



Mucus-Associated Bacteria from Two Freshwater Fish Species (*Monopterus albus* and *Oreochromis niloticus*) and Their Antibacterial Activities

Siti Suhaila Binti Harith^{1*}, Nor Alya Syamimie Binti Sulaiman¹ & Nurshafiqah Binti Mohd Shahrin¹

¹Faculty of Applied Sciences, Universiti Teknologi MARA Cawangan Pahang Kampus Jengka, 26400 Bandar Tun Abdul Razak Jengka, Pahang Darul Makmur, Malaysia.

Received 22 July 2026; Revised 27 October 2026; Accepted 28 October 2026

Published 30 October 2026

DOI: 10.24191/bioenv.v4i3.170

ABSTRACT. The increasing prevalence of antibiotic-resistant pathogens has intensified the search for antimicrobial agents from natural sources. This study isolated and characterised bacteria associated with the skin mucus of *Monopterus albus* (Asian swamp eel) and *Oreochromis niloticus* (Nile tilapia) and evaluated their antibacterial potential. Six bacterial isolates were obtained from three individuals of each fish species. The isolates were characterised using colony morphology, Gram staining, motility, starch and gelatine hydrolysis, and methyl red and Voges–Proskauer tests. Antibacterial activity was initially screened using the cross-streak method, followed by disc diffusion assays of co-culture-derived supernatants against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. Two isolates exhibited the most promising activity. The supernatant from the co-culture involving Isolate 1 showed inhibition against *E. coli*, whereas that involving Isolate 2 inhibited *S. aureus*. Both isolates were characterised as Gram-negative, rod-shaped bacteria. These findings identify freshwater fish mucus as a source of bacterial isolates warranting further investigation for antibacterial activity. However, because the tested supernatants originated from co-cultures, the inhibitory effects could not be attributed solely to the mucus-associated isolates. Further studies should establish isolate identity, determine the origin of the inhibitory activity, and characterise the active compounds.

Key words: Fish mucus, Antimicrobial activity, Freshwater microbiota, Asian swamp eel, Nile tilapia

1. INTRODUCTION

Monopterus albus, commonly known as the Asian swamp eel, belongs to the Synbranchidae family (Cheng et al., 2021). *Oreochromis niloticus*, commonly known as Nile tilapia, is a freshwater fish found in the wild in Africa and the Middle East but farmed throughout the world for culinary purposes. Both have positive aquacultural characteristics such as their tolerance to poor water quality and the fact that they eat a wide range of natural food organisms. Therefore, it is not surprising that *M. albus* and *O. niloticus* are ranked among the most reared freshwater fishes globally and they have served as a source of high protein food in tropical aquaculture (Antonio-Estrada et al., 2024; Nurfaidah et al., 2021).

M. albus and *O. niloticus* face major health challenges in aquaculture due to bacterial pathogens that lead to disease outbreaks and economic losses. The problem of bacterial diseases continues to be significant in eel and tilapia aquaculture, as it determines the welfare of the fish and its survival. Bacterial pathogens reported in tilapia include *Streptococcus* spp., whereas pathogenic strains of *Aeromonas hydrophila* have been associated with disease in Asian swamp eels (El-Sayed et al., 2022; Wang et al., 2025).

The impacts include such as a decrease in the rate of growth, increased expenditures on treating diseases, and a

*Corresponding author: Tel.: +60 94602622.
E-mail address: ssuhaila@uitm.edu.my (Siti Suhaila)

substantial decline in farm profitability (Rodríguez-Hernández et al., 2025). Due to the close relationship between the occurrence of a disease and the exposure to microbes coupled with the state of the environment, strategies that strengthen natural defence mechanisms are gaining further attention. Reviews of aquaculture research highlight an increase in the need to develop microbial-based approaches to promote fish health (Kuebutornye et al., 2019). These reasons justify research on naturally occurring bacteria possessing antibacterial potential as an effective approach to disease management.

The mucus covers the epidermis and helps protect fish against pathogens, reduce friction during swimming, and maintain ionic balance. Moreover, the skin is colonized with a diverse community of microbes that can help in host defense as it can prevent colonization by pathogenic bacteria such as *A. hydrophila* and *Streptococcus iniae*. In addition to protective and microbial functions, the epidermis participates in wound repair, regeneration as well as communication with the immune system by releasing antimicrobial peptides, and signalling molecules (Ellis 2001).

Previous studies have reported that fish mucus offers the initial shield to safeguard them against pathogens, including viruses, bacteria, and fungi that could lead to illness. Fish possess a comparatively less developed adaptive immune system and therefore depend largely on innate immune mechanisms, among which the mucosal barriers play an important protective role (Bhatnagar & Budhalia, 2022). The physical barrier of fish uses mucus to cleanse the pathogens that adhere to the fish surface (Sridhar et al., 2021). Subsequently, being a chemical barrier, it can trap biomolecules that can fight the infections that invade the mucosa (Dash et al., 2018). Different environmental conditions in the host provoke the formation of the mucus, such as the existence of the contaminants and pathogen infection, as well as dietary deficiency and the dysfunction of the immune system (Díaz-Puertas et al., 2023).

Many studies have established that bacteria present on fish skin can make antimicrobial compounds that add to the host defence against the pathogenic microorganisms. They can produce bioactive materials, including bacteriocins, peptides and secondary metabolites working to avoid the growth of harmful bacteria, fungi and other microorganisms (Uniacke-Lowe et al., 2024). Some of the examples are *Pseudomonas* sp, *Citrobacter* sp., *Burkholderia* sp., *Serratia* sp., and *Chromobacterium* sp., *Yersinia* sp., *Vibrio* sp. and *Edwardsiella* sp. (Korzekwa et al., 2022; Ali et al., 2023). Although the antimicrobial properties of fish mucus have been investigated, comparatively less is known about the culturable bacteria associated with the mucus of *Monopterus albus* and *Oreochromis niloticus* and their ability to produce extracellular antibacterial substances. Previous studies have primarily focused on the antimicrobial activity of crude or extracted mucus, while the antibacterial potential of metabolites secreted by mucus-associated bacterial isolates remains less well characterised. Information on the inhibitory activity of cell-free supernatants derived from these bacteria against common pathogenic bacteria is still limited.

However, the search for novel antimicrobial agents remains a continual challenge, as pathogenic microorganisms continuously undergo genetic adaptation to ensure their survival and bypass existing treatment strategies. Therefore, this study aimed to isolate and identify the mucus-associated bacteria from *M. albus* and *O. niloticus* and evaluate

their potential antibacterial activity against common pathogenic bacteria.

2. METHODOLOGY

The samples of three *Monopterus albus* were obtained from local eel sellers, with the eels are originally collected from swamp in Jengka 21, Pahang, Malaysia whereas three freshwater tilapia *Oreochromis niloticus* collected from a local fish farmer in Kampung Temau, Raub, Pahang. All fish were obtained alive and appeared healthy at the time of collection. Following transportation to the laboratory, the fish were maintained for 1–2 days prior to mucus sampling using water collected from the same source where the fish were obtained. During this holding period, the fish were kept alive under laboratory conditions to minimize handling stress before mucus collection. Meanwhile, three pathogenic bacterial strains (*Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*) obtained from the laboratory stock culture were used for antibacterial activity evaluation.

2.1. Bacterial isolation and characterization

Samples were collected aseptically from the skin mucus of three individuals of each species *Monopterus albus* and *Oreochromis niloticus* using sterile cotton swabs. The swabs were carefully applied to the mucus and streaked onto Nutrient Agar (NA) plates and the plates were incubated at 30 °C for 24 hours. Distinguishable colonies were isolated for further investigation and preserved as stock cultures. The purified isolates underwent standard microbiological analysis, including morphological characterization and Gram stain and a few selected biochemical tests to distinguish and screen their different properties among isolates (Whitman, 2015).

2.2. Preparation of Antibacterial Compound Solution

The experiment aimed to induce antibacterial agents in isolated bacterial cultures against the tested bacteria *E. coli*, *B. subtilis* and *S. aureus*. Co-cultures were prepared by culturing isolated bacteria with one of the three tested bacteria in a conical flask containing 50 mL of nutrient broth. The co-culture medium was incubated at 35°C for 48 hours. Then, the broth was centrifuged at 8000 rpm for 20 minutes to obtain cell-free supernatant of co-culture (CFS-Co). CFS-Co was tested for its antibacterial activities against the same bacteria to which it was exposed while preparing CFS-Co. CFS-*E. coli* was prepared using *E. coli*, CFS-*S. aureus* was prepared using *S. aureus*, and CFS-*B. subtilis* was prepared using *B. subtilis*.

2.3. Determination of Potential Antimicrobial Activities

Antibacterial screening was performed to assess the inhibitory capacity of the bacterial isolates derived from both freshwater fish mucus against the selected test bacteria. Antibacterial activity was assessed using cross-streak and disc diffusion assays. The antibacterial activity of mucus-associated bacterial isolates was preliminarily screened using the cross-streak method. Each purified bacterial isolate was streaked as a straight line along the centre of an agar plate. The test bacteria, including *E. coli*, *S. aureus*, and *B. subtilis*, were then streaked perpendicular to the primary streak of the mucus-associated isolate. The plates were incubated at 35 °C for 24 hours. Antagonistic activity was assessed by observing the inhibition of test bacterial growth near the primary streak. The presence of a clear inhibition zone or restricted growth of the test bacteria was considered indicative of potential antibacterial

activity of the mucus-associated isolate (Suman & Yugandhar, 2021).

The second method is The Kirby-Bauer disc diffusion. This is usually adopted to study the possible applications of bacterial extracts in preventing the growth of selected microorganisms. A Nutrient Agar plate was smeared with the tested microorganisms. Using sterile forceps, the discs were placed on the agar surface, and one was an antibiotic disc, which is streptomycin as a positive control, and sterile broth as a negative control. 25µL of CFS-Co broth was placed in each disc. The plates were incubated for 18 to 24 hours in an incubator set at 37°C. The diameter of the inhibitory zones surrounding each disc, measured in millimetres, is used to calculate the antibacterial activity following incubation.

3. RESULTS AND DISCUSSION

3.1. Isolation and characterization of skin-associated bacteria.

A total of 6 bacterial isolates with distinguishable colony morphologies were obtained. Of the six bacterial isolates obtained, only two exhibited potential antibacterial activity, with one isolate originating from the mucus of *Monopterus albus* and the other from *Oreochromis niloticus*. These two isolates were selected for further characterization in this study (Table 1). Isolate 1 consisted of short Gram-negative rods, whereas Isolate 2 consisted of longer Gram-negative rods. Isolate 1 formed purple colonies, whereas Isolate 2 formed opaque white colonies (Figure 1). Isolate 1 produced purple-pigmented colonies, which may resemble those of *Chromobacterium violaceum*. Nevertheless, further biochemical and molecular analyses are required to determine its taxonomic identity. Both isolates formed circular colonies with entire margins, convex elevations, and smooth surfaces. The first isolate was short rod, non-motile, positive for gelatin hydrolysis and negative for starch hydrolysis. The second isolate was relatively long, non-motile, positive for gelatin hydrolysis and starch hydrolysis. The positive gelatin hydrolysis observed in both isolates may indicate that the isolate possesses physiological characteristics commonly associated with antimicrobial-producing bacteria, supporting its potential as a source of antimicrobial compounds (Gan et al., 2021; Song et al., 2023). Both bacterial isolates showed negative results for the Methyl Red (MR) test and the Voges–Proskauer (VP) test.

Table 1. The characteristics of two isolated bacteria.

Characteristics		Isolate 1	Isolate 2
Colony morphology	Color	Purple	Opaque white
	Elevation	Convex	Convex
	Margin	Entire	Entire
	Form	Circular	Circular
Cell morphology	Shape	Short Rod	Long Rod
	Motility	Non-motile	Non-motile
Biochemical test	Gelatin Hydrolysis	Positive	Positive
	Starch Hydrolysis	Negative	Positive
	Methyl Red (MR)	Negative	Negative
	Voges-Proskauer (VP)	Negative	Negative

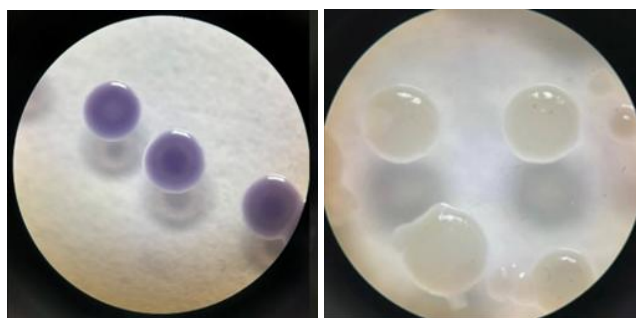


Figure 1. Isolates growth on Nutrient Agar with different colony morphology: Isolate 1 (left), Isolate 2 (right).

3.2 Preliminary screening

Figure 2, the presence of inhibition zones at the intersection between the bacterial isolate 1 and the three tested bacteria (*E. coli*, *S. aureus*, and *B. subtilis*) indicates that some isolates could produce antimicrobial compounds that inhibited bacterial growth.

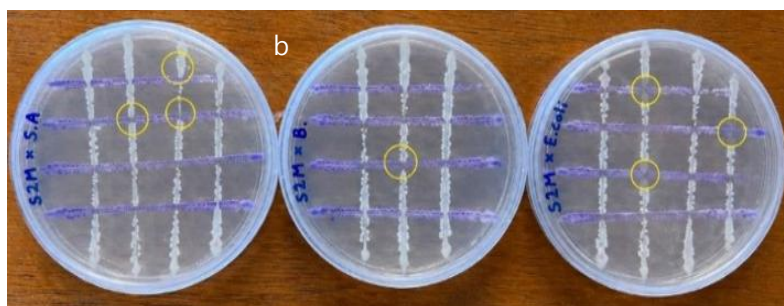


Figure 2. Representative image of the Cross streak: Inhibition zone of potential antibacterial action of Isolate 1 on *S. aureus* (a), *B. subtilis* (b), *E. coli* (c).

In Table 2, summarize results of One Way ANOVA run to examine the effect of different isolates on its antibacterial activities against three tested bacteria measured through the zone of inhibition (mm). CFS-Isolate 1 have antimicrobial activity against *E. coli* (15.50 ± 0.55) the large zone of inhibition but not for *B. subtilis* and *S. aureus*. While for CFS-isolate 2, the antimicrobial activity only positive against *S. aureus* (11.00 ± 0.89) but not *E. coli* and *B. subtilis*. CFS-isolate 3, 4, 5 and 6 dot not posed and significant differences of the antimicrobial activity with control negative. One-Way ANOVA was run separately for each different tested bacteria and the analysis was performed at $\alpha = 0.05$ with $N = 6$. Statistical analysis revealed that there is significant differences exist among sample against all three tested bacteria. Tested against *E. coli*, Tukey's HSD test revealed significant differences between CFS-Isolate 1, compared to the Negative control and Positive control ($p=0.01$). For *B. subtilis*, Tukey's HSD test revealed a significant difference between CFS-Isolate 4 (7.17 ± 0.76 mm) and the negative control (6.00 ± 0.00 mm, $p = 0.035$). However, CFS-Isolate 4 was not selected for further characterization due to its relatively small inhibition zone, as priority was given to isolates with stronger antibacterial activity. Whereas for *S. aureus*, Tukey's HSD test revealed significant differences between CFS-Isolate 2 and the negative control ($p=0.05$). However, in all Tukey's test, CFS-co culture differed significantly from positive controls. The cell-free supernatants obtained from the co-cultivation of Isolates 1 and 2 exhibited antibacterial activity against *E. coli* and *S. aureus*, respectively, as indicated by the observed zones of inhibition.

Co-culture systems have been shown to produce extracellular antimicrobial metabolites in inhibiting *B. subtilis* and other Gram-positive bacteria (Tran et al., 2025; Sun et al., 2022). Co-culture supernatants involving Isolates 1 and 2 showed the most promising inhibitory activity against *E. coli* and *S. aureus*, respectively. Further experiments are required to identify the inhibitory substances and determine their microbial origin.

Table 2. One-Way ANOVA of mean zone of inhibition (mm) of Cell Free Supernatant (CFS) against respective co-culture bacteria.

	Tested Bacteria		
	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>
CFS-Isolate 1	15.50 ± 0.55 ^b	6.83 ± 0.75 ^{bc}	6.83 ± 0.75 ^c
CFS-Isolate 2	6.67 ± 0.52 ^{cd}	6.50 ± 0.55 ^{bc}	11.00 ± 0.89 ^b
CFS-Isolate 3	7.00±0.63 ^c	7.00±0.63 ^{bc}	6.83 ± 0.41 ^c
CFS-Isolate 4	7.17 ± 0.75 ^c	7.17 ± 0.76 ^b	7.00 ± 0.63 ^c
CFS-Isolate 5	6.83 ± 0.41 ^{cd}	6.17 ± 0.41 ^c	6.17 ± 0.41 ^c
CFS-Isolate 6	6.00 ± 0.00 ^{cd}	6.33 ± 0.52 ^c	6.33 ± 0.52 ^c
Streptomycin (Positive control)	17.17 ± 0.75 ^a	17.68 ± 0.82 ^a	17.33 ± 0.52 ^a
Sterile Broth (Negative control)	6.00 ± 0.00 ^d	6.00 ± 0.00 ^c	6.00 ± 0.00 ^c

Note: Values are mean ± SD. Within each column, means sharing a superscript letter are not significantly different according to Tukey's HSD test at $\alpha = 0.05$.

4. CONCLUSION

Six bacterial isolates were obtained from the skin mucus of *Monopterus albus* and *Oreochromis niloticus*. Two isolates, one from each fish species, showed promising antibacterial activity during preliminary screening and were selected for further characterisation. Both were characterised as Gram-negative, rod-shaped bacteria. Supernatants obtained from co-cultures of Isolate 1 with *Escherichia coli* and Isolate 2 with *Staphylococcus aureus* produced inhibition zones of 15.50 ± 0.55 mm and 11.00 ± 0.89 mm against the respective test bacteria. These findings provide preliminary evidence of antibacterial activity associated with co-cultures involving fish mucus-derived bacteria. However, the inhibitory effects cannot be attributed solely to the mucus-associated isolates. Further studies using appropriate monoculture controls, molecular identification, and chemical characterisation are needed to determine the origin of the activity and identify the inhibitory compounds.

ACKNOWLEDGEMENTS

The authors would like to thank Universiti Teknologi MARA Cawangan Pahang for technical assistance and laboratory support.

AUTHOR CONTRIBUTIONS

Siti Suhaila conceived and designed the study. Nor Alya Syamimie Binti Sulaiman and Nurshafiqah Binti Mohd Shahrin performed the experiments and collected the data. All authors carried out the statistical analysis and drafted the manuscript. Siti Suhaila contributed to the revision and approved the final version.

FUNDINGS

The study received no external funding.

DECLARATION OF GENERATIVE AI IN THE WRITING PROCESS

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with language editing, including grammar and clarity improvement. The authors carefully reviewed and revised the content as necessary and took full responsibility for the final version of the publication.

DATA AVAILABILITY

Not applicable.

COMPETING INTEREST

The authors declare that there are no competing interests.

COMPLIANCE OF ETHICAL STANDARDS

Not applicable.

SUPPLEMENTARY MATERIAL

No supplementary material is associated with this article.

REFERENCES

- Ali, S., Nasir, K. K. M., Ullah, W., Rauf, M., Zaman, S., & Ullah, D. F. (2023). Bactericidal activities and biochemical analysis of skin mucus of Cyprinid fish. *Journal of King Saud University – Science*, 35(6), 102731. <https://doi.org/10.1016/j.jksus.2023.102731>
- Antonio-Estrada, C., Cruz-Domínguez, E., Martínez-López, M., Matías-Pérez, D., & García-Montalvo, I. A. (2024). *Oreochromis niloticus* is a blue economy alternative for the Papaloapan region of the state of Oaxaca, Mexico. *Frontiers in Sustainable Food Systems*, 8, 1322838. <https://doi.org/10.3389/fsufs.2024.1322838>
- Bhatnagar, A., & Budhalia, K. (2022). Biochemical components, immune characteristics and antimicrobial activity of epidermal mucus of the three Indian major carp species. *Egyptian Journal of Aquatic Biology and Fisheries*, 26(4), 1273–1288. <https://doi.org/10.21608/ejabf.2022.256741>
- Cheng, H., He, Y., & Zhou, R. (2021). Swamp eel (*Monopterus albus*). *Trends in Genetics*, 37, 1137–1138. <https://doi.org/10.1016/j.tig.2021.09.007>
- Dash, S., Das, S. K., Samal, J., & Thatoi, H. N. (2018). Epidermal mucus, a major determinant in fish health: A review. *Iranian Journal of Veterinary Research*, 19(2), 72–81. <https://pmc.ncbi.nlm.nih.gov/articles/PMC6056142/>

- Díaz-Puertas, R., Adamek, M., Mallavia, R., & Falco, A. (2023). Fish skin mucus extracts: An underexplored source of antimicrobial agents. *Marine Drugs*, 21(6), 350. <https://doi.org/10.3390/md21060350>
- Ellis, A. E. (2001). Innate host defense mechanisms of fish against viruses and bacteria. *Developmental & Comparative Immunology*, 25(8–9), 827–839. [https://doi.org/10.1016/s0145-305x\(01\)00038-6](https://doi.org/10.1016/s0145-305x(01)00038-6)
- El-Sayed, A.-F. M., Nasr-Allah, A. M., Dickson, M., & Gilmour, C. (2022). Analysis of aquafeed sector competitiveness in Egypt. *Aquaculture*, 547, 737486. <https://doi.org/10.1016/j.aquaculture.2021.737486>
- Gan, B. H., Gaynord, J., Rowe, S. M., Deingruber, T., & Spring, D. R. (2021). The multifaceted nature of antimicrobial peptides: Current synthetic chemistry approaches and future directions. *Chemical Society Reviews*, 50(13), 7820–7880. <https://doi.org/10.1039/d0cs00729c>
- Korzekwa, K., Gomulka, P., Nowosad, J., Kucharczyk, D. J., Targońska, K., Czarkowski, T. K., Żarski, D., Horváth, L., Müller, T., Kujawa, R., Łuczyński, M. J., Łuczyńska, J., Abdel-Latif, H. M. R., & Kucharczyk, D. (2022). Bacterial survey of skin ulcers in *Anguilla anguilla* L. females during artificial maturation under controlled conditions. *Aquaculture Reports*, 25, 101259. <https://doi.org/10.1016/j.aqrep.2022.101259>
- Kuebutornye, F. K. A., Abarike, E. D., & Lu, Y. (2019). A review on the application of *Bacillus* as probiotics in aquaculture. *Fish & Shellfish Immunology*, 87, 820–828. <https://doi.org/10.1016/j.fsi.2019.02.010>
- Nurfaidah, Metusalach, M., Sukarno, & Mahendradatta, M. (2021). Protein and albumin contents in several freshwater fish species of Makassar, South Sulawesi, Indonesia. *International Food Research Journal*, 28(4), 745–751. <https://doi.org/10.47836/ifrj.28.4.11>
- Rodríguez-Hernández, M., Martínez-Castellanos, G., Maria, C. L., Reyes-Gonzalez, D., & Moreno, R. (2025). Production costs and growth performance of tilapia (*Oreochromis niloticus*) in intensive production systems: A review. *Sustainability*, 17(4), 1745. <https://doi.org/10.3390/su17041745>
- Song, P., Zhang, X., Wang, S., Xu, W., Wang, F., Fu, R., & Wei, F. (2023). Microbial proteases and their applications. *Frontiers in Microbiology*, 14, 1236368. <https://doi.org/10.3389/fmicb.2023.1236368>
- Sun, Y., Shi, X., Xing, Y., Ren, X. X., Zhang, D. Y., Li, X., Xiu, Z. L., & Dong, Y. S. (2022). Co-culture of *Aspergillus sydowii* and *Bacillus subtilis* induces the production of antibacterial metabolites. *Fungal Biology*, 126(4), 320–332. <https://doi.org/10.1016/j.funbio.2022.01.002>
- Suman, V., & Yugandhar, N. M. (2021). Characterization of pectinase and antibacterial activity by using *Nocardiosis dasnonivelli* S10 isolated from marine samples. *Journal of Cardiovascular Disease Research*, 12(1), 616–631. <https://doi.org/10.48047/>
- Tiralongo, F., Messina, G., Lombardo, B. M., Longhitano, L., Li-Volti, G., & Tibullo, D. (2020). Skin mucus of marine fish as a source for the development of antimicrobial agents. *Frontiers in Marine Science*, 7, 541853. <https://doi.org/10.3389/fmars.2020.541853>
- Tran, C., Addison, R. S., Cock, I. E., Chen, X., & Feng, Y. (2025). Co-culturing *Bacillus* strains for enhanced antimicrobial activity. *Antibiotics*, 14(9), 908. <https://doi.org/10.3390/antibiotics14090908>
- Uniacke-Lowe, S., Stanton, C., Hill, C., & Ross, R. P. (2024). The marine fish gut microbiome as a source of novel bacteriocins. *Microorganisms*, 12(7), 1346. <https://doi.org/10.3390/microorganisms12071346>
- Wang, Y., Qiu, J., Wang, Y., Zhou, Z., Zhang, Y., Li, M., Zhang, X., Chen, X., Yuan, Q., Ayana, G. U., Zhou, W., & Zhang, Q. (2025). Identification, characteristics and whole genome analysis of a pathogenic *Aeromonas hydrophila* ST2616 from diseased Asian swamp eel (*Monopterus albus*) with hemorrhagic sepsis. *Aquaculture Reports*, 42, 102805. <https://doi.org/10.1016/j.aqrep.2025.102805>

Whitman, W. B. (Ed.). (2015). *Bergey's manual of systematics of archaea and bacteria*. John Wiley & Sons.
<https://doi.org/10.1002/9781118960608>



© 2026 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND) license (<http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>).