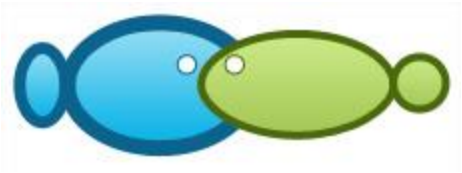

Growth kinetics and antibiotic production of *Bacillus* sp. isolated from salted anchovy wastewater

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Abstract. This study examined the growth kinetics and antibiotic production of *Bacillus* sp. isolated from salted anchovy (*Stolephorus* sp.) wastewater, a protein-rich and saline environment. The isolate exhibited typical *Bacillus* characteristics: gram-positive, rod-shaped, motile, and endospore-forming, with hydrolytic enzyme activities including amylolytic, proteolytic, lipolytic, cellulolytic, and fermentative functions. Antibiotic filtrates were produced using different protein substrates skim milk, soy milk, egg yolk, and peptone under varying salinity levels (0‰, 1‰, 2‰, 5‰) with zinc supplementation. Skim milk provided the optimal nitrogen source, yielding the largest inhibition zones against *Staphylococcus aureus* (25 mm) and *Escherichia coli* (22 mm). Salinity significantly affected antibiotic activity, with 5‰ NaCl producing the highest inhibition zones of 14 mm (*S. aureus*) and 10 mm (*E. coli*). These results indicate that both the quality of nitrogen substrates and environmental salinity critically influence secondary metabolite production in *Bacillus* sp. The study highlights the potential of utilizing salted fish-processing wastewater as a sustainable substrate for microbial antibiotic biosynthesis, offering an eco-friendly approach to valorise industrial waste while producing bioactive compounds.
Keywords: inhibition zone, protein substrates, saline wastewater.

Introduction. Fish processing industries are among the major contributors to environmental pollution due to the high volume of organic waste they generate, which can deteriorate water quality, increase biochemical oxygen demand (BOD), and threaten aquatic ecosystems. In Indonesia, approximately 30 – 40 % of total fish production equivalent to about 8.6 million tons in 2021 becomes waste. Of this, nearly 2 million tons remain unutilized and are discarded as by-products, including fish viscera, bones, scales, and wastewater. Such waste poses significant environmental challenges, including eutrophication, odour generation, and the proliferation of pathogenic microorganisms. Consequently, developing effective strategies to valorise fish processing waste is both an environmental necessity and an economic opportunity (Margariti, 2021; Saad Abdelkarim, 2020).

One promising approach for valorisation is the utilization of microbial metabolites produced by antagonistic microorganisms, which can generate bioactive compounds such as antibiotics, enzymes, and other secondary metabolites. Antibiotics, in particular, play a crucial role in controlling microbial contamination and can be harnessed for biomedical and aquaculture applications. The production of antibiotics can occur either directly or indirectly. Direct production often involves co-culturing potential antibiotic-producing bacteria with target pathogens or applying the disc diffusion method to evaluate inhibitory effects. Indirect production, on the other hand, relies on optimization processes in controlled culture conditions that promote the accumulation of antibiotic filtrates, which are subsequently tested for antimicrobial activity against indicator bacteria. The disc diffusion method has been widely applied in numerous studies to quantify antibacterial potency. For instance, disc diffusion assays were utilized to evaluate marine bacteria and their antibacterial activity against common pathogens (McCormick et al., 2014).

Members of the genus *Bacillus* are particularly notable for their ability to produce a broad spectrum of peptide-based antibiotics with diverse modes of action. *Bacillus brevis* produces gramicidin and tyrocidine, *Bacillus polymyxa* synthesizes polymyxin, and *Bacillus subtilis* and *Bacillus licheniformis* generate bacitracin. Antibiotics, like tyrothricin, tyrocidine, gramicidin, bacitracin, polymyxin, and viomycin, are primarily derived from amino acid precursors through complex ribosomal and non-ribosomal biosynthetic pathways. Antibiotic biosynthesis in *Bacillus* is regulated bioenergetically by intrinsic genetic factors, while extrinsic environmental parameters including nutrient composition, temperature, pH, oxygen availability, salinity, and fermentation duration significantly influence production yield (Ramdhani et al., 2023). In particular, *B. subtilis* demonstrates multiple antagonistic mechanisms, such as antibiosis, competitive exclusion, and growth promotion, with bacitracin production effectively inhibiting Gram-positive bacteria by disrupting cell wall synthesis.

Optimization of antibiotic production against pathogens such as *Staphylococcus aureus* and *Escherichia coli* has been shown to depend on several parameters. Wulandari and Sulistyani (2016) reported that Actinomycetes isolate AL35 exhibited the highest inhibitory activity on the second day of incubation. Similarly, Warsi and Sulistyani (2018) found that the peak production of secondary metabolites against methicillin-resistant *S. aureus* (MRSA) occurred during the early exponential phase of bacterial growth, highlighting the critical influence of incubation time on metabolite accumulation (Selvaraj et al., 2023).

Recent studies have focused on isolating *Bacillus* species from fish-processing wastewater due to its rich nutrient content and high salinity, which favour the growth of halotolerant and metabolically versatile strains. Several *Bacillus* isolates obtained from salted anchovy (*Stolephorus* sp.) wastewater exhibited moderate to strong antibacterial activity against *S. aureus* and *E. coli*. Fourteen isolates were recovered from both washing and boiling wastewater, demonstrating that wastewater streams from salted fish processing are a valuable source of antibiotic-producing bacteria. The saline-rich and protein-dense environment of such wastewater likely induces stress responses in bacteria, triggering secondary metabolite production as a survival mechanism (Tong et al., 2023).

Despite extensive studies on antibiotic optimization in *Bacillus* and other bacteria, information remains limited regarding isolates derived from fish-processing wastewater, particularly in terms of the influence of nitrogen source composition, salinity, and incubation conditions on antibiotic yield. Therefore, the present study aims to investigate the growth kinetics and antibiotic production of *Bacillus* sp. isolated from salted anchovy wastewater, with a specific focus on the efficiency of nitrogen sources in enhancing antibiotic biosynthesis. This research further explores how variations in nitrogen substrates, incubation parameters, and salinity levels affect the production of antibiotic filtrates, providing insights into the potential valorisation of high-protein, saline-rich fish-processing waste through microbial metabolite exploitation (Shobha et al., 2020).

Material And Method

Materials and equipment. The equipment used in this study included an autoclave, analytical balance, Petri dishes, Erlenmeyer flasks, test tubes, vortex mixer, inoculating loop, pH meter, graduated cylinders, Bunsen burner, funnels, drop pipettes, hot plate, magnetic stirrer, incubator, plastic wrap, cotton buds, microcentrifuge tubes (Eppendorf type), labelling paper, filter paper, sprayer, storage box, tissue, cotton, sterile gauze, orbital shaker, gloves, face masks, and writing tools. The materials consisted of 70% ethanol, spiritus, and distilled water (aquadest). Chloramphenicol was used as a positive control antibiotic. The bacterial isolate employed in this experiment was an antagonistic strain belonging to the genus *Bacillus* sp., previously identified as having potential antibacterial activity.

Isolation of bacteria from saline fish-processing wastewater. Bacterial isolation began with the collection of 1 mL of saline fish-processing wastewater, which was then transferred into a test tube containing 9 mL of sterile distilled water and homogenized to

achieve a 10^{-1} dilution. Subsequently, 0.1 mL of the 10^{-1} suspension was mixed with 9.9 mL of standardized bacterial suspension of *Staphylococcus aureus* and *Escherichia coli*, adjusted to 0.5 McFarland standard. Each bacterial isolate was combined and poured into sterile Petri dishes, followed by Pour Plate inoculation on nutrient agar (NA) medium. The plates were incubated at 37°C for 24 hours (Yee et al., 2019). Isolation was performed based on colonies that appeared most frequently and exhibited the largest clear zones. Pure isolates were obtained by re-isolating the colonies and streaking them on slanted NA medium for further purification.

Partial characterization of bacteria from saline fish-processing wastewater. Aseptic technique was used to spread one loopful of bacterial suspension onto a glass slide pre-treated with a drop of distilled water, followed by fixation over a spirit lamp flame. The smear was stained with crystal violet for 1 minute and rinsed with running water while holding the slide at an angle to avoid water retention. Lugol's iodine solution was applied for 1 minute, followed by another rinse. Decolorization was performed using 96 % ethanol for 10 – 20 seconds. Finally, the smear was counterstained with safranin for 10 – 30 seconds, rinsed with running water, air-dried, and observed under a microscope (Elrefaey et al., 2022).

Spore staining was performed to observe endospores within bacterial cells. Endospores are released as free spores when vegetative cells age and lyse, and they exhibit high resistance under extreme environmental conditions. A thin smear was aseptically prepared on a glass slide from the bacterial suspension and fixed over a spirit lamp flame. Malachite green was applied to the smear and left for 20 minutes without heating. The smear was then rinsed with running water at an angle and dried with blotting paper. A few drops of safranin were added and left for 30 seconds, followed by another rinse with running water and drying. The prepared smear was then ready for microscopic observation (Pushpamalini et al., 2020).

Motility testing was conducted to assess bacterial movement, particularly for Gram-positive, rod-shaped, non-spore-forming isolates. Motility medium was used for the assay. Aseptic inoculation was performed using a loop to stab the bacterial suspension into the prepared motility medium. The inoculated medium was incubated at 37°C for 48 hours. Positive motility was indicated by bacterial growth spreading from the stab line, whereas negative motility was indicated by growth confined to a single line without spreading (Kolahalam et al., 2019).

The 3 % KOH test was conducted to confirm the Gram reaction of bacterial isolates following Gram staining. A loopful of bacterial suspension was placed on a glass slide, and 3 % KOH solution was added. The reaction was observed for changes in viscosity: a mucilaginous (slimy) reaction indicates Gram-negative bacteria, whereas the absence of slime indicates Gram-positive bacteria (Pillai et al., 2020).

In-vitro potential assessment of bacterial isolates from salted anchovy wastewater. The isolate index was determined to evaluate the natural ability of bacteria to degrade starch, cellulose, protein, fermentable substrates, and lipids, as well as their antibiotic activity in salted anchovy wastewater. Each isolate was aseptically sampled using a loop, adjusted to a final volume of 10 mL with sterile distilled water in a test tube, and serially diluted from 10^{-1} to 10^{-7}. The suspensions were homogenized using a vortex mixer. One millilitre of the 10^{-5} to 10^{-7} dilutions was pipetted and poured into Petri dishes containing specific media to assess enzymatic or metabolic indices as follows:

1. Proteolytic index (IP): skim milk agar (SMA)
2. Fermentative index (IF): glucose peptone agar (GPA) supplemented with calcium carbonate ($CaCO_3$)
3. Amylolytic index (IA): rice starch agar (APB)
4. Lipolytic index (IL): modified nutrient agar (NA)
5. Cellulolytic index (IS): carboxymethyl cellulose (CMC) agar
6. The plates were incubated at 37°C for 48 hours.

7. The isolate indices were calculated based on the methods of Jamilah et al. (2009), using the ratio of halo zone (HZ) to colony zone (CZ) as follows:

$$IA, IS, IP, IF, IL = \frac{HZ}{CZ}$$

8. Where IA = amylolytic index, IS = cellulolytic index, IP = proteolytic index, IF = fermentative index, IL = lipolytic index, and antibiosis index as described by (Nurmiati et al., 2024). The measurement of bacteria-free zones (halo zones) is illustrated in Figure 1.

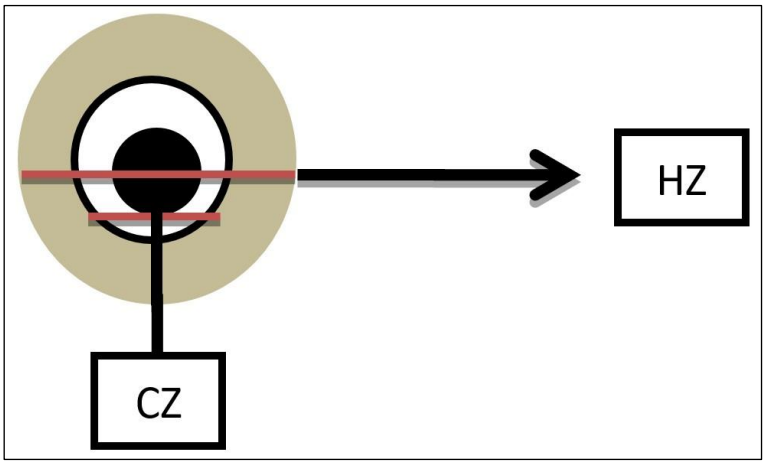

Figure 1. Measurement of bacteria-free zone (halo zone)

Notes:
IK = isolate with large halo and small colony
IB = isolate with large halo and large colony
HZ = diameter of halo zone
CZ = diameter of colony

Growth curve of Bacillus sp*. *isolates. To determine the bacterial growth curve, 100 mL of sterile Starter II antibiotic medium was prepared in a 250 mL Erlenmeyer flask and inoculated with 1 – 2 loopfuls of *Bacillus* sp. isolates. The culture was incubated at room temperature with agitation at 120 rpm for 24 hours. Subsequently, 5 mL of the inoculum was transferred into 95 mL of production medium in a 250 mL Erlenmeyer flask. Samples of 1 mL were taken every 2 hours to measure bacterial cell density using a spectrophotometer at a wavelength of 600 nm. Sampling was continued until a decrease in bacterial growth was observed. The growth curve was then constructed to analyse the growth pattern of the bacteria over the incubation period.

Preparation of seed cultures (Starter I and Starter II). Seed cultures of potential antibiotic-producing *Bacillus* sp. isolates were prepared by transferring one loopful of bacterial culture into 100 mL of liquid medium containing 1 g glucose and 1 g peptone (Meevootisom et al., 1983). The medium was homogenized using a vortex mixer and incubated at 37°C for 24 hours. The resulting culture was designated as Starter I. Starter II was prepared by inoculating 5% (v/v) of Starter I into a fresh 100 mL liquid medium containing the same glucose-peptone composition, followed by incubation in a shaking incubator at room temperature (28°C) and 120 rpm for 24 hours (Hoehler & Jørgensen, 2013).

Antibiotic filtrate production. For antibiotic filtrate production, 10 mL of Starter II was inoculated into various protein substrates, including skim milk (SK), soybean powder milk (KD), egg yolk (KT), and peptone (P). The cultures were incubated in a shaking incubator at 28°C with 120 rpm agitation for different time intervals. Samples were collected

periodically every 4 hours for a total of five samplings, centrifuged at 10,000 rpm for 10 minutes, and the resulting supernatants (antibiotic filtrates) were used for subsequent optimization studies. The antibacterial activity of the filtrates was evaluated using the disc diffusion method.

Evaluation of different protein substrates in antibiotic filtrate production against test bacteria. The effect of different protein substrates on antibiotic filtrate activity against *Staphylococcus aureus* and *Escherichia coli* was assessed to identify the optimal protein source, indicated by the largest inhibition zone (IZD). Sterile filter paper discs were immersed in the supernatants obtained from each substrate, placed on Petri dishes containing nutrient agar (NA) previously inoculated with the test bacteria, and incubated. Each treatment was performed in triplicate to ensure reproducibility.

Optimization of salted anchovy wastewater media, 24-hour incubation, for antibiotic filtrate production against test bacteria. The experiment was performed using salted anchovy wastewater as the culture medium with four salinity variations: 100% (control), 75%, 50%, and 25% salt concentration. Cultures were incubated in a shaking incubator at room temperature (28°C) with 120 rpm agitation, following the optimal incubation time determined in previous optimization studies. After incubation, the cultures containing different starter doses and protein substrates were centrifuged to separate the supernatant (antibiotic filtrate) from the crude fraction. Sterile filter paper discs were then immersed in the supernatant and placed on Petri dishes containing Nutrient Agar (NA) previously inoculated with *Staphylococcus aureus* and *Escherichia coli*. The plates were incubated, and each treatment was performed in triplicate. The procedure for applying antibiotic filtrates with different salinity levels.

The inhibition zone diameters (IZD) formed were measured and presented in tables and diagrams, representing the optimization of various salt concentrations in protein substrates for antibiotic filtrate production against the test bacteria.

Optimization of salinity for antibiotic filtrate production. The bacterial isolate was inoculated (1 %, v/v) into skim milk broth (10 %, w/v; pH 6.23) containing different NaCl concentrations (0 – 5 %, w/v) and supplemented with $ZnSO_4 \cdot 7H_2O$ (0.01 %, w/v). Cultures were incubated at 30 °C and 150 rpm for 16 h. The cultures were centrifuged (10,000 × g, 15 min), and the supernatants were filtered through a 0.22 µm membrane to obtain cell-free antibiotic filtrates. Antibacterial activity was evaluated by the agar well diffusion method against the test bacteria, and inhibition zones were measured after 24 h of incubation at 37 °C. All experiments were performed in triplicate.

Data analysis. All data obtained were analyzed descriptively and presented in the form of tables and diagrams. Tables summarized the inhibition zone diameters (IZD) of the antibiotic filtrates, while diagrams provided visual comparisons of the IZD values obtained for each treatment.

Results and Discussion

Morphological characteristics and in-vitro antibacterial potential of Bacillus sp. The observed morphological and microscopic characteristics of the isolate were typical of the *Bacillus* genus, which is well recognized for its ability to produce diverse extracellular enzymes and bioactive metabolites. The Gram-positive, rod-shaped, motile, and endospore-forming traits indicate that this bacterium is well adapted to survive under saline and nutrient-limited conditions, as found in anchovy wastewater. Such physiological resilience allows *Bacillus* species to maintain metabolic activity during stress and to synthesize secondary metabolites, including antimicrobial compounds (Ramyabharathi et al., 2020).

The positive results for amylolytic, proteolytic, lipolytic, cellulolytic, and fermentative activities suggest that the isolate secretes multiple hydrolytic enzymes capable of degrading various organic substrates. This enzymatic diversity may contribute to efficient

nutrient utilization during fermentation, thereby enhancing antibiotic biosynthesis (Hamouda et al., 2020).

Previous studies have shown that extracellular enzyme production in *Bacillus* spp. is often associated with antimicrobial potential, as protease and lipase activities are linked to the synthesis of peptide-based or lipid derived antibiotics. These observations support the hypothesis that enzymatic versatility is one of the key adaptive and functional traits of *Bacillus* species inhabiting saline or marine environments. The morphological, cellular, and enzymatic features of the isolate were consistent with previously described *Bacillus* species recovered from saline environments (Marlida et al., 2023). Overall, the results confirm that the isolate possesses both structural and metabolic traits favourable for antibiotic production and survival in hypersaline ecological niches.

Nitrogen source efficiency and antibiotic yield of Bacillus sp. isolated from salted anchovy wastewater. The efficiency of different nitrogen sources significantly influenced the antibiotic yield of *Bacillus* sp. isolated from salted anchovy wastewater. Among the protein-based substrates tested, skim milk provided the most favourable condition for antibiotic production, producing inhibition zones of 25 mm against *Staphylococcus aureus* and 22 mm against *Escherichia coli*. In contrast, peptone supplementation resulted in the smallest inhibition zones, indicating minimal antibiotic synthesis. These results demonstrate that the biochemical nature of the nitrogen source plays a critical role in modulating secondary metabolite production by *Bacillus* species (Al-Askar et al., 2023).

Skim milk, composed mainly of casein and whey proteins, offers a balanced profile of amino acids and peptides that can be easily hydrolysed by extracellular proteases produced by *Bacillus*. The rapid release of amino nitrogen supports cellular metabolism and provides key precursors for antibiotic biosynthesis (Ramezani Farani et al., 2023).

The availability of both carbon and nitrogen in optimal ratios likely enhances enzymatic activities involved in peptide-based antibiotic formation. This observation is consistent with reports that readily digestible organic nitrogen sources tend to enhance antibiotic yield in *Bacillus* and other soil or marine isolates (Figure 2). Soy milk yielded moderate inhibition activity (16 mm for *S. aureus* and 15 mm for *E. coli*), which can be attributed to its more complex glycoprotein structure. Plant-derived proteins typically contain higher carbohydrate content and anti-nutritional factors such as trypsin inhibitors, which may reduce nitrogen assimilation efficiency. Therefore, even though soy milk is rich in protein, its limited digestibility may restrict the supply of free amino nitrogen for secondary metabolism. Egg yolk produced relatively low antibacterial activity (13 mm and 10 mm).

The high lipid concentration in this substrate may hinder oxygen transfer and reduce the metabolic rate of bacterial cells, thereby limiting antibiotic synthesis. Furthermore, lipids can alter medium viscosity and interfere with nitrogen uptake, leading to lower biosynthetic efficiency. Peptone, despite being a common nitrogen supplement, generated the lowest yield (7 mm and 6 mm), suggesting that its simple peptide composition lacks the balanced amino acid spectrum required for efficient antibiotic biosynthesis. Overall, the trend observed indicates that nitrogen complexity and bioavailability strongly determine antibiotic productivity. Protein substrates that can be easily hydrolyzed into amino acids particularly those rich in essential nitrogen compounds enhance the biosynthetic capacity of *Bacillus* sp. (Wang et al., 2020).

The results also highlight that nitrogen utilization efficiency is not solely dependent on total protein concentration, but also on the structural characteristics and digestibility of the protein source. Similar observations were noted in other marine derived *Bacillus* isolates, where organic nitrogen sources supported higher antibiotic yields than inorganic or poorly digestible substrates. Therefore, the superior performance of skim milk as a nitrogen source reflects its biochemical compatibility with the metabolic requirements of *Bacillus* sp. in antibiotic biosynthesis. This finding suggests that optimizing nitrogen source quality, rather than merely quantity, is a key strategy for improving antibiotic production efficiency in microbial fermentation systems (Zhu et al., 2023).

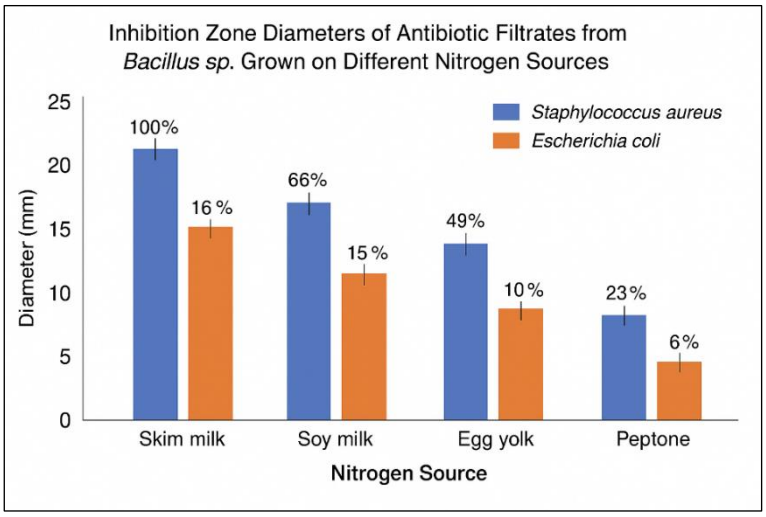

Figure 2. Inhibition zone diameters of antibiotic filtrates produced by *Bacillus* sp. grown on different protein-based nitrogen sources against test bacteria.

Optimization of salinity at 16-hour incubation (skim milk substrate; pH 6.23) with Zn trace element in antibiotic filtrate production against test bacteria. The optimization of salinity in antibiotic filtrate, under the best conditions of incubation time, pH, and trace element supplementation, was carried out to determine the optimal salinity level for antibiotic production against the test bacteria *Staphylococcus aureus* and *Escherichia coli*. This experiment was conducted using four different salt concentrations: 0‰, 1‰, 2‰, and 5‰. The assay employed 5 mm diameter disc paper. The results of this test are presented in Figure 3.

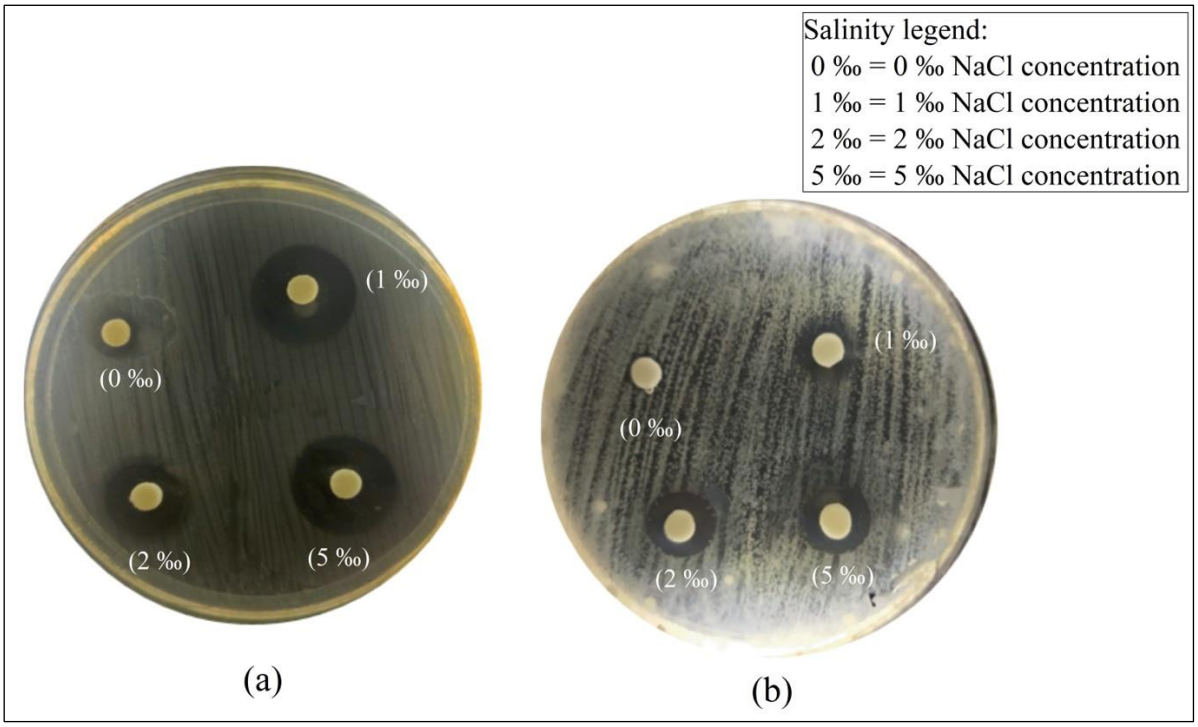

Figure 3. Inhibition zone diameter (IZD) of antibiotic filtrate at different salinity levels against test bacteria (a - *Staphylococcus aureus*; b - *Escherichia coli*; 0 ‰ = 0 ‰ NaCl concentration; 1 ‰ = 1 ‰ NaCl concentration; 2 ‰ = 2 ‰ NaCl concentration; 5 ‰ = 5 ‰ NaCl concentration).

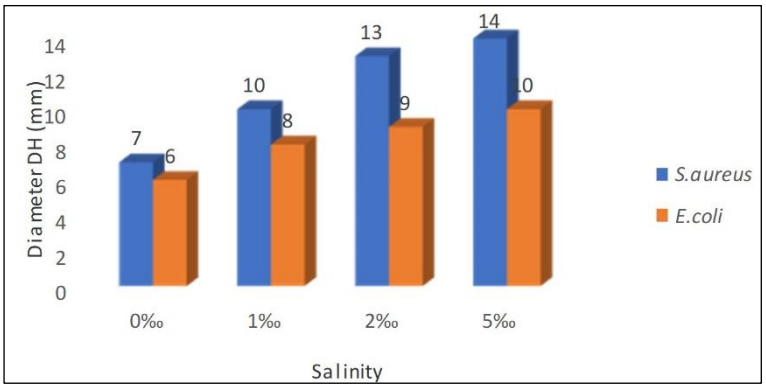

Figure 4. Inhibition zone diameter (IZD) of antibiotic filtrate at different salinity levels against test bacteria.

Different salinity levels in the skim milk (SK) substrate, with an incubation time of 16 hours, pH 6.73, and zinc (Zn) trace element, affected the formation of the inhibition zone (IZ). Among all tested salinity levels, some produced moderate IZD values. Based on Figure 22, the results indicate that the SK substrate with 16-hour incubation, pH 6.73, Zn trace element, and 5 ‰ salinity is likely the optimal salinity for antibiotic filtrate production against the two test bacteria. This conclusion is supported by the observation that the largest IZD was formed at 5‰ salinity compared to the other salinity levels. Subsequently, the diameters of the inhibition zones of the antibiotic filtrates at different salinity levels were measured, and the results are presented in Figure 4.

Based on the diagram, for the test bacterium *Staphylococcus aureus*, the highest inhibition zone diameter (IZD) of the antibiotic filtrate, 14 mm, was observed at 16-hour incubation, pH 6.73, Zn trace element, and 5‰ salinity. This was followed by 2‰ salinity, which produced an IZD of 13 mm, then 1‰ salinity with 10 mm, and 0‰ salinity, which only formed an IZD of 7 mm. These results indicate that the antibiotic filtrate forms larger inhibition zones at higher salinity levels (Mathivanan & Rajaram, 2014).

For the test bacterium *Escherichia coli*, the highest IZD of 10 mm was obtained under the same conditions (16-hour incubation, pH 6.73, Zn trace element) at 5‰ salinity. This was followed by 2‰ salinity with an IZD of 9 mm, and 1‰ salinity with 8 mm. At 0‰ salinity, no inhibition zone was formed; only the 5 mm disc diameter was measured. An additional 1 mm was added to account for the disc itself, resulting in a total of 6 mm, with the disc surface free from bacterial growth.

Salinity significantly affects the growth of *Bacillus* sp. isolates that produce antibiotics by inducing various stress responses and physiological changes. Ayaz et al. (2022) reported that salt-tolerant *Bacillus* sp. show optimal growth at 15 ppt salinity. High salinity also leads to the accumulation of compatible solutes, affecting cell wall metabolism, motility, and sporulation in *Bacillus subtilis*. Moreover, increased salinity triggers ribonuclease biosynthesis and influences motility regulation through signal transduction systems in *Bacillus pumilus* (Ripolles-Avila et al., 2019).

Several *Bacillus* species have adaptive mechanisms that allow them to remain active under high salinity conditions, including the production of osmoregulatory molecules such as betaine and proline, which help maintain cell turgor pressure and enzyme stability under high-salinity stress (Atikij et al., 2019).

Conclusions. The present study demonstrates that *Bacillus* sp. isolated from salted anchovy wastewater exhibits significant antibiotic-producing potential, with growth and secondary metabolite synthesis strongly influenced by substrate type, nitrogen source quality, and environmental salinity. Among the tested protein substrates, skim milk proved to be the most effective nitrogen source, supporting the highest antibiotic yield against *Staphylococcus aureus* and *Escherichia coli*. Salinity also played a critical role, with 5‰ NaCl producing the largest inhibition zones, indicating that halotolerance and stress adaptation mechanisms enhance antibiotic biosynthesis. The isolate's enzymatic versatility, including amylolytic, proteolytic, lipolytic, cellulolytic, and fermentative activities, further contributes to efficient nutrient utilization and metabolite production. These findings

highlight the feasibility of using protein-rich, saline fish-processing wastewater as a sustainable and eco-friendly medium for microbial antibiotic production, providing both environmental and biotechnological benefits. Future studies could focus on scaling up production and characterizing the specific bioactive compounds for potential applications in aquaculture and pharmaceutical industries.

Authors Contributions. Conceptualization: NN. Methodology: FAF, TWE. Formal Analysis: FAF, TWE. Investigation: FAF. Data Curation: FAF. Writing - Original Draft: NN. Writing - Review & Editing: NN, TWE. Supervision: NN.

Conflict of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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