for surviving the harsh conditions of the gastrointestinal tract. Compared to other probiotic strains, such as *Bacillus subtilis*, which typically tolerate higher bile salt concentrations, *P. alcaligenes* SG03 shows comparable resilience, making it a promising candidate for gut-related applications.

The pH resistance test was conducted with various pH levels of 2, 3, 4, and 5, along with different incubation times. The results showed that as the pH increased, the CFU of *P. alcaligenes* SG03 also increased. pH 5 was found to be the optimal pH, with CFU values of $8.67\times10^{12}$ (18 hours), $14.67\times10^{12}$ (24 hours), and $20\times10^{12}$ (48 hours). This suggests that the strain can survive in mildly acidic environments, which is a desirable trait for probiotics intended to colonize the host's digestive system. The effect of pH on the CFU of *P. alcaligenes* SG03 can be seen in Figure 8.

The bioactivity assay was conducted to assess the antioxidant and anti-inflammatory activities produced by *P. alcaligenes* SG03. The results showed that as the incubation time increased, both antioxidant and anti-inflammatory activities of *P. alcaligenes* SG03 also increased. After 48 hours of incubation, the highest antioxidant activity (53.00%) and anti-inflammatory activity (60.33%) were observed. These activities are likely mediated by the production of bioactive metabolites, such

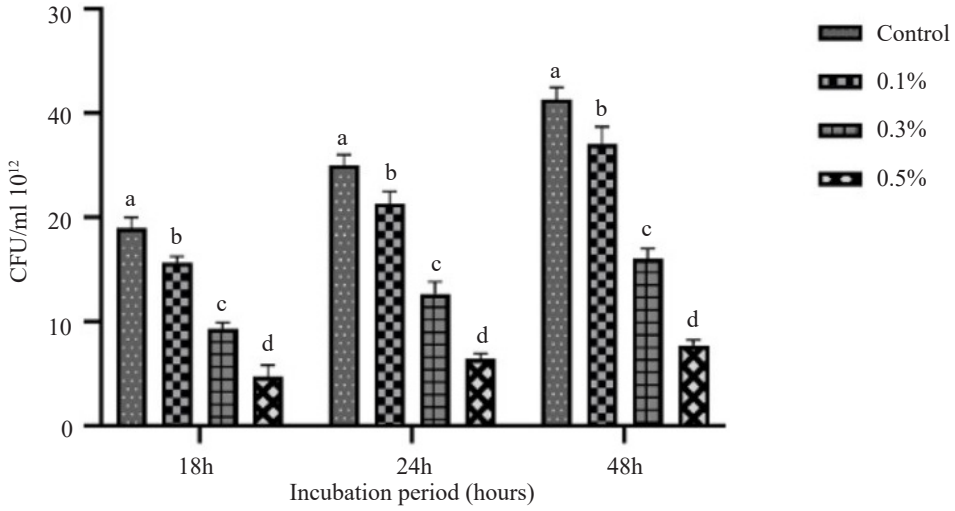


Figure 7. Bile salt resistance test of *P. alcaligenes* SG03 isolates with various concentrations and incubation times. The average values with different letters indicate significant differences ($p<0.05$) based on the DMRT test

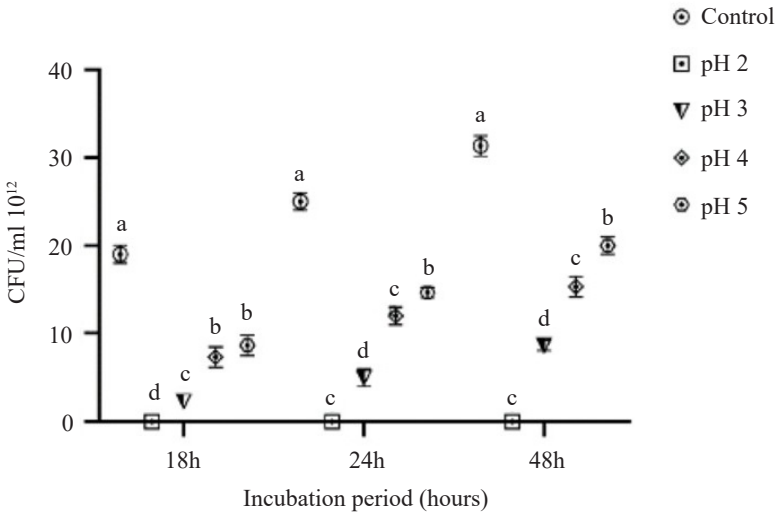


Figure 8. pH resistance test of *P. alcaligenes* SG03 isolates with various levels and incubation times. The average values with different letters indicate significant differences ($p<0.05$) based on the DMRT test

as exopolysaccharides (EPS) or enzymes like superoxide dismutase (SOD), which neutralize free radicals and modulate inflammatory pathways. The strain's ability to enhance antioxidant and anti-inflammatory responses highlights its potential for applications in functional foods and therapeutics. The antioxidant and anti-inflammatory activity data for *P. alcaligenes* SG03 can be seen in Figure 9.

The antibiotic resistance test showed that *P. alcaligenes* SG03 is sensitive to tetracycline, amoxicillin, cefixime, streptomycin, and chloramphenicol (Table 2). This sensitivity reduces the risk of horizontal gene transfer, making the strain a safer probiotic candidate. Additionally, the strain exhibited proteolytic activity but lacked lipolytic and hemolytic properties (Table 3). The proteolytic activity suggests the production of enzymes like proteases, which can aid in nutrient acquisition and host digestion. The absence of lipolytic and hemolytic activities further supports the strain's safety profile, as these traits are often associated with pathogenic potential.

Based on the test results, *P. alcaligenes* SG03 is sensitive to Tetracycline, Amoxicillin, Cefixime, Streptomycin, and Chloramphenicol. Additionally, tests were conducted for proteolytic, lipolytic activities, and hemolysis. The results showed that *P. alcaligenes* SG03 exhibits proteolytic activity but does not have lipolytic or hemolytic properties.

## 4. Discussion

*P. alcaligenes* SG03 holds great potential as a probiotic in shrimp farming due to its ability to enhance health and resistance to pathogens. One of its main capabilities is the production of antimicrobial compounds, such as bacteriocins and lipopeptides, which effectively inhibit the growth of pathogens like *Vibrio* spp. This is particularly important in shrimp farming, where bacterial infections often cause significant losses (Oni *et al*. 2022). However, while genome analysis suggests the presence of genes like bacA and lpa for these compounds, experimental validation is needed to confirm their production and efficacy. Additionally, *P. alcaligenes* SG03 improves digestion through proteolytic enzymes, though lipolytic activity was not observed, contrasting with other probiotics like *Bacillus subtilis* (Lestari *et al*. 2024). Further studies are required to confirm its role in nutrient absorption and SCFA production, as these

Table 3. Results of proteolytic, lipolytic, and hemolytic activity testing of *P. alcaligenes* SG03

| Types of testing | Activity |
|---|---|
| Proteolytics | + |
| Lipolytics | No activity |
| Haemolysis | No activity |

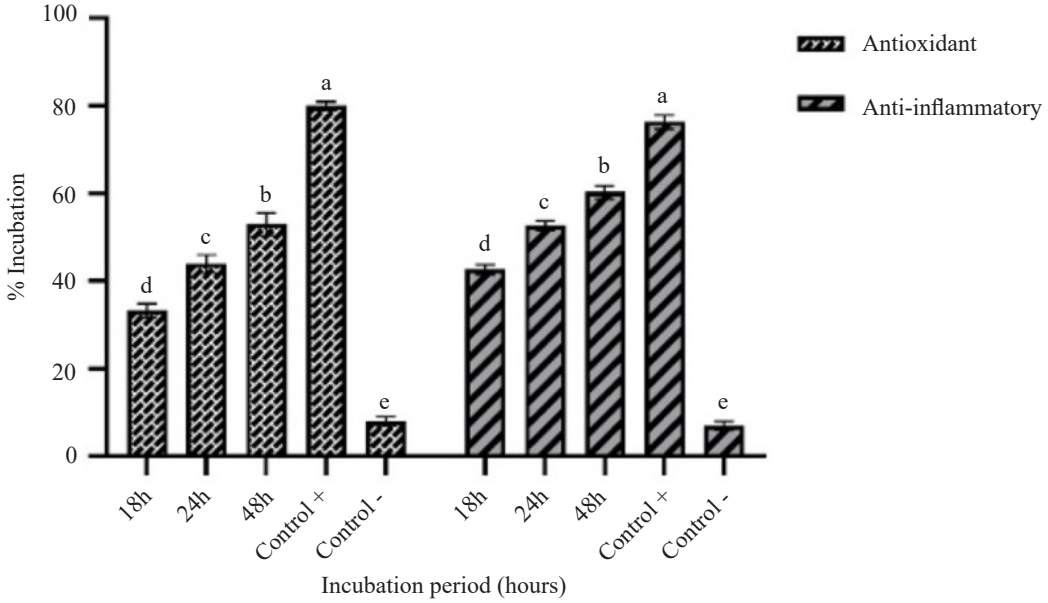


Figure 9. Antioxidant and anti-inflammatory activity test of *P. alcaligenes* SG03 isolates with various incubation times. The average values with different letters indicate significant differences ($p<0.05$) based on the DMRT test

benefits are well-documented in *Lactobacillus* spp. but less so in *Pseudomonas* species (Rauf *et al*. 2022). By improving digestive efficiency, this bacterium can contribute to the overall growth and health of shrimp. *P. alcaligenes* SG03 also produces short-chain fatty acids (SCFAs) through fermentation, which serve as an energy source and have anti-inflammatory effects that support gastrointestinal integrity (Mezo *et al*. 2022).

Probiotic-related genes in *P. alcaligenes* SG03 can be analyzed using COGs and KEGG to understand their biological functions. COGs categorize genes into functional groups, such as Carbohydrate Metabolism (G) and Amino Acid Metabolism (E). At the same time, KEGG maps genes to specific metabolic pathways like lactate fermentation and fatty acid biosynthesis. For example, genes involved in lactate fermentation can be linked to energy metabolism pathways, illustrating how *P. alcaligenes* SG03 generates lactic acid as a primary metabolic product. This functional annotation provides insights into the strain's probiotic mechanisms, such as its ability to modulate microbiota health and host immune responses. Additionally, genes involved in antimicrobial peptide production can be mapped to pathways in KEGG, highlighting their role in pathogen inhibition. The antimicrobial activity of *P. alcaligenes* SG03 is a key feature of its probiotic application. Genes involved in bacteriocin and lipopeptide biosynthesis enable it to combat pathogens like *Vibrio* spp. and *Aeromonas* spp. (Dimkić *et al*. 2022). While these genomic predictions are promising, biochemical assays are necessary to confirm the production and functionality of these compounds. Bacteriocins disrupt cell wall synthesis, while lipopeptides disrupt bacterial membranes, leading to cell death (Lakshmanan *et al*. 2020). Additionally, *P. alcaligenes* SG03 stimulates immune responses, such as hemocyte activity and cytokine production, though these claims require further experimental validation. Comparative studies with other probiotics could highlight its unique advantages in immune modulation (Kwon *et al*. 2021).

Genes involved in exopolysaccharide (EPS) synthesis, such as epsB and epsC, enhance the probiotic potential of *P. alcaligenes* SG03 by promoting biofilm formation and gut colonization (Boopathi *et al*. 2022). These biofilms protect the bacteria from environmental stress and strengthen the epithelial barrier, the first line of defense against pathogens. Additionally, EPS can modulate the mucosal immune response by enhancing mucin secretion, which is essential for maintaining gut integrity. This mechanism is particularly beneficial in shrimp farming, where gut health is critical for disease resistance. *P. alcaligenes* SG03's ability to withstand high salinity is mediated by genes like otsA (trehalose biosynthesis) and proC (proline biosynthesis), which protect cells from osmotic stress (Phour *et al*. 2020; Aboyadak *et al*. 2024). These adaptations are crucial for survival in shrimp farming environments, where salinity levels can fluctuate. Additionally, ion transport mechanisms, regulated by genes like nhaA, help maintain ionic balance under high salinity. Comparing these traits with other probiotics, such as *Lactobacillus* spp., which are less salt-tolerant, underscores the competitive advantage of *P. alcaligenes* SG03 in aquaculture.

Acid resistance genes, such as rpoS and arcA, enable *P. alcaligenes* SG03 to survive in low pH conditions. The rpoS gene regulates stress response genes, while arcA is involved in the arginine deiminase pathway, which generates ATP and aids pH regulation (Hall *et al*. 2018; Li *et al*. 2020). These mechanisms are essential for the strain's survival in acidic environments, such as the shrimp digestive tract. Comparative studies with other probiotics could further highlight its resilience under acidic conditions. In addition to acid resistance, *P. alcaligenes* SG03 exhibits resistance to antibiotics, heavy metals, and oxidative stress. Genes like gshA and cysK enhance glutathione production, protecting cells from oxidative damage (Vasoya *et al*. 2019). The copZ gene regulates copper homeostasis, while zwf produces NADPH to counteract oxidation caused by heavy metals (Sada *et al*. 2022; Rebelo *et al*. 2023). These traits, combined with its ability to form biofilms, make *P. alcaligenes* SG03 highly resilient to environmental stress. This study demonstrates that *P. alcaligenes* SG03 holds promising potential as a probiotic with several desirable characteristics. Osmotolerance testing revealed optimal growth at 4% glucose, while temperature tolerance testing identified 30°C as the optimal temperature. The strain also exhibited high CFU counts at 2% NaCl and 0.1% bile salt, demonstrating its resilience in harsh conditions. Bioactivity testing showed increasing antioxidant (53.00%) and anti-inflammatory (60.33%) activities over time, highlighting its potential for functional applications. However, further experimental validation is needed to confirm its antimicrobial compound production and immune-stimulating properties.

## Acknowledgements

The author extends gratitude to the Beasiswa Pendidikan Indonesia (BPI) from the Ministry of Education, Culture, Research, and Technology (Kemendikbudristek) for providing financial assistance for tuition, living expenses, and research costs during the author's studies at IPB University (2021-2024) and the National Research and Innovation Agency (BRIN) for research facilities to support the progress of this research.

## References

Aboyadak, I.M., Abdel-Tawwab, M., Ali, N.G., 2024. Identification and florfenicol-treatment of *pseudomonas putida* infection in gilthead seabream (*Sparus aurata*) fed on tilapia-trash-feed. *BMC Veterinary Research*. 20, 1-11. https://doi.org/10.1186/s12917-024-04004-z

Akinyemi, M.O., Ogunremi, O.R., Adeleke, R.A., Ezekiel, C.N., 2024. Probiotic potentials of lactic acid Bacteria and yeasts from Raw Goat milk in Nigeria. *Probiotics Antimicrob Proteins*. 16, 163-180.

Anita, U.E., Akan, O.D., Stephen, N.U., Eno-Ibanga, C.K., Akpan, N.G., 2018. Isolation and screening of yeast isolates indigenous palm wine for ethanol production. *Philip J Sci*. 147, 411-417.

Boopathi, S., Vashisth, R., Mohanty, A.K., Jia, A.Q., Sivakumar, N., Alharbi, N.S., Arockiaraj J., 2022. Investigation of interspecies crosstalk between probiotic *Bacillus subtilis* BR4 and *Pseudomonas aeruginosa* using metabolomics analysis. *Microbial Pathogenesis*. 166, 105542. https://doi.org/10.1016/j.micpath.2022.105542

Dimkić, I., Janakiev, T., Petrović, M., Degrassi, G., Fira, D., 2022. Plant-associated *Bacillus* and *Pseudomonas* antimicrobial activities in plant disease suppression via biological control mechanisms - A review. *Physiological and Molecular Plant Pathology*. 117, 101754. https://doi.org/10.1016/j.pmpp.2021.101754

Ekawati, N., Mutiara, I., Hertati, A., Kusdianawati, Mustopa, A.Z., Fatimah, Manguntungi, B., Elviantari, A., 2024. Biodiversity and probiotic potential of yeasts isolated from Sumbawa horse milk. *Molecular Biology Reports*. 51, 911. https://doi.org/10.1007/s11033-024-09828-4

Fang, Z., Sun, D., Gao, J., Guo, M., Sun, L., Wang, Y., Gooneratne, R., 2020. An acylase from *Shewanella putrefaciens*presents a *Vibrio parahaemolyticus* acylhomoserine lactone-degrading activity and exhibits temperature-, pH- and metal-dependences. *Acta Alimentaria*. 49, 375-381. https://doi.org/10.1556/066.2020.49.4.2

Fernandes, S., Kerkar, S., Leitao, J., Mishra, A., 2019. Probiotic role of salt pan bacteria in enhancing the growth of whiteleg shrimp, *Litopenaeus vannamei*. *Probiotics and Antimicrobial Proteins*. 11, 1309-1323. https://doi.org/10.1007/s12602-018-9503-y

Fidien, K.A., Manguntungi, B., Sukmarini, L., 2021. Diversity analysis, identification, and bioprospecting of lactic acid bacteria (lab) isolated from sumbawa horse milk. *Biodiversitas*. 22, 3333-3340. https://doi.org/10.13057/biodiv/d220639

Garbi, M.I., Mohammed, S.F., Magzoub, A.A., Hassabelrasoul, R.M., Saleh, M.S., Badri, A.M., Ibrahim, I.T., Elshikh, A.A., Kabbashi, A.S., 2017. *In vitro* anti-inflammatory properties of methanolic extract of *Hibiscus sabdariffa* flowers. *International Journal of Home Science*. 3, 234-237.

González-Palacios, C., Fregeneda-Grandes, J.M., Aller-Gancedo, J.M., 2020. Possible mechanisms of action of two *Pseudomonas* fluorescens isolates as probiotics on saprolegniosis control in rainbow trout (*Oncorhynchus mykiss*Walbaum). *Animals*. 10, 1507. https://doi.org/10.3390/ani10091507

Gupta, D.S., Kumar, M.S., 2022. The implications of quorum sensing inhibition in bacterial antibiotic resistance—with a special focus on aquaculture. *Journal of Microbiological Methods*. 203, 106602. https://doi.org/10.1016/j.mimet.2022.106602

Hall, C.W., Hinz, A.J., Gagnon, L.B.P., Zhang, L., Nadeau, J.P., Copeland, S., Mah, T.F., 2018. *Pseudomonas aeruginosa* biofilm antibiotic resistance gene ndvB expression requires the RpoS stationary-phase sigma factor. *Applied and Environmental Microbiology*. 84. https://doi.org/10.1128/AEM.02762-17

Hertati, A., Mustopa, A.Z., Faiz, M., Ekawati, N., Manguntungi, B., Fatimah., Meilina, L., Tisnadjaja., 2023. Potential of *Lactococcus lactis* strain LAC3 isolated from Indonesian traditional fermented buffalo milk (Dadih) as antioxidant and antidiabetic agents. In: *AIP Conference Proceedings*. American Institute of Physics Inc. 30.

Kaktcham, P.M., Kujawska, M., Kouam, E.M.F., Piame, L.T., Tientcheu, M.L.T., Mueller, J., Felsl, A., Truppel, B.A., Ngoufack, Z., Hall, L.J., 2025. Genomic insights into the beneficial potential of Bifidobacterium and Enterococcus strains isolated from Cameroonian infants. *Microbial Genomics*. 11, 001354. https://doi.org/10.1099/mgen.0.001354

Kwon, H., Hall, D.R., Smith, R.C., 2021. Prostaglandin E2 signaling mediates oenocytoid immune cell function and lysis, limiting bacteria and Plasmodium oocyst survival in Anopheles gambiae. *Frontiers in Immunology*. 12, 680020. https://doi.org/10.3389/fimmu.2021.680020

Lakshmanan, R., Kalaimurugan, D., Sivasankar, P., Arokiyaraj, S., Venkatesan, S., 2020. Identification and characterization of *Pseudomonas aeruginosa* derived bacteriocin for industrial applications. *International Journal of Biological Macromolecules*. 165, 2412-2418. https://doi.org/10.1016/j.ijbiomac.2020.10.126

Lestari, S.D., Hussain, N., Hussin, A.S.M., Mustafa, S., Sew, Y.S., 2024. *Bacillus* multi-strain from malaysian fish sauces demonstrating proteolytic, lipolytic, esterolytic and glutamic-acid production activities. *Applied Food Biotechnology*. 11, e26-e26.

Li, W., Yang, L., Nan, W., Lu, J., Zhang, S., Ujiroghene, O.J., Lv, J., 2020. Whole-genome sequencing and genomic-based acid tolerance mechanisms of *Lactobacillus delbrueckii* subsp. bulgaricus LJJ. *Applied Microbiology and Biotechnology*. 104, 7631-7642. https://doi.org/10.1007/s00253-020-10788-5

Lin, S.J., Huang, J.Y., Le, P.T., Lee, C.T., Chang, C.C., Yang, Y.Y., Wang, H.C., 2022. Expression of the AHPND toxins PirA vp and PirB vp is regulated by components of the *Vibrio parahaemolyticus* quorum sensing (QS) system. *International Journal of Molecular Sciences*. 23, 2889. https://doi.org/10.3390/ijms23052889

Lubis, A.R., Sumon, M.A.A., Dinh-Hung, N., Dhar, A.K., Delamare-Debouteville, J., Kim, D.H., Brown, C.L., 2024. Review of quorum-quenching probiotics: A promising non-antibiotic-based strategy for sustainable aquaculture. *Journal of Fish Diseases*. e13941. https://doi.org/10.1111/jfd.13941

Mezo, E., Balogh, F.H., Horvath, I.M., Bufa, A., Marosvolgi, T., Kocsis, B., Makszin, L., 2022. Effect of culture conditions on fatty acid profiles of bacteria and lipopolysaccharides of the genus *Pseudomonas*-GC-MS analysis on ionis liquid-based column. *Molecules*. 27, 1-21. https://doi.org/10.3390/molecules27206930

Mizan, M.F.R., Ashrafudoulla, M., Hossain, M.I., Cho, H.R., Ha, S.D., 2020. Effect of essential oils on pathogenic and biofilm-forming *Vibrio parahaemolyticus* strains. *Biofouling*. 36, 467-478. https://doi.org/10.1080/08927014.2020.1772243

Muche, N., Geremew, T., Jiru, T.M., 2023. Isolation and characterization of potential probiotic yeasts from Ethiopian injera sourdough. *3 Biotech*. 13. https://doi.org/10.1007/s13205-023-03729-2

Nurjannah, K.A.I., Mustopa, A.Z., Masniawati, A., 2024. Nutritional profiling and in silico analysis of pharmacological activi ties from local rice Pulu Mandoti fermented with *Pleurotus* spp. *World J Microbiol Biotechnol*. 40, 6. https://doi.org/10.1007/ s11274-024-03979-z

Oni, F.E., Esmaeel, Q., Onyeka, J.T., Adeleke, R., Jacquard, C., Clement, C., Höfte, M., 2022. *Pseudomonas* lipopeptide-mediated biocontrol: Chemotaxonomy and biological activity. *Molecules*. 27, 372. https://doi.org/10.3390/molecules27020372

Phan, T.T.C., Vu, U.N., Pham, N.T., Vu, H.H., Huynh, G.T., 2022. Evaluation of *Pseudomonas stutzeri* AM1 and *Pseudomonas oleovorans* ST1.1 isolated from shrimp pond sediments as probiotics for whiteleg shrimp, *Litopenaeus vannamei* culture. *International Journal of Aquatic Biology*. 10, 201-208.

Phour, M., Sindhu, S.S., 2020. Amelioration of salinity stress and growth stimulation of mustard (*Brassica juncea* L.) by salt-tolerant *Pseudomonas* species. *Applied Soil Ecology*. 149, 103518. https://doi.org/10.1016/j.apsoil.2020.103518

Rauf, A., Khalil, A.A., Rahman, U.U., Khalid, A., Naz, S., Shariati, M.A., Rebezov, M., Urtecho, E.Z., Albuquerque, R.D.D.G.D., Anwar, S., Alamri, A., Saini, R.K., Rengasamy, K.R.R., 2022. Recent advances in the therapeutic application of short-chain fatty acids (SCFAs): An updated review. *Critical Reviews in Food Science and Nutrition*. 62, 6034-6054. https://doi.org/10.1080/10408398.2021.1895064

Reina, J.C., Torres, M., Llamas, I., 2019. *Stenotrophomonas maltophilia* AHL-degrading strains isolated from marine invertebrate microbiota attenuate the virulence of *Pectobacterium carotovorum* and *Vibrio coralliilyticus*. *Marine Biotechnology*. 21, 276-290. https://doi.org/10.1007/s10126-019-09879-w

Rusmana, I., Hastuti, Y.P., Alfisah, R.K., Akhdiya, A., 2022. Characteristics and activity of anti-quorum sensing *Bacillus* spp. isolated from *Penaeus vannamei* shrimp ponds. *HAYATI Journal of Biosciences*. 29, 97-106. https://doi.org/10.4308/hjb.29.1.97-106

Rebelo, A., Almeida, A., Peixe, L., Antunes, P., Novais, C., 2023. Unraveling the role of metals and organic acids in bacterial antimicrobial resistance in the food chain. *Antibiotics*. 12, 1474. https://doi.org/10.3390/antibiotics12091474

Sada, M., Kimura, H., Nagasawa, N., Akagawa, M., Okayama, K., Shirai, T., Murakami, K., 2022. Molecular evolution of the *Pseudomonas aeruginosa* DNA gyrase gyrA gene. *Microorganisms*. 10, 1660. https://doi.org/10.3390/microorganisms10081660

Saputra, A., Maftuch, M., Andayani, S., Yanuhar, U., 2023. Pathogenicity of *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease (AHPND) in shrimp (*Litopenaeus vannamei*) in Serang, Banten, Indonesia. *Biodiversitas Journal of Biological Diversity*. 24. https://doi.org/10.13057/biodiv/d240451

Sylvere, N., Mustopa, A.Z., Budiarti, S., Hertati, A., Handayani, I., 2023. Whole-genome sequence analysis and probiotic characteristics of *Lactococcus lactis* Subsp. lactis strain Lac3 isolated from traditional fermented buffalo milk (Dadih). *JGEB*. 21, 49-52. https://doi.org/10.1186/s43141-023-00503-y

Tran, K., Le-Thi, L., Vo, H., Dinh-Thi, T., Nguyen-Thi, T., Phan, N.H., Nguyen, K., 2023. Probiotic properties and safety evaluation in the invertebrate model host *Galleria mellonella* of the *Pichia kudriavzevii* YGM091 strain isolated from fermented goat milk. *Probiotics and Antimicrobial Proteins*. 16, 1288-1303. https://doi.org/10.1007/s12602-023-10114-1

Vasoya, M., 2019. The role of glutathione-encoding genes (gshA-gshB) in oxidative stress and antibiotic susceptibility in *Pseudomonas aeruginosa* [Master's thesis]. The Center Brooklyn: Long Island University.

Zhang, S., Yang, Q., Eggermont, M., Defoirdt, T., 2023. Quorum-sensing interference in vibrios. *Reviews in Aquaculture*. 15, 1452-1466. https://doi.org/10.1111/raq.12787