

**MAKERERE**



**UNIVERSITY**

COLLEGE OF NATURAL SCIENCES

SCHOOL OF BIOSCIENCES

DEPARTMENT OF ZOOLOGY, ENTOMOLOGY AND FISHERIES SCIENCES

# **Inhibition Potential of Gut-Derived Bacteria from Nile Tilapia Against *Aeromonas hydrophila***

OBEDI DANIEL JESSE

23/U/16439/PS

2300716439

**SUPERVISOR: DR. PETER AKOLL**

A RESEARCH DISSERTATION SUBMITTED TO THE DEPARTMENT OF ZOOLOGY,  
ENTOMOLOGY AND FISHERIES SCIENCES, COLLEGE OF NATURAL SCIENCES,  
MAKERERE UNIVERSITY IN PARTIAL FULFILLMENT FOR THE AWARD OF  
BACHELOR OF SCIENCE DEGREE IN FISHERIES AND AQUACULTURE

**SEPTEMBER 2026**

## Declaration

I, **OBEDI DANIEL JESSE** do hereby declare that the content in this research dissertation is original and has never been submitted by anyone else to any institution for any academic award and all information used to support my work has been acknowledged by referencing.

Signature: .....  ..... Date: ..... 3rd September, 2026

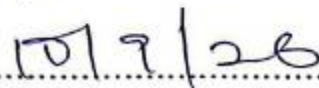
## Approval

I affirm that I have guided the research process and this research dissertation has been submitted after my approval.

### Supervisor

DR. PETER AKOLL

Signature: ..... 

Date: ..... 

Department of Zoology, Entomology and Fisheries sciences  
College of Natural sciences  
Makerere University

## **Dedication**

I dedicate this work to my parents Mr. & Mrs. David Ogwang, to all my siblings and BFA class of 2027 Makerere University.

## **Acknowledgements**

First and foremost, I convey my gratitude and glorification to the Almighty God for the grace of life and seeing this research through to the very end.

In a special way, I deeply appreciate my father Mr. David Ogwang for financing this study all the way through and also extend deep gratitude to my mother Mrs. Rosemary Ogwang for her encouragement and support.

Great thanks and appreciation to my supervisor Dr. Peter Akoll for his guidance and contribution to this study. His guidance surely was a lamp along the path of this study. The department of zoology, entomology and fisheries sciences is also deeply appreciated for allowing the execution of this study at the department microbiological laboratory and for assistance with the equipments used during the study.

Enormous appreciation and gratitude goes to my research assistant, Mr. Luka Odur for his contributions and efforts across all activities conducted during this study.

Great thanks to Dr. Perpetra Akite, Dr. Robinson Odong, Mr. Andrew Byamugisha, Ms. Florence Aijo, Ms. Winfred Nantege, Mr. Stephen Alaphi, Mr. Patrick Katende, Mr. John P. Emejje, Mr. Charles Bbombokka and Mr. Arnold Ssebwato in their respective capacities, for their individual services and contributions to the fulfilment of this study.

# Table of Contents

|                                                                           |     |
|---------------------------------------------------------------------------|-----|
| Declaration .....                                                         | i   |
| Approval .....                                                            | ii  |
| Dedication .....                                                          | iii |
| Acknowledgements .....                                                    | iv  |
| Abstract .....                                                            | x   |
| CHAPTER 1. INTRODUCTION .....                                             | 1   |
| 1.1. Background .....                                                     | 1   |
| 1.2. Statement of the problem .....                                       | 2   |
| 1.3. Objectives of the study.....                                         | 3   |
| 1.3.1. General objective .....                                            | 3   |
| 1.3.2. Specific objectives .....                                          | 3   |
| 1.4. Hypotheses.....                                                      | 3   |
| 1.5. Significance of the study.....                                       | 3   |
| CHAPTER 2. LITERATURE REVIEW .....                                        | 4   |
| 2.1. Morphology, ecology and environmental persistence. ....              | 4   |
| 2.2. Clinical signs in cultured fish caused by <i>A. hydrophila</i> ..... | 4   |
| 2.3. Pathogenicity of <i>Aeromonas hydrophila</i> .....                   | 5   |
| 2.4. Probiotic bacteria and Nile tilapia.....                             | 5   |
| 2.5. Representative probiotic isolates in Tilapia .....                   | 6   |
| 2.6. In vitro inhibitory effects against <i>A. hydrophila</i> . ....      | 6   |
| 2.7. Mechanisms of Pathogen Inhibition .....                              | 7   |
| CHAPTER 3. MATERIALS AND METHODS .....                                    | 8   |
| 3.1. Study area.....                                                      | 8   |
| 3.2. Experimental design.....                                             | 8   |
| 3.2.1. Sample collection.....                                             | 8   |
| 3.2.2. Gut extraction.....                                                | 8   |
| 3.2.3. Primary culture and colony differentiation .....                   | 8   |
| 3.2.4. Subculture and isolation of pure colonies .....                    | 9   |
| 3.2.5. Identification of bacterial species .....                          | 9   |
| 3.3. In-vitro antagonistic activity assays.....                           | 9   |
| 3.3.1. Preparation of pathogenic lawn .....                               | 9   |

|                                                                        |    |
|------------------------------------------------------------------------|----|
| 3.3.2. Cell-Free Supernatant (CFS) inhibition assay .....              | 9  |
| 3.3.3. Satellitism inhibition assay .....                              | 10 |
| 3.4. Data analysis .....                                               | 10 |
| CHAPTER 4. RESULTS .....                                               | 11 |
| 4.1. Diversity and composition of gut Isolates .....                   | 11 |
| 4.1.1. Species richness .....                                          | 11 |
| 4.1.2. Relative abundance of bacterial Species .....                   | 11 |
| 4.2. Antagonistic activity of bacterial isolates .....                 | 12 |
| CHAPTER 5. DISCUSSION .....                                            | 13 |
| 5.1. Taxonomy, occurrence, and one-health of the isolates .....        | 13 |
| 5.2. Diversity and dominance of the gut microbiota .....               | 15 |
| 5.3. Isolates antagonistic activity against <i>A. hydrophila</i> ..... | 16 |
| 5.4. Assay and methodological limitations .....                        | 17 |
| Conclusion .....                                                       | 18 |
| Recommendation .....                                                   | 18 |
| References .....                                                       | 19 |
| APPENDICES .....                                                       | a  |

**List of figures**

|                                                         |    |
|---------------------------------------------------------|----|
| Figure 1. Plates showing inhibition assay results. .... | 12 |
|---------------------------------------------------------|----|

## List of Tables

|                                                                      |    |
|----------------------------------------------------------------------|----|
| Table 1. Relative abundance (%) of bacterial species by sample ..... | 11 |
|----------------------------------------------------------------------|----|

## **List of appendices**

|                                                          |   |
|----------------------------------------------------------|---|
| Appendix 1. Gut extraction.....                          | a |
| Appendix 2. Primary culture plates growth. ....          | a |
| Appendix 3. Subcultured isolations. ....                 | a |
| Appendix 4. CFS inhibition assay setup inoculation. .... | b |
| Appendix 5. Table showing inhibition assay results. .... | b |

## Abstract

Bacterial diseases, particularly caused by *Aeromonas hydrophila*, remain a major constraint to Nile tilapia (*Oreochromis niloticus*) aquaculture. Management of infections relies on application of antibiotics, resulting in accelerated development and spread of antimicrobial resistance. The present study assessed the diversity and probiotic potential against *A. hydrophila* of gut-derived bacteria from Nile tilapia, using and the cell-free supernatant and satellitism assays. Mature Nile tilapia individuals from three aquaculture farms were dissected, and gut bacteria isolated and identified using culture conventional culture techniques and the VITEK 2 Compact system respectively. Nine bacterial species, namely *Staphylococcus gallinarum*, *S. sciuri*, *Micrococcus luteus*, *Kocuria kristinae*, *Bacillus subtilis*, *Aeromonas veronii*, *A. sobria*, *Rhizobium radiobacter*, and *Plesiomonas shigelloides*, were recorded. None of the five selected gram-positive isolates — *S. gallinarum*, *M. luteus*, *K. kristinae*, *B. subtilis*, and *S. sciuri* — showed inhibitory activity against *A. hydrophila* probably due to inhibitions associated with culture media and conditions. The study recommends improvement in culture techniques that have the potential of maintaining the antagonist activities.

# CHAPTER 1. INTRODUCTION

## 1.1. Background

Aquaculture is a critical sector in global food production (Khan *et al.*, 2011; Anderson *et al.*, 2017; Cantrell, 2023). Aquatic animals including fish are rich in bioavailable proteins and highly demanded to meet growing global population nutritional demand (Khan *et al.*, 2011; Anderson *et al.*, 2017; Cantrell, 2023). Global aquaculture production of animal species accounted for 51%, and Africa contributed 0.8% and 1.9% in 2022 and 2024 respectively (FAO, 2024). Uganda, second largest producer of Nile tilapia in sub-Saharan Africa after Egypt produced 101,377 metric tonnes with 15,000 tonnes from aquaculture (FAO, 2022). Nile tilapia (*Oreochromis niloticus*) accounted for 70% of Uganda's total aquaculture production (FAO, 2022). *Oreochromis niloticus* is a highly cultivated species in tropical and sub-tropical counties globally with production in over 90 countries (Prabu *et al.*, 2019; El-Sayed & Fitzsismmons, 2023). Merits of *O. niloticus* like rapid growth, adaptability to diverse environmental conditions, relative tolerance to diseases and economic value have led to adoption (FAO, 2021; El-Sayed & Fitzsismmons, 2023). However, Nile tilapia culture expansion continues to be hampered by bacterial diseases (Haenen *et al.*, 2023).

Global annual production loss exceeding USD 6 billion is majorly from bacterial infections (Maezono *et al.*, 2025). Among the main bacterial diseases impacting tilapia aquaculture include streptococcosis, Motile Aeromonad Septicaemia (MAS), francisellosis, columnaris, edwardsiellosis and vibriosis (Haenen *et al.*, 2023) caused by *Streptococcus agalactiae* and *S. iniae*, *Francisella orientalis*, *Flavobacterium columnare*, *Vibrio vulnificus*, motile *Aeromonas* spp. respectively (Beleke *et al.*, 2019; Haenen *et al.*, 2023). *Aeromonas hydrophila* is an important etiological agent of motile aeromonad septicemia (MAS), ulcerative disease, and hemorrhagic septicemia in Nile tilapia (Pridgeon & Klesius, 2011). Pool prevalence of *A. hydrophila* was reported at 36.0% across Africa (Zhou *et al.*, 2023) and at 43.8% in Ugandan fish farms (Wamala *et al.*, 2018), which implies an important infectious disease. Stress factors like elevated temperatures, high stocking densities foster vulnerability to opportunistically pathogenic *A. hydrophila* (Awan *et al.*, 2018; El-Banna *et al.*, (2022), causing average mortality rates of 30% (Beaz-Hidalgo & Figueras, 2013). Beyond direct mortalities, infections lead to reduced fish performance in feeding and growth, resulting in downgraded product quality (Zhou *et al.*, 2023).

Consequently, treatment and prophylactic disease management strategies are utilized to counteract and prevent bacterial diseases.

Intensification of aquaculture production to meet global demand has exacerbated usage of antibiotics to counteract bacterial diseases (Cabello, 2006; Schar *et al.*, 2020; Quesada *et al.*, 2013). Commonly used antibiotics in aquaculture worldwide are tetracycline, oxolinic acid, flumequine, sarafloxacin, enrofloxacin, amoxicillin, erythromycin, sulfadimethoxine, ormetoprim and florfenicol (Arthur *et al.*, 2019). However, imprudent usage of antibiotics has driven antimicrobial resistance (AMR) which lead to treatment failures (Wamala *et al.*, 2018). In response, alternative strategies to reduce antibiotics dependence include bacteriophage therapy, vaccination strategies, quorum quenching, prebiotics, chicken egg yolk antibody (IgY), plant therapy and probiotics are being explored (Bondad-Reantaso *et al.*, 2023). Due to environmental concerns, attention has shifted toward biological alternatives like probiotics that promote health in Nile tilapia culture.

Probiotics are beneficial bacteria that can improve and sustain health of Nile tilapia in aquaculture (Irianto & Austin, 2002). Species in the genera *Bacillus*, *Streptococcus*, *Enterococcus*, *Lactobacillus*, and *Lactococcus* have probiotic potential (Irianto & Austin, 2002). Probiotics improve resistance against major bacterial pathogens of tilapia, including *S. agalactiae* and *A. hydrophila* (Phuoc & Hai, 2022). Furthermore, dietary supplementation with probiotics showed enhanced growth performance, feed conversion efficiency, and innate immune responses in Nile tilapia (Santos *et al.*, 2023; Panase *et al.*, 2023). In Uganda, research on probiotics in aquaculture is in early stages hence a knowledge gap.

## **1.2. Statement of the problem**

Bacterial diseases remain a major constraint in aquaculture production, leading to significant economic losses and compromised fish health. Disease management has largely relied on the use of antibiotics for treatment and prevention. However, the widespread misuse of antibiotics drives development of antimicrobial resistance which results in disease treatment failure. In response, alternatives have been studied including probiotics, vaccines, immunostimulants, phages and herbal interventions. Probiotics have emerged as a promising alternative strategy for disease management, offering the potential to enhance host immunity, improve gut microbial balance, and inhibit pathogenic bacteria. Despite these advantages, Ugandan isolates remain unexplored, hence

a knowledge gap underscoring the need for systematic investigation of the probiotic potential of gut-dwelling bacteria as a sustainable and effective alternative to antibiotics.

### **1.3. Objectives of the study**

#### **1.3.1. General objective**

To assess the potential of gut bacteria in *O. niloticus* to inhibit *Aeromonas hydrophila* for adoption as probiotics for sustainable disease management in Uganda.

#### **1.3.2. Specific objectives**

1. To determine the diversity of cultivable bacteria from the gut of Nile tilapia.
2. To determine the in-vitro inhibition levels of the isolated bacteria against *Aeromonas hydrophila*.

### **1.4. Hypotheses**

1. The gut of Nile tilapia does not harbor diverse bacterial species.
2. The isolates have no significant inhibitory effect on *Aeromonas hydrophila*.

### **1.5. Significance of the study**

This study contributes to the exploration of probiotic-based pathogen and disease management strategies in addressing the growing challenge of *Aeromonas hydrophila* in Uganda's aquaculture, by identifying probiotic species capable of inhibiting the pathogen. In doing so, the findings support the development of sustainable, eco-friendly alternatives to antibiotics, reducing the risk of antimicrobial resistance while enhancing fish health and productivity. More broadly, the study contributes to SDG 2 (Zero Hunger) and SDG 3 (Good Health and Well-Being) by advancing probiotic-based alternatives to antibiotics that support sustainable aquaculture productivity and help combat antimicrobial resistance.

## CHAPTER 2. LITERATURE REVIEW

### 2.1. Morphology, ecology and environmental persistence

*Aeromonas hydrophila* is a Gram-negative, facultative anaerobic, motile rod belonging to the family *Aeromonadaceae*, and it is one of the most significant motile aeromonads associated with bacterial infections in freshwater fish globally (Janda & Abbott, 2010; Igbinosa *et al.*, 2012). *A. hydrophila* is naturally found in pond water, sediments, biofilms, and the skin, gills, and intestines of healthy or diseased fish (Austin & Austin, 2016; Ninh *et al.*, 2021). Adaptability allows *A. hydrophila* to survive in diverse salinities, temperatures, and nutrient levels, enabling persistence and dissemination within aquaculture systems (Beaz & Figueras, 2013). Though naturally part of the aquatic microbiota, pathogenicity of *A. hydrophila* is induced under stressful conditions such as overcrowding, temperature fluctuations, poor water quality, or nutritional stress that suppress host immunity (Rasmussen *et al.*, 2016; Ninh *et al.*, 2021).

Environmental stressors compromise fish mucosal barriers, increasing vulnerability to *A. hydrophila* infection and septicemia (Rauta *et al.*, 2011; Stratev & Odeyemi, 2016). Motility, biofilm formation, and secretion of extracellular enzymes such as proteases and lipases enhance *A. hydrophila* colonization and resistance to disinfection (Beaz & Figueras, 2013; Hossain *et al.*, 2018). Consequently, *A. hydrophila* remains one of the most economically important bacterial pathogens in aquaculture, contributing to severe production losses and increased antibiotic use, particularly in species like Nile tilapia (*O. niloticus*), African catfish (*Clarias gariepinus*), and common carp (*Cyprinus carpio*) (Wamala *et al.*, 2018; Adah *et al.*, 2024).

### 2.2. Clinical signs in cultured fish caused by *A. hydrophila*

*A. hydrophila* is the major causative agent of Motile Aeromonad Septicemia (MAS), an acute or chronic systemic bacterial infection affecting a broad range of freshwater fish, including tilapia, catfish, and carp (Austin & Austin, 2016; Wamala *et al.*, 2018). Acute infections may cause up to 100% mortality within days, often with few external signs, while chronic cases exhibit external hemorrhages, ulcers, and fin rot (Beaz & Figueras, 2013; Rasmussen *et al.*, 2016). Common clinical features include petechial hemorrhages, abdominal distension, exophthalmia, darkened coloration, and erratic swimming behavior (Stratev & Odeyemi, 2016; Igbinosa *et al.*, 2012).

Internally, infected fish display hepatomegaly, splenomegaly, and hemorrhagic lesions on visceral organs, consistent with systemic bacterial invasion (Beaz & Figueras, 2013; Rauta *et al.*, 2011).

Histopathological observations reveal severe hepatic necrosis, renal tubular damage, and splenic congestion, resulting from extracellular toxins such as hemolysins, aerolysin, and proteases that degrade host tissues and impair immunity (Hossain *et al.*, 2018; Rasmussen *et al.*, 2016). In Uganda, *A. hydrophila* outbreaks are frequent in farmed tilapia and catfish, causing high mortalities and economic losses due to poor fish quality and market rejection (Wamala *et al.*, 2018; Adah *et al.*, 2024). Co-infections with *Pseudomonas* and *Flavobacterium* species further complicate disease management (Wamala *et al.*, 2020). Environmental stressors such as poor water quality and temperature fluctuations (25–30°C) are strongly linked to MAS outbreaks (Nhin *et al.*, 2021). Recurrence of infections despite antibiotic interventions highlights the need for sustainable prevention strategies such as probiotics and improved biosecurity (Hoseinifar *et al.*, 2018; Calcagnile *et al.*, 2024).

### **2.3. Pathogenicity of *Aeromonas hydrophila***

The pathogenicity of *A. hydrophila* involves diverse virulence factors, host susceptibility, and environmental stressors. Critical determinants include aerolysin and hemolysin toxins, responsible for cell lysis and tissue necrosis leading to hemorrhagic lesions and septicemia (Rasmussen *et al.*, 2016; Beaz & Figueras, 2013). Enzymes such as proteases, lipases, and elastases contribute to host tissue degradation and immune evasion, while outer membrane proteins and flagellar structures enhance adhesion and motility on mucosal surfaces (Igbinosa *et al.*, 2012; Hossain *et al.*, 2018). Additionally, *A. hydrophila* synthesizes siderophores to acquire iron under limiting conditions and forms biofilms that protect against phagocytosis and antimicrobial agents (Stratev & Odeyemi, 2016; Adah *et al.*, 2024). Environmental stress, including high temperatures, poor water quality, and handling, upregulates virulence genes, intensifying disease outbreaks in aquaculture (Wamala *et al.*, 2018; Calcagnile *et al.*, 2024). These adaptive mechanisms enable *A. hydrophila* to persist as both a commensal and an opportunistic pathogen, making it a recurrent cause of Motile Aeromonad Septicemia and a persistent threat to sustainable fish production.

### **2.4. Probiotic bacteria and Nile tilapia**

The gastrointestinal tract of Nile tilapia (*Oreochromis niloticus*) harbors a diverse consortium of microorganisms that contribute significantly to host nutrition, digestion, and immunity. Within

this microbial community, probiotic bacteria defined as live microorganisms which when administered in adequate amounts, confer health benefits to the host and play a pivotal role in maintaining intestinal homeostasis and preventing pathogen colonization (Cavalcante *et al.*, 2020). The most frequently reported probiotic genera in tilapia include *Bacillus*, *Lactobacillus* (or *Lactiplantibacillus*), *Paenibacillus*, *Pediococcus*, *Pseudomonas*, and the yeast *Saccharomyces* (Hoseinifar *et al.*, 2024). *Bacillus* species, particularly *B. subtilis*, *B. amyloliquefaciens*, and *B. velezensis*, are preferred for the production of spores that survive pelleting and storage, while *Lactobacillus* spp. and *Pediococcus* spp. are valued for their capacity to colonize the gut mucosa and stimulate immune responses (Kuebutornye *et al.*, 2020). Such microbial taxa are often isolated from the intestine or pond water of healthy tilapia and screened for probiotic potential through a series of in vitro and in vivo assays.

## **2.5. Representative probiotic isolates in Tilapia**

Several strains have been identified with strong probiotic potential in tilapia culture. For instance, *Paenibacillus ehimensis* isolated from tilapia pond water, was reported to enhance fish growth, innate immune parameters, and survival following *Aeromonas hydrophila* challenge (Chen *et al.*, 2019). Likewise, indigenous *Bacillus* spp. such as *B. subtilis* and *B. amyloliquefaciens* have demonstrated broad-spectrum antibacterial activity and immunostimulatory effects when incorporated into tilapia diets (Macias *et al.*, 2024; Nayak *et al.*, 2024). Lactic acid bacteria such as *Lactiplantibacillus plantarum* can modulate gut microbial balance, improve feed utilization, and reduce infection severity upon pathogen exposure (Butprom *et al.*, 2013; Ren *et al.*, 2013). These findings emphasize the potential of both spore-forming and non-spore-forming probiotic taxa in the maintenance of tilapia health.

## **2.6. In vitro inhibitory effects against *A. hydrophila***

In vitro inhibition assays are often employed to screen for antagonistic probiotic strains. Common methods include agar well diffusion, spot-on-lawn, cell-free supernatant (CFS) testing, and co-culture assays. Several tilapia-derived isolates of *Bacillus*, *Paenibacillus*, and *Lactobacillus* have shown pronounced inhibitory effects against *A. hydrophila* under laboratory conditions (Chen *et al.*, 2019; Wang *et al.*, 2023). For example, *B. subtilis* isolates reduced pathogen growth by over 50% in co-culture, while CFS from *L. plantarum* disrupted *A. hydrophila* membrane integrity, leading to cell lysis and decreased cultivability (Ren *et al.*, 2013; Wang *et al.*, 2023). The

magnitude of inhibition often declines following neutralization or protease treatment, suggesting that both acidic and proteinaceous metabolites contribute to the observed antagonism (Midhun *et al.*, 2017).

## **2.7. Mechanisms of Pathogen Inhibition**

The inhibitory activity of probiotic bacteria against *A. hydrophila* is attributed to multiple, often overlapping mechanisms. First, several isolates produce bacteriocins or antimicrobial peptides, which exert bactericidal effects through pore formation or interference with cell wall synthesis; these are typically heat-stable yet protease-sensitive, confirming their proteinaceous nature (Chen *et al.*, 2019). Second, organic acid production, particularly lactic and acetic acids by lactic acid bacteria, lowers environmental pH and restricts the growth of Gram-negative bacteria such as *A. hydrophila* (Ren *et al.*, 2013). Third, extracellular hydrolytic enzymes such as proteases, lipases, and chitinases secreted by *Bacillus* and *Paenibacillus* species degrade bacterial biofilms and interfere with pathogenic adhesion (Lin *et al.*, 2022).

Furthermore, some probiotics exhibit competitive exclusion, effectively occupying mucosal attachment sites and depriving the pathogen of essential nutrients (Kuebutornye *et al.*, 2020). Emerging studies have also highlighted quorum-quenching activity, where probiotic metabolites degrade *A. hydrophila* signaling molecules, thereby suppressing virulence gene expression (Xu *et al.*, 2023). In addition, certain metabolites disrupt biofilm formation and increase pathogen susceptibility to environmental stresses and antimicrobial compounds (Wang *et al.*, 2023).

## **CHAPTER 3. MATERIALS AND METHODS**

### **3.1. Study area**

The experimental research was conducted at the Department of Zoology, Entomology and Fisheries Sciences microbiology laboratory, GPS coordinates 0°20'11.3"N 32°33'57.9"E in College of Natural Sciences, Makerere University. Makerere University is situated on Makerere hill, University parish, Kawempe North constituency in Kawempe division, Kampala district, Uganda.

### **3.2. Experimental design**

A two-objective qualitative research laboratory-based experiment isolated bacteria from the gut of healthy Nile tilapia and evaluated the inhibitory activity against *A. hydrophila* in-vitro. Primary and subcultures were done in triplicate while the antagonistic assays were done in duplicates and results were statistically analyzed using R studio and Microsoft excel programs.

#### **3.2.1. Sample collection**

Three healthy Nile tilapia denoted PA, KJ and TI, weighing  $537 \pm 106.1$  grams with total length of  $30.1 \pm 1$  centimeters, were collected aseptically from three unbiased selected aquaculture farms in Wakiso district using sterile scoop nets and euthanized using clove oil. Samples were placed in cooler box and properly iced to minimize microbial alteration and growth during transit to microbiology laboratory. All consequent handling procedures were conducted aseptically to prevent external contamination.

#### **3.2.2. Gut extraction**

Prior to dissection, fish samples were first surface-sterilized around incision area using 70% ethanol to eliminate surface contaminants. The abdominal cavity was subsequently opened along the ventral midline under aseptic conditions, and the entire gut carefully excised using sterile surgical forceps and scissors. The excised gut was stretched out on the dissection tray and mid gut located (Appendix 1).

#### **3.2.3. Primary culture and colony differentiation**

The mid guts were aseptically harvested independently from each of the three fish guts, thoroughly ground in a mortar and macerated then transferred independently into individually labelled sterile tubes containing sterile alkaline peptone water. Each sample was thoroughly vortexed and the

resultant supernatant from each independently processed sample was subsequently subjected to standard microbiological analysis. Using sterile swab sticks, the resultant supernatant was streaked onto fresh Tryptic Soy Agar plates, which were incubated aerobically at 30°C for 48 hours to facilitate bacterial growth (Appendix 2).

#### **3.2.4. Subculture and isolation of pure colonies**

Distinct bacterial colonies were differentiated based on macroscopic morphology including shape, margin, elevation, and color. Colonies were aseptically picked and streaked on fresh agar plates to obtain pure isolates (Appendix 3). The purity of each isolate was confirmed through consistent colony morphology and Gram-staining results. Pure isolates were preserved on blood base agar and nutrient agar at 4 °C for further identification and functional assays.

#### **3.2.5. Identification of bacterial species**

Pure isolates were subjected to biochemical characterization using the VITEK® 2 Compact system version 08.01 at Kiruddu national referral hospital. The system generated biochemical profiles automatically interpreted to species level, allowing identification of the species isolated. Bacterial diversity and richness of the mid gut bacterial community were assessed based on VITEK® 2 identification outcomes across all three fish samples.

### **3.3. In-vitro antagonistic activity assays**

#### **3.3.1. Preparation of pathogenic lawn**

*Aeromonas hydrophila* was obtained from College of Veterinary medicine, Animal resources and Biosecurity microbiology laboratory stock culture in Makerere University and confirmed using standard biochemical and morphological characteristics. The pathogen was maintained on Tryptic Soy Agar (TSA) at 4 °C and sub-cultured prior to use in inhibition assays. Using sterile swab sticks, fresh Mueller-Hinton agar plates were surface spread with a wet lawn of *A. hydrophila* at 10<sup>6</sup> CFU/ml compared with McFarland standard for the inhibition assays. Pathogenic species isolates I<sub>6</sub>, I<sub>7</sub>, I<sub>8</sub> and I<sub>9</sub> identified were excluded from the inhibition assays.

#### **3.3.2. Cell-Free Supernatant (CFS) inhibition assay**

The antimicrobial activity of CFS was assessed using the agar well diffusion method. Isolates were inoculated into Tryptic Soy Broth and incubated at 30°C for 48 hours. The cultures were then centrifuged at 5000 rpm for 10 minutes, and the supernatant filtered through 0.22 µm membranes

filter to obtain sterile cell-free supernatants (CFS). Four shallow wells (7 mm diameter) were bored halfway into Mueller-Hinton agar previously seeded with a wet lawn of *A. hydrophila* ( $10^6$  CFU/ml). Two wells were filled with 100  $\mu$ L of CFS, one with 30 $\mu$ g tetracycline antibiotic solution as the positive control and one was filled with sterile distilled water as a negative control and plates incubated at 30 °C for 24 hours (Appendix 4). Zones of inhibition around the wells were measured in millimeters as an indicator of inhibitory activity.

### 3.3.3. Satellitism inhibition assay

Inhibition satellitism method was used to evaluate the direct antagonistic interaction between isolate candidates and *A. hydrophila* on a cell-to-cell basis. Mueller-Hinton agar plates seeded with wet lawn of *A. hydrophila* ( $10^6$  CFU/ml) were streaked across the surface to introduce the isolates. Inoculated plates were incubated at 30 °C for 24 hours, and the presence of visible inhibition zones or satellite growth suppression around the isolate streaks were recorded as evidence of antagonism. The intensity and consistency of inhibition zones were measured in millimeters and compared across inhibition assay plates to identify the most effective probiotic candidates. Only Isolates 1 – 5 were tested against the pathogen in the antagonism assays whereas isolates 6 – 9 were exempted due to their pathogenicity record.

## 3.4. Data analysis

Data was entered and managed in Microsoft Excel and analyses were performed in R Studio (v2026.06.0+242), with outputs presented in tabular form. Diversity of bacteria in the gut of Nile tilapia was recorded as species richness of distinct bacterial species identified across each sample. Relative abundance of identified bacterial species per sample was calculated using formula;

$$\text{Relative Abundance} = (\text{Number of colonies of isolate } x \div \text{Total number of colonies of all isolates in sample } y) \times 100\%.$$

Invitro inhibition zone diameters (mm) of *A. hydrophila* observed from both the Cell-Free Supernatant assay and the inhibition satellitism assay constituted the primary outcome variable for specific objective two. One-way ANOVA with Tukey's HSD post-hoc were rendered inapplicable because the dataset exhibited no variance. Results were instead reported descriptively as mean  $\pm$  standard deviation (Appendix 5).

## CHAPTER 4. RESULTS

### 4.1. Diversity and composition of gut Isolates

#### 4.1.1. Species richness

Nine bacteria species were identified across the three samples, with at least six cultivable species recorded from TI and KJ, while five from PA. The bacteria consisted of five gram-positive species: *Staphylococcus gallinarum*, *Micrococcus luteus*, *Kocuria kristinae*, *Bacillus subtilis* and *Staphylococcus sciuri* and four gram-negative species namely; *Aeromonas veronii*, *Aeromonas sobria*, *Rhizobium radiobacter* and *Pleisomonas shigelloides* (Table 1).

#### 4.1.2. Relative abundance of bacterial Species

Three species *S. gallinarum*, *K. kristinae* and *S. sciuri* were ubiquitous across all samples. Two species were found in only two samples: *M. luteus* was present in PA (16.4%) and KJ (11.8%) but absent from TI, while *A. veronii* occurred in TI (21.3%) and KJ (51.6%) but was absent from PA. The remaining four species were sample-specific. *R. radiobacter* (32.8%) and *P. shigelloides* (21.3%) occurred only in TI, *A. sobria* (52.9%) occurred only in PA, and *B. subtilis* (10.8%) occurred only in KJ. All three of the species that occurred across the samples were gram-positive, whereas three of the four sample-specific species were gram-negative. *A. sobria* recorded the highest sample percentage abundance unlike *S. gallinarum* which recorded the least in this study.

Table 1. Relative abundance (%) of bacterial species by sample

| Bacterial species                | Gram reaction | TI    | PA    | KJ    |
|----------------------------------|---------------|-------|-------|-------|
| <i>Staphylococcus gallinarum</i> | +             | 3.3%  | 3.9%  | 5.4%  |
| <i>Staphylococcus sciuri</i>     | +             | 10.9% | 7.7%  | 4.3%  |
| <i>Kocuria kristinae</i>         | +             | 10.4% | 19.2% | 16.1% |
| <i>Micrococcus luteus</i>        | +             | —     | 16.4% | 11.8% |
| <i>Bacillus subtilis</i>         | +             | —     | —     | 10.8% |
| <i>Aeromonas veronii</i>         | -             | 21.3% | —     | 51.6% |
| <i>Aeromonas sobria</i>          | -             | —     | 52.9% | —     |
| <i>Rhizobium radiobacter</i>     | -             | 32.8% | —     | —     |
| <i>Pleisomonas shigelloides</i>  | -             | 21.3% | —     | —     |

## 4.2. Antagonistic activity of bacterial isolates

No measurable zone of inhibition was recorded across both satellitism and cell free supernatant assays for all tested bacteria hence no significant inhibitory effect against *A. hydrophila*. Positive control (tetracycline) produced consistent zone of inhibition diameter  $\pm$  SD of  $21.7 \pm 1.1$  mm, while the negative control showed no inhibition.

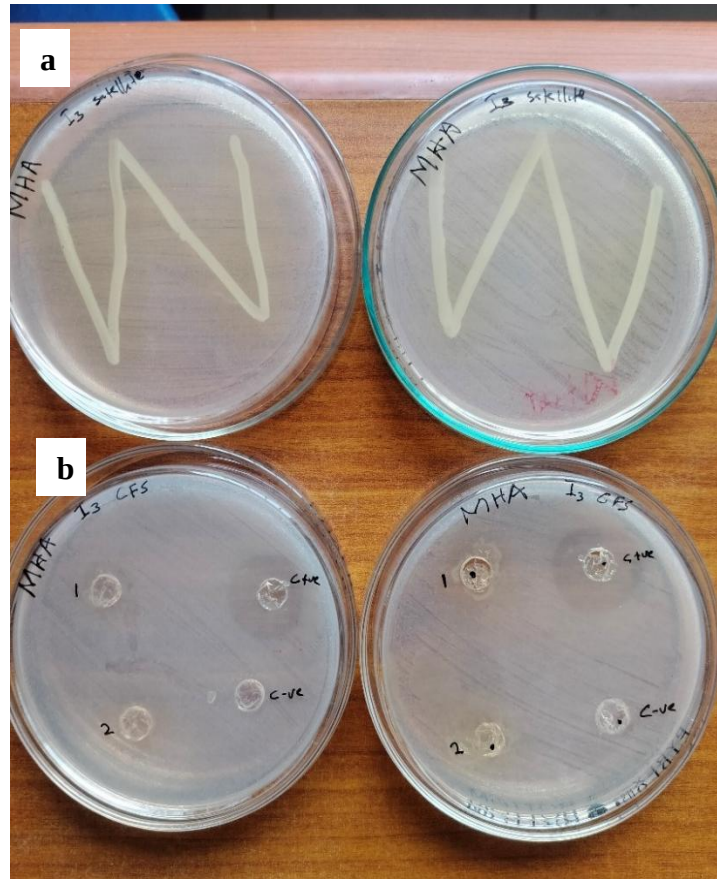


Figure 1. Plates showing inhibition assay results, (a) satellitism assay and (b) shows cell free supernatant assay post incubation with only positive control well exhibiting a halo.

## CHAPTER 5. DISCUSSION

### 5.1. Taxonomy, occurrence, and one-health of the isolates

The diversity of bacteria reflects poor health in fish (Xavier *et al.*, 2024; Diwan *et al.*, 2022). Several bacteria identified carry direct implications for health risk, as several of the recovered taxa are linked to zoonotic potential and antimicrobial resistance in tilapia (Haenen *et al.*, 2023). Presence of opportunistic bacteria signals a heightened risk of disease outbreak under stressful rearing conditions which favor pathogen proliferation in fish (Xavier *et al.*, 2024; dos Santos *et al.*, 2023). The present study recorded nine bacterial species from Nile tilapia gut belonging to three phyla. Phylum Bacillota comprised *Staphylococcus gallinarum*, *Staphylococcus sciuri*, and *Bacillus subtilis*; Phylum Actinomycetota comprised *Micrococcus luteus* and *Kocuria kristinae*; and Phylum Pseudomonadota comprised *Aeromonas veronii*, *Aeromonas sobria*, *Rhizobium radiobacter*, and *Plesiomonas shigelloides*. These three phyla are consistently reported as core constituents of the Nile tilapia gut microbiota across independent studies (Hossain *et al.*, 2021).

*Staphylococcus* species within the Phylum Bacillota, Class Bacilli, Order Bacillales, Family Staphylococcaceae (Devriese *et al.*, 1983) exhibit pathogenic potential that varies considerably by species, ranging from harmless skin commensals to opportunistic pathogens capable of infection (Becker *et al.*, 2014). *Staphylococcus gallinarum* has been isolated from aquatic environments and retail market fish (Devriese *et al.*, 1983; Cole *et al.*, 2021.) with no documented fish pathogenicity, but carries human relevance as a rare opportunistic pathogen causing bacteraemia in immunocompromised patients (Yu *et al.*, 2008; Morfín-Otero *et al.*, 2012). In contrast, *Staphylococcus sciuri*, is highly pathogenic agent in fish causing disease outbreaks characterised by superficial haemorrhage, liver damage, and significant mortality in hybrid sturgeon, (Zhang *et al.*, 2022), though no equivalent pathogenicity study has yet been conducted in Nile tilapia specifically. Human health relevance of *S. sciuri* lies less in direct infection but more as a reservoir of the *mecA* methicillin-resistance gene, a silent contributor to antimicrobial-resistance (Becker *et al.*, 2014).

*Bacillus subtilis* belongs to the Family Bacillaceae within the Phylum Bacillota and Class Bacilli, Order Bacillales (Bhandari *et al.*, 2013). *B. subtilis* is ubiquitous in soil, water, and the gastrointestinal tracts of animals and is frequently isolated from the intestines of healthy fish (Earl

*et al.*, 2008). No fish pathogenicity is documented for *B. subtilis* but rather improves fish growth and disease resistance as a probiotic (Chen *et al.*, 2019; Lin *et al.*, 2022). Dietary supplementation with *B. subtilis* improved growth, immune function, digestive enzyme activity, and antioxidant status through secretion of antimicrobial peptides and bacteriocins that inhibit pathogens and support gut microbial balance *in vivo* (Yu *et al.*, 2025; Olmos *et al.*, 2020). Furthermore, *B. subtilis* holds Qualified Presumption of Safety (QPS) since 2007, reflecting a similarly low disease-risk profile in humans (EFSA Panel on Biological Hazards, 2008).

*Micrococcus luteus* occurs as a ubiquitous commensal of skin, mucosae, soil, and water (Nouioui *et al.*, 2018) and in fish tissues (Abd El-Rhman *et al.*, 2009), belonging to the Phylum Actinomycetota, Class Actinomycetes, Order Micrococcales and Family Micrococcaceae. *M. luteus* is an opportunistic pathogen that induce gill hyperplasia, hepatic/renal degeneration, and mortality in farmed Nile tilapia (Suresh *et al.*, 2025). Despite inducing pathologies in fish, *M. luteus* can inhibit growth of *A. hydrophila* (Abd El-Rhman *et al.*, 2009). In humans, *M. luteus* is an established cause of bloodstream infection in immunocompromised patients (Zhu *et al.*, 2021).

*Kocuria kristinae* is an emerging fish bacteria pathogen particularly to marine species (Meng *et al.*, 2023). *Kocuria kristinae* has been documented as a rare, catheter-associated cause of urinary tract infection in humans (Tewari *et al.*, 2013) and confirmed pathogenicity to marine fish causing dose-dependent mortality (Meng *et al.*, 2023).

*Aeromonas veronii* and *Aeromonas sobria*, in the Phylum Pseudomonadota, Class Gammaproteobacteria, Order Aeromonadales and Family Aeromonadaceae, are closely related (Janda & Abbott, 2010; Sherif & Kassab, 2023). *A. veronii* occurs in freshwater and sediment, colonizing the gut of both healthy and diseased fish (Janda & Abbott, 2010). *A. veronii* causes motile Aeromonad septicaemia with haemorrhagic lesions, fin/tail rot, and abdominal distension, and outbreaks reaching 100% mortality in tilapia (dos Santos *et al.*, 2023). In humans, *A. veronii* is a recognized zoonotic pathogen causing sepsis and gastroenteritis, particularly in vulnerable groups (Janda & Abbott, 2010). Similarly, *A. sobria* is widely distributed across aquatic products and shows notable environmental resilience (Igbinosa *et al.*, 2012; Liu *et al.*, 2025), and is established as the cause of tail-rot disease in juvenile Nile tilapia, with an experimentally determined LD<sub>50</sub> (Li & Cai, 2011). Clinical manifestations extend to extensive haemorrhage of the gills, heart, liver, spleen, and intestines, reflecting a systemic haemorrhagic septicaemia (Liu *et*

*al.*, 2025). In humans, *A. sobria* is clinically relevant as one of the principal *Aeromonads* responsible for gastroenteritis, wound infections, and septicaemia (Janda & Abbott, 2010).

*Plesiomonas shigelloides*, of Order Enterobacterales and Family Enterobacteriaceae, is the sole member of genus *Plesiomonas* (Janda *et al.*, 2016). The species has been recoded recently and an emerging pathogen in commercial catfish aquaculture (Mohammed *et al.*, 2026). In humans, *P. shigelloides* is a well-established pathogen causing gastroenteritis typically linked to consumption of contaminated aquatic food (Janda *et al.*, 2016).

On the other hand, *Rhizobium. radiobacter* belongs to Class Alphaproteobacteria, Order Hyphomicrobiales in the Family Rhizobiaceae (Flores-Félix *et al.*, 2020). *R. radiobacter* occurs predominantly as a soil- and plant-associated organism, primarily in terrestrial than aquatic environments (Liu *et al.*, 2025). There is no documented pathogenicity of the bacterial to aquatic hosts. However, the species is recognized as an opportunist bacteria causing bacteraemia and peritonitis in immunocompromised patients and poss a serious human risk associated with catheter operations (Liu *et al.*, 2025).

## **5.2. Diversity and dominance of the gut microbiota**

Six cultivable species were isolated from two samples and five species from one sample making nine isolates across all three samples. Species richness outcomes of this study reflect that the culture-dependent approach used recovers only a small proportion of the taxa rather than a definitive measure of true diversity (Kanika *et al.*, 2025). In a comparable setting, high-throughput 16S rRNA sequencing of shrimp pond water resolved 2,498 unique taxonomic units, yet only 15 isolates were ultimately recovered by culturing (Ali *et al.*, 2022). Species recovered here therefore plausibly represent a fraction of the bacteria actually present in the tilapia gut, since culture-dependent methods consistently miss taxa that molecular techniques can detect (Ali *et al.*, 2022). Individual host variation and the specific aquaculture environment from which each sample was drawn also contribute to the species richness observed (Kokou *et al.*, 2018). Furthermore, culture-system types also shape gut bacterial community structure in farmed fish species (Vala *et al.*, 2024). Gut samples of *Pangasius pangasius* cultured in biofloc, pond and cage systems yielded significant differences between genera which highlights the culture environmental influence on gut bacterial diversity (Vala *et al.*, 2024).

Numerical dominance of *Aeromonas veronii* and *Aeromonas sobria* (51.6% and 52.9%, respectively), is a consistently recognized status of the bacteria as ubiquitous, opportunistic inhabitants of fish gut community (Janda & Abbott, 2010). Similar pattern was reported by Newaj-Fyzul *et al.* (2008), who found *Aeromonas* spp. as the second most prevalent genus (44.0%) in tilapia fish-slurry isolates. Both species possess a broad genomic and physiological toolkit that favours proliferation including large complements of virulence and upto 60 antimicrobial-resistance genes across 22 drug classes (da Silva Maia *et al.*, 2023). This competitive advantage is reflected in field data showing *A. veronii* as the single most common species among motile aeromonads recovered from septicemic fish (Beaz-Hidalgo & Figueras, 2013), and in documented outbreaks in which it reached 100% mortality in farmed tilapia (dos Santos *et al.*, 2023). *A. sobria* shows a parallel pattern, tolerating a wide range of pH, temperature, and salinity conditions that allow for persistence and dominance across diverse aquatic niches (Igbinosa *et al.*, 2012). Combined dominance of the bacteria in two of the three samples reflects an opportunistic bloom favored by environmental ubiquity and a high fitness (Janda & Abbott, 2010), which is also broadly consistent with patterns reported elsewhere in tilapia gut/organ surveys (Chitambo *et al.*, 2023; Wu *et al.*, 2020)

### **5.3. Isolates antagonistic activity against *A. hydrophila***

Five gram positive isolates namely; *S. gallinarum*, *S. sciuri*, *K. kristinae*, *M. luteus* and *B. subtilis* were tested against *A. hydrophila* and no visible inhibition zone, as a sign of antagonism, was recorded in this study. Consistently with other studies, *S. gallinarum*, *S. sciuri* and *K. kristinae* have no reported antagonistic or immunostimulatory probiotic activity, in for instance, a meta-analysis pooling data from 67 studies (Saba *et al.*, 2024) and systematic reviewed data from 79 studies on probiotics for disease management (Ntakirutimana *et al.*, 2025) in Nile tilapia comparable aquaculture species. However, *S. gallinarum*, *S. sciuri*, and *K. kristinae* have each been subjected to probiotic-relevant screening in non-piscine contexts namely; fermented foods (Dhanya Raj *et al.*, 2023), dairy products (syn. *Mammaliicoccus sciuri*; Naqqash *et al.*, 2022), and the bovine reproductive tract (Styková *et al.*, 2016) respectively. The bacteria rather exhibit pathogenic implications in fish than beneficial ones especially *S. sciuri* and *K. kristinae* (Zhang *et al.*, 2022); Meng *et al.*, 2023). Inhibitory mechanisms documented for established tilapia probiotics are largely genus-specific: *Bacillus* spp. reduced *A. hydrophila* growth by over 50% in co-culture through bacteriocins and hydrolytic-enzyme production (Chen *et al.*, 2019; Lin *et al.*,

2022). Lactic acid bacteria such as *Lactiplantibacillus plantarum* achieved inhibition through organic-acid-mediated pH suppression and cell-free-supernatant-induced membrane disruption (Ren *et al.*, 2013; Wang *et al.*, 2023) and quorum-quenching activity against *Aeromonas* signaling molecules (Xu *et al.*, 2023).

*M. luteus* and *B. subtilis* on the other hand, have documented antagonism against *Aeromonas* pathogens specifically in Nile tilapia. *M. luteus* isolated from tilapia gonads and intestine has previously produced a 4 cm inhibition zone against *A. hydrophila*, and reduced experimental mortality from 80–90% to 25% following a challenge (Abd El-Rhman *et al.*, 2009). *Bacillus* is the genus most consistently linked to *A. hydrophila* inhibition in the wider literature (Chen *et al.*, 2026). However, antagonism is a strain-specific outcome traceable to variable biosynthetic gene cluster (BGC) content and expression; among 111 *Bacillus*-like strains screened in a study, a single isolate, *B. velezensis* FJAT-57093 showed strong inhibitory activity against *A. hydrophila* (Chen *et al.*, 2026). Furthermore, Kieseewalter *et al.* (2021) isolated 23 *B. subtilis* strains from 11 sampling sites and found that nonribosomal peptide production varied even among strains co-isolated from the same soil sample. Failure of pathogen inhibition could therefore be a result of protocol used in this study (Chen *et al.*, 2026).

#### **5.4. Assay and methodological limitations**

The absence of inhibitory activity in *M. luteus* and *B. subtilis* is also attributed to protocol limitations rather than a true absence of antagonistic capacity. Chen *et al.* (2026) used near-identical conditions to obtain CFS from *B. velezensis* against *A. hydrophila*, yet raw supernatant was only weakly active limiting even in a confirmed producer until concentrated to 40 mg/mL. Medium choice compounds this across both isolates, since Mueller-Hinton agar, while diffusion-optimal, is nutritionally leaner than the enriched media shown to maximize metabolite output elsewhere (Kieseewalter *et al.*, 2021). Optimal *B. subtilis* antimicrobial yield further required an agitated, nutrient-defined medium (corn flour, soybean meal, NaCl; 220 rpm), conditions markedly richer than the static TSB used here (Wu *et al.*, 2023). Even bypassing supernatant concentration entirely, live-cell screening recovered antagonism in fewer than half of *Bacillus* isolates tested by Zhang *et al.* (2019), confirming strain-level variability independent of assay format. *M. luteus* required marine broth at 37 °C for up to seven days (Dugyala & Kuthadi, 2024), and the pigment-

associated active compound recovered from the cell pellet rather than filtered supernatant (Majeed, 2017), both excluded by the present 48-hour, 0.22  $\mu\text{m}$ -filtered CFS format.

## Conclusion

This study isolated and Identified bacteria from the gut of three randomly sampled Nile tilapia, and tested for their probiotic potential against gram negative pathogen *A. hydrophila*. Nine cultivable bacteria species were isolated and species richness in the samples ranged 5 to 6, while *A. veronii* and *A. sobria* dominated relative abundance, together reflecting a modest diversity, opportunist-favoring gut community consistent with compromised host health rather than a stable, beneficial microbiota. Tested isolates exhibited no antagonism invitro against the pathogen and results attributed to combination of strain-level variability and protocol-related limitations — unconcentrated supernatant, suboptimal culture medium, and short harvest duration — that likely masked strain-specific antagonistic potential.

## Recommendation

Future probiotic-screening work with these isolates should directly address the protocol gaps identified in this study rather than repeat a single generic protocol across all isolates. Cell-free supernatants should be concentrated before testing — via acid precipitation, lyophilization, or ultrafiltration — since raw, unconcentrated supernatant has been shown to underestimate activity even in a confirmed *Aeromonas hydrophila*-inhibiting strain (Chen et al., 2026). Growth conditions should also be tailored per isolate rather than standardized: *B. subtilis* metabolite yield responds strongly to agitated, nutrient-defined media (Wu et al., 2023), while *M. luteus* requires substantially longer incubation in enriched marine-type broth to reach peak activity (Dugyala & Kuthadi, 2024). Because *M. luteus* antibacterial activity has been linked to a cell-associated pigment rather than a freely secreted compound (Majeed, 2017), future assays should include a solvent-extracted cell-pellet fraction alongside filtered supernatant. Diffusion-based assays alone are also insufficient, since they cannot detect non-diffusible mechanisms; isolates should additionally be screened for competitive exclusion and mucosal adhesion (Knipe et al., 2021). Finally, richer culture media more representative of literature-optimized conditions (Kiesewalter et al., 2021) and live-cell screening formats (Zhang et al., 2019) should be used alongside CFS assays, since strain-level variability persists even when concentration and medium limitations are addressed.

## References

- Abd El-Rhman, A. M., Khattab, Y. A. E., & Shalaby, A. M. E. (2009). *Micrococcus luteus* and *Pseudomonas* species as probiotics for promoting the growth performance and health of Nile tilapia, *Oreochromis niloticus*. *Fish & Shellfish Immunology*, 27(2), 175–180. <https://doi.org/10.1016/j.fsi.2009.03.020>
- Adah, D. A., Ajoa, A. J., & Olobo, J. O. (2024). *Molecular characterization and antibiotic resistance of Aeromonas species isolated from farmed Clarias in Uganda*. *Journal of Aquatic Animal Health*, 36(2), 145–157.
- Akoll, P., & Mwanja, W. (2012). Fish health status, research and management in East Africa: past and present. *African Journal of Aquatic Science*, 37(2), 117–129. <https://doi.org/10.2989/16085914.2012.694628>
- Ali, S., Xie, J., Zada, S., Hu, Z., Zhang, Y., Cai, R., & Wang, H. (2022). Bacterial community structure and bacterial isolates having antimicrobial potential in shrimp pond aquaculture. *AMB Express*, 12, 76. <https://doi.org/10.1186/s13568-022-01423-9>
- Anderson, J. L., Asche, F., Garlock, T., & Chu, J. (2017). *Aquaculture: Its role in the future of food*. World Agricultural Resources and Food Security: International Food Security. Emerald Publishing Limited.
- Arthur, R., Alday-Sanz, V., Zarza, C., Karunasagar, I., Hine, M., Hurchzermeyer, D., ... & Reantaso, M. B. (2019). Recommendations for prudent and responsible use of veterinary medicines in aquaculture. *FAO Technical Guidelines for Responsible Fisheries. Aquaculture Development*, 1-35.
- Austin, B., & Austin, D. A. (2016). *Bacterial Fish Pathogens: Disease of Farmed and Wild Fish* (6th ed.). Springer.
- Awan, F., Dong, Y., Wang, N., Liu, J., Ma, K., & Liu, Y. (2018). The fight for invincibility: environmental stress response mechanisms and *Aeromonas hydrophila*. *Microbial pathogenesis*, 116, 135-145.
- Beaz-Hildago, R., & Figueras, M. J. (2013). *Aeromonas* spp. whole genomes and virulence factors implicated in fish disease. *Journal of Fish Diseases*, 36(4), 371–388. <https://doi.org/10.1111/jfd.12025>
- Becker, K., Heilmann, C., & Peters, G. (2014). Coagulase-negative staphylococci. *Clinical Microbiology Reviews*, 27(4), 870–926. <https://doi.org/10.1128/CMR.00109-13>

- Bekele, B., Workagegn, K. B., & Natarajan, P. (2019). Prevalence and antimicrobial susceptibility of pathogenic bacteria in Nile tilapia, *Oreochromis niloticus* L. *International Journal of Aquaculture and Fishery Sciences*, 5(4), 22-26.
- Bhandari, V., Ahmod, N. Z., Shah, H. N., & Gupta, R. S. (2013). Molecular signatures for *Bacillus* species: Demarcation of the *Bacillus subtilis* and *Bacillus cereus* clades in molecular terms and proposal to limit the placement of new species into the genus *Bacillus*. *International Journal of Systematic and Evolutionary Microbiology*, 63(7), 2712–2726. <https://doi.org/10.1099/ijs.0.048488-0>
- Bondad-Reantaso, M. G., MacKinnon, B., Karunasagar, I., Fridman, S., & Subasinghe, R. (2023). Review of alternatives to antibiotic use in aquaculture. *Reviews in Aquaculture*, 15(4), 1421–1451. <https://doi.org/10.1111/raq.12786>
- Butprom, P., Phumkhachorn, P., & Rattanachaikunsopon, P. (2013). Effect of *Lactobacillus plantarum* C014 on innate immune parameters and disease resistance of hybrid tilapia (*Oreochromis niloticus* × *O. aureus*). *Fish & Shellfish Immunology*, 35(2), 599–606.
- Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental microbiology*, 8(7), 1137-1144.
- Calcagnile, M., Tredici, S. M., & Alifano, P. (2024). A comprehensive review on probiotics and their use in aquaculture: Biological control, efficacy, and safety through the genomics and wet methods. *Heliyon*, 10(24).
- Cantrell, D. (2023). Aquaculture: Sustainable food production. In *The Palgrave Handbook of Global Sustainability* (pp. 679-701). Cham: Springer International Publishing.
- Cavalcante, R. B., Lima, F. R. S., Batista, J. M., & de Souza, R. L. (2020). Probiotics, prebiotics and synbiotics for Nile tilapia: Growth and disease resistance—a review. *Aquaculture Reports*, 16, 100243.
- Chakraborty, N., Das, B. K., Bera, A. K., Borah, S., Mohanty, D., Yadav, A. K., Kumar, J., Koushlesh, S. K., Chanu, T. N., Panda, S. P., & Vallangi, R. (2022). Co-Prevalence of Virulence and Pathogenic Potential in Multiple Antibiotic Resistant *Aeromonas* spp. from Diseased Fishes with In Silico Insight on the Virulent Protein Network. *Life*, 12(12), 1979. <https://doi.org/10.3390/life12121979>

- Chen, S. W., Wang, P. C., Tsai, M. A., & Chen, S. C. (2019). Dietary administration of probiotic *Paenibacillus ehimensis* NPUST1 enhances growth, immunity, and resistance against *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 511, 734236.
- Chen, Y., Li, S., Li, W., Zheng, X., Chen, M., Liu, X., Lan, J., & Wang, J. (2026). Probiotic properties in vitro of *Bacillus velezensis* FJAT-57093 with antibacterial activity against the aquatic pathogen *Aeromonas hydrophila*. *Microorganisms*, 14(1), 41. <https://doi.org/10.3390/microorganisms14010041>
- Chitambo, B., Munyeme, M., & Hang'ombe, B. (2023). Identifying bacteria with public health significance from farmed Nile tilapia (*Oreochromis niloticus*), Zambia. *International Journal of Microbiology*, 2023, 6650378. <https://doi.org/10.1155/2023/6650378>
- Cole, J., Sudha, S., Shome, B. R., Sivaraman, G. K., Holmes, M. A., & Muneeb, K. H. (2021). Virulence and intermediate resistance to high-end antibiotic (teicoplanin) among coagulase-negative staphylococci sourced from retail market fish. *Archives of Microbiology*, 203, 5695–5702. <https://doi.org/10.1007/s00203-021-02558-2>
- da Silva Maia, J. C., de Albuquerque Silva, G. A., de Barros Cunha, L. S., Gouveia, G. V., Góes-Neto, A., Brenig, B., Araújo, F. A., Aburjaile, F., Ramos, R. T. J., Soares, S. C., de Carvalho Azevedo, V. A., da Costa, M. M., & de Simoni Gouveia, J. J. (2023). Genomic characterization of *Aeromonas veronii* provides insights into taxonomic assignment and reveals widespread virulence and resistance genes throughout the world. *Antibiotics*, 12(6), 1039. <https://doi.org/10.3390/antibiotics12061039>
- Devriese, L. A., Poutrel, B., Kilpper-Bälz, R., & Schleifer, K. H. (1983). *Staphylococcus gallinarum* and *Staphylococcus caprae*, two new species from animals. *International Journal of Systematic Bacteriology*, 33(3), 480–486. <https://doi.org/10.1099/00207713-33-3-480>
- Dhanya Raj, C. T., Kandaswamy, S., Suryavanshi, M. V., Ramasamy, K. P., Rajasabapathy, R., & Arthur James, R. (2023). Genomic and metabolic properties of *Staphylococcus gallinarum* FCW1 MCC4687 isolated from naturally fermented coconut water towards GRAS assessment. *Gene*, 867, Article 147356. <https://doi.org/10.1016/j.gene.2023.147356>
- Diwan, A. D., Harke, S. N., Gopalkrishna, & Panche, A. N. (2022). Aquaculture industry prospective from gut microbiome of fish and shellfish: an overview. *Journal of animal physiology and animal nutrition*, 106(2), 441-469 <https://doi.org/10.1111/jpn.13619>

- dos Santos, S. B., Alarcon, M. F., Ballaben, A. S., Harakava, R., Galetti, R., Guimarães, M. C., Natori, M. M., Takahashi, L. S., Ildefonso, R., & Rozas-Serri, M. (2023). First report of *Aeromonas veronii* as an emerging bacterial pathogen of farmed Nile tilapia (*Oreochromis niloticus*) in Brazil. *Pathogens*, 12(8), 1020. <https://doi.org/10.3390/pathogens12081020>
- Dugyala, Y. B., & Kuthadi, S. K. (2024). Antibacterial activity of *Micrococcus luteus* YSD1 isolated from Velankanni water source, Tamil Nadu. *Acta Scientific Biotechnology*, 5(2), 26–33.
- Earl, A. M., Losick, R., & Kolter, R. (2008). Ecology and genomics of *Bacillus subtilis*. *Trends in Microbiology*, 16(6), 269–275. <https://doi.org/10.1016/j.tim.2008.03.004>
- EFSA Panel on Biological Hazards (BIOHAZ). (2008). The maintenance of the list of QPS microorganisms intentionally added to food or feed: Scientific opinion of the Panel on Biological Hazards. *EFSA Journal*, 6(12), 923. <https://doi.org/10.2903/j.efsa.2008.923>
- El-Banna, N. I., Hafez, E. E., & Abdel-Salam, H. M. (2022). Antimicrobial resistance of *Aeromonas hydrophila* isolated from fish farms in Africa: A growing challenge. *Microorganisms*, 13(3), 485. <https://doi.org/10.3390/microorganisms13030485>
- El-Sayed, A. F. M., & Fitzsimmons, K. (2023). From Africa to the world—The journey of Nile tilapia. *Reviews in Aquaculture*, 15, 6-21.
- FAO (2021). *The State of World Fisheries and Aquaculture 2021*. Food and Agriculture Organization of the United Nations.
- FAO (2022). *Fisheries and aquaculture country Profile - Uganda*. <https://www.fao.org/fishery/en/facp/uga>
- FAO (2024). *The State of World Fisheries and Aquaculture 2021. Blue transformation in action*. Food and Agriculture Organization of the United Nations.
- Flores-Félix, J. D., Menéndez, E., Peix, A., García-Fraile, P., & Velázquez, E. (2020). History and current taxonomic status of genus *Agrobacterium*. *Systematic and Applied Microbiology*, 43(1), 126046. <https://doi.org/10.1016/j.syapm.2019.126046>
- Haenen, O. L., Dong, H. T., Hoai, T. D., Crumlish, M., Karunasagar, I., Barkham, T., ... & Bondad-Reantaso, M. G. (2023). Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance. *Reviews in Aquaculture*, 15, 154-185.
- Hoseinifar, S. H., Doan, H. V., Harikrishnan, R., & Ringø, E. (2024). Promising probiotic candidates for sustainable aquaculture: A review. *Frontiers in Microbiology*, 15, 1448753.

- Hoseinifar, S. H., Sun, Y. Z., Wang, A., & Zhou, Z. (2018). Probiotics as means of diseases control in aquaculture: A review. *Frontiers in Microbiology*, 9, 2429. <https://doi.org/10.3389/fmicb.2018.02429>
- Hossain, M. J., *et al.* (2019). Virulent *Aeromonas hydrophila* outbreaks in the U.S. catfish industry: Causes and consequences. *World Aquaculture Magazine*, 50(3), 35–38.
- Hossain, S., Rahman, M., & Kibria, A. S. M. (2018). Pathogenicity and molecular identification of *Aeromonas hydrophila* isolated from diseased tilapia in Bangladesh. *Aquaculture Reports*, 12, 25–32.
- Hossain, T. J., Das, M., Ali, F., Chowdhury, S. I., & Zedny, S. A. (2021). Substrate preferences, phylogenetic and biochemical properties of proteolytic bacteria present in the digestive tract of Nile tilapia (*Oreochromis niloticus*). *AIMS Microbiology*, 7(4), 528–545. <https://doi.org/10.3934/microbiol.2021032>
- Igbinosa, I. H., Igumbor, E. U., Aghdasi, F., Tom, M., & Okoh, A. I. (2012). Emerging *Aeromonas* species infections and their significance in public health. *The Scientific World Journal*, 2012, 625023. <https://doi.org/10.1100/2012/625023>
- Irianto, A., & Austin, B. (2002). Probiotics in aquaculture. *Journal of fish diseases*, 25(11), 633–642. <https://doi.org/10.1046/j.1365-2761.2002.00422.x>
- Janda, J. M., & Abbott, S. L. (2010). The genus *Aeromonas*: taxonomy, pathogenicity, and infection. *Clinical Microbiology Reviews*, 23(1), 35–73. <https://doi.org/10.1128/CMR.00039-09>.
- Janda, J. M., Abbott, S. L., & McIver, C. J. (2016). *Plesiomonas shigelloides* revisited. *Clinical Microbiology Reviews*, 29(2), 349–374. <https://doi.org/10.1128/CMR.00103-15>
- Kanika, N. H., Liaqat, N., Chen, H., Ke, J., Lu, G., Wang, J., & Wang, C. (2025). Fish gut microbiome and its application in aquaculture and biological conservation. *Frontiers in Microbiology*, 15, 1521048. <https://doi.org/10.3389/fmicb.2024.1521048>
- Khan, M. A., Khan, S., & Miyan, K. (2011). Aquaculture as a food production system: a review. *Biology and medicine*, 3(2), 291–302.
- Kiesewalter, H. T., Lozano-Andrade, C. N., Wibowo, M., Strube, M. L., Maróti, G., Snyder, D., Jørgensen, T. S., Larsen, T. O., Cooper, V. S., Weber, T., & Kovács, Á. T. (2021). Genomic and chemical diversity of *Bacillus subtilis* secondary metabolites against plant pathogenic fungi. *mSystems*, 6(1), e00770–20. <https://doi.org/10.1128/mSystems.00770-20>

- Knipe, H., Temperton, B., Lange, A., Bass, D., & Tyler, C. R. (2021). Probiotics and competitive exclusion of pathogens in shrimp aquaculture. *Reviews in Aquaculture*, 13(1), 324–352. <https://doi.org/10.1111/raq.12477>
- Kokou, F., Sasson, G., Nitzan, T., Doron-Faigenboim, A., Harpaz, S., Cnaani, A., & Mizrahi, I. (2018). Host genetic selection for cold tolerance shapes microbiome composition and modulates its response to temperature. *eLife*, 7, e36398. <https://doi.org/10.7554/eLife.36398>
- Kuebutornye, F. K. A., Abarike, E. D., Lu, Y., Wang, Z., Chi, S., & Li, X. (2020). Effects of host-associated *Bacillus* species on mucosal immunity and gut health of Nile tilapia and its resistance against *Aeromonas hydrophila*. *Aquaculture*, 525, 735289.
- Li, Y. & Cai, S.-H. (2011). Identification and pathogenicity of *Aeromonas sobria* on tail-rot disease in juvenile tilapia *Oreochromis niloticus*. *Current Microbiology*, 62(2), 623–627. <https://doi.org/10.1007/s00284-010-9753-8>
- Lin, C. C., Chen, J. C., Chen, Y. C., & Hsieh, Y. T. (2022). Functional characterization of *Bacillus*-derived hydrolytic enzymes against fish pathogenic bacteria. *Aquaculture Research*, 53(5), 1897–1907.
- Liu, M., Jiang, X., Pi, Y., Ren, X., Chen, M., Tang, S., Dai, X., Wu, Y., Guo, Y., Zhang, X., Luo, Z., & Xie, X. (2025). *Agrobacterium radiobacter* bacteremia in a gastric cancer patient: A case report and literature review. *Infection and Drug Resistance*, 18, 6279–6288. <https://doi.org/10.2147/IDR.S556428>
- Macias, L., Hernández, R., Jiménez, A., & Martínez, M. (2024). Assessment of *Bacillus* species capacity to improve resistance of Nile tilapia to *Aeromonas hydrophila* under soybean meal challenges. *Frontiers in Cellular and Infection Microbiology*, 14, 135473. <https://doi.org/10.3389/fcimb.2024.135473>
- Maezono, M., Nielsen, R., Buchmann, K. and Nielsen, M. (2025), The Current State of Knowledge of the Economic Impact of Diseases in Global Aquaculture. *Rev Aquac*, 17: e70039. <https://doi.org/10.1111/raq.70039>
- Majeed, H. Z. (2017). Antimicrobial activity of *Micrococcus luteus* carotenoid pigment. *Al-Mustansiriyah Journal of Science*, 28(1), 64. <https://doi.org/10.23851/mjs.v28i1.314>

- Meng, X., Chen, F., Xiong, M., Hao, H., & Wang, K.-J. (2023). A new pathogenic isolate of *Kocuria kristinae* identified for the first time in the marine fish *Larimichthys crocea*. *Frontiers in Microbiology*, 14, 1129568. <https://doi.org/10.3389/fmicb.2023.1129568>
- Midhun, S. J., Philip, R., Bright Singh, I. S., & Arun, A. B. (2017). Antibacterial activity and probiotic characterization of *Paenibacillus ehimensis* NPUST1 isolated from tilapia pond water. *Aquaculture Reports*, 8, 79–85. <https://doi.org/10.1016/j.aqrep.2017.01.004>
- Mohamed, R. A., Abd-Elghany, S. M., & El-Gohary, F. A. (2021). Occurrence and molecular identification of *Aeromonas hydrophila* in fish and fish products in Egypt. *Advances in Veterinary Research*, 9(2), 101–110.
- Mohammed, H. H., ElBanna, N. I., Erdogan, O., Aarattuthodi, S., Tekedar, H. C., Abdelhamed, H., & Díaz-Delgado, J. (2026). *Plesiomonas shigelloides* as an emerging pathogen in catfish aquaculture: A case from a South Texas commercial farm. *Microorganisms*, 14(1), 144. <https://doi.org/10.3390/microorganisms14010144>
- Morfín-Otero, R., Martínez-Vázquez, M. A., López, D., Rodríguez-Noriega, E., & Garza-González, E. (2012). Isolation of rare coagulase-negative isolates in immunocompromised patients: *Staphylococcus gallinarum*, *Staphylococcus pettenkoferi* and *Staphylococcus pasteurii*. *Annals of Clinical & Laboratory Science*, 42(2), 182–185.
- Naqqash, T., Wazir, N., Aslam, K., Shabir, G., Tahir, M., & Shaikh, R. S. (2022). First report on the probiotic potential of *Mammaliicoccus sciuri* isolated from raw goat milk. *Bioscience of Microbiota, Food and Health*, 41(4), 149–159. <https://doi.org/10.12938/bmfh.2021-022>
- Nayak, A., Dey, P., Behera, T., & Swain, P. (2024). In vitro determination of probiotic efficacy of *Bacillus subtilis* isolates: Antagonism against fish pathogens including *Aeromonas hydrophila*. *Journal of Fish Diseases*, 47(2), 201–213.
- Newaj-Fyzul, A., Mutani, A., Ramsubhag, A., & Adesiyun, A. (2008). Prevalence of bacterial pathogens and their anti-microbial resistance in tilapia and their pond water in Trinidad. *Zoonoses and Public Health*, 55(4), 206–213. <https://doi.org/10.1111/j.1863-2378.2007.01098.x>
- Nhinh, D. T., Le, D. V., Van, K. V., Huong Giang, N. T., Dang, L. T., & Hoai, T. D. (2021). Prevalence, virulence gene distribution and alarming multidrug resistance of *Aeromonas hydrophila* associated with disease outbreaks in freshwater aquaculture. *Antibiotics*, 10(5), 532. <https://doi.org/10.3390/antibiotics10050532>.

- Nouioui, I., Carro, L., García-López, M., Meier-Kolthoff, J. P., Woyke, T., Kyrpides, N. C., Pukall, R., Klenk, H.-P., Goodfellow, M., & Göker, M. (2018). Genome-based taxonomic classification of the phylum Actinobacteria. *Frontiers in Microbiology*, 9, 2007. <https://doi.org/10.3389/fmicb.2018.02007>
- Ntakirutimana, R., Rahiman, K. M., Farhath, A. A., Lovejan, M., & Jijina, M. P. (2025). From antibiotics to probiotics: sustainable disease management in Nile Tilapia aquaculture. *Advanced Gut & Microbiome Research*, 2025(1), 5144977. <https://doi.org/10.1155/agm3/5144977>
- Olmos, J., Acosta, M., Mendoza, G., & Pitones, V. (2020). *Bacillus subtilis*, an ideal probiotic bacterium to shrimp and fish aquaculture that increase feed digestibility, prevent microbial diseases, and avoid water pollution. *Archives of microbiology*, 202(3), 427-435. <https://doi.org/10.1007/s00203-019-01757-2>
- Panase, A., Thirabunyanon, M., Promya, J., & Chitmanat, C. (2023). Influences of *Bacillus subtilis* and fructooligosaccharide on growth performances, immune responses, and disease resistance of Nile tilapia, *Oreochromis niloticus*. *Frontiers in Veterinary Science*, 9, 1094681. <https://doi.org/10.3389/fvets.2022.1094681>
- Phuoc, L. H., & Hai, T. N. (2022). Effects of dietary supplementation with probiotics and prebiotics on growth, physiological condition, and resistance to pathogens challenge in Nile tilapia (*Oreochromis niloticus*). *Fishes*, 7(5), 273. <https://doi.org/10.3390/fishes7050273>
- Prabu, E., Rajagopalsamy, C.B.T., Ahilan, B., Jeevagan, I.J.M.A., & Renuhadevi, M., (2019). Tilapia an Excellent Candidate Species for World Aquaculture: A Review. *Annual Research & Review in Biology*, 114.
- Pridgeon, J. W., & Klesius, P. H. (2011). Major bacterial diseases in aquaculture and their vaccine development. *CAB Reviews*, 6(48), 1–16.
- Quesada, S. P., Paschoal, J. A. R., & Reyes, F. G. R. (2013). Considerations on the aquaculture development and on the use of veterinary drugs: special issue for fluoroquinolones—a review. *Journal of food science*, 78(9), R1321-R1333.
- Rasmussen, C. R., Figueras, M. J., McGarey, D., & Liles, M. R. (2016). Virulence factors of *Aeromonas hydrophila*: In the wake of reclassification. *Frontiers in Microbiology*, 7, 1337. <https://doi.org/10.3389/fmicb.2016.01337>

- Rauta, P. R., Nayak, B., & Das, S. (2011). Immune responses and expression profiles of cytokine genes in *Labeo rohita* following *Aeromonas hydrophila* infection. *Fish & Shellfish Immunology*, 30(3), 953–959.
- Ren, P., Fu, S., Liu, H., Wang, Y., & Xu, W. (2013). Ex vivo antagonism of *Lactobacillus plantarum* subsp. *plantarum* JCM 1149 against *Aeromonas hydrophila* in hybrid tilapia. *Fish & Shellfish Immunology*, 35(3), 845–852.
- Saba, A. O., Yasin, I. S. M., & Azmai, M. N. A. (2024). Meta-analyses indicate that dietary probiotics significantly improve growth, immune response, and disease resistance in tilapia. *Aquaculture International*, 32(4), 4841–4867. <https://doi.org/10.1007/s10499-024-01404-8>
- Santos, V. M., Achilonu, I., & Chibanga, J. (2023). Effect of *Bacillus* probiotics on the immunological responses of Nile tilapia (*Oreochromis niloticus*): A review. *Fishes*, 8(7), 366. <https://doi.org/10.3390/fishes8070366>
- Schar, D., Klein, E. Y., Laxminarayan, R., Gilbert, M., & Van Boeckel, T. P. (2020). Global trends in antimicrobial use in aquaculture. *Scientific Reports*, 10, 21878. <https://doi.org/10.1038/s41598-020-78849-3>
- Sherif, A. H., & Kassab, A. S. (2023). Multidrug-resistant *Aeromonas* bacteria prevalence in Nile tilapia broodstock. *BMC Microbiology*, 23, 92. <https://doi.org/10.1186/s12866-023-02827-8>
- Siangpro, N., Chuakrut, S., Sirimanapong, W., Tanasupawat, S., Phongsopitanun, W., Meksiriporn, B., Boonnorat, J., Sarin, S., Kucharoenphaibul, S., & Jutakanoke, R. (2023). *Lactiplantibacillus argentoratensis* and *Candida tropicalis* isolated from the gastrointestinal tract of fish exhibited inhibitory effects against pathogenic bacteria of Nile tilapia. *Veterinary Sciences*, 10(2), 129. <https://doi.org/10.3390/vetsci10020129>
- Stratev, D., & Odeyemi, O. A., (2016). Antimicrobial resistance of *Aeromonas hydrophila* isolated from different food sources: A mini-review. *Journal of Infection and Public Health*, 9(5), 535–544.
- Styková, E., Nemcová, R., Gancarčíková, S., Valocký, I., & Lauková, A. (2016). Bovine vaginal strain *Kocuria kristinae* and its characterization. *Folia Microbiologica*, 61(3), 243–248. <https://doi.org/10.1007/s12223-015-0431-x>

- Suresh, K., Pillai, D., Soni, M., Rathlavath, S., & Narshivudu, D. (2025). *Micrococcus luteus*, an emerging opportunistic pathogen in farmed Nile tilapia, *Oreochromis niloticus* in Andhra Pradesh, India. *Aquaculture International*, 33, 51. <https://doi.org/10.1007/s10499-024-01761-4>
- Tewari, R., Dudeja, M., Das, A. K., & Nandy, S. (2013). *Kocuria kristinae* in catheter-associated urinary tract infection: A case report. *Journal of Clinical and Diagnostic Research*, 7(8), 1692–1693.
- Vala, F. D., Lende, S. R., Solanki, V. K., Jora, K., Desai, R., Sharma, P., Nathani, N., & Mootapally, C. (2024). Melodious tuning of microbial dynamics in biofloc, cage, and pond culturing systems: A study on *Pangasius pangasius* fish gut microbiome. *Frontiers in Microbiology*, 15, 1434312. <https://doi.org/10.3389/fmicb.2024.1434312>
- Wamala, S. P., Mugimba, K. L., Mutoloki, S., Evensen, Ø., & Byarugaba, D. K. (2018). *Occurrence and antibiotic susceptibility of fish bacteria in Uganda: Implications for aquaculture biosecurity*. *Journal of Fish Diseases*, 41(6), 1–12.
- Wamala, S. P., Mugimba, K. L., Mutoloki, S., Evensen, Ø., & Byarugaba, D. K. (2020). Multilocus sequence analysis reveals high genotypic diversity of *Aeromonas hydrophila* infecting fish in Uganda. *Aquaculture*, 526, 735428.
- Wang, D., Li, X., Chen, L., Zhang, H., & Liu, Y. (2023). Unraveling the antibacterial mechanism of *Lactobacillus plantarum* cell-free supernatant against *Aeromonas hydrophila*: Membrane disruption and metabolite effects. *International Journal of Food Microbiology*, 388, 110203.
- Wu, D., Fu, L., Cao, Y., Dong, N., & Li, D. (2023). Genomic insights into antimicrobial potential and optimization of fermentation conditions of pig-derived *Bacillus subtilis* BS21. *Frontiers in Microbiology*, 14, Article 1239837. <https://doi.org/10.3389/fmicb.2023.1239837>
- Wu, F., Chen, B., Liu, S., Xia, X. J., Gao, L. L., Zhang, X. Y., & Pan, Q. (2020). Effects of woody forages on biodiversity and bioactivity of aerobic culturable gut bacteria of tilapia (*Oreochromis niloticus*). *PLOS ONE*, 15(7), e0235560. <https://doi.org/10.1371/journal.pone.0235560>

- Xavier, R., Severino, R., & Silva, S. M. (2024). Signatures of dysbiosis in fish microbiomes in the context of aquaculture. *Reviews in Aquaculture*, 16(2), 706–731. <https://doi.org/10.1111/raq.12862>
- Xu, W., Lin, Y., Zhang, S., Li, T., & Zhao, X. (2023). Selective antagonism of *Lactiplantibacillus plantarum* and *Pediococcus acidilactici* against *Aeromonas* spp.: Quorum-quenching and inhibition mechanisms. *Microbiology Spectrum*, 11(5), e01567-23.
- Yu, D., Chen, Y., Pan, Y., Li, H., McCormac, M. A., & Tang, Y.-W. (2008). *Staphylococcus gallinarum* bacteremia in a patient with chronic hepatitis B virus infection. *Annals of Clinical & Laboratory Science*, 38(4), 401–404.
- Yu, H., Nazir, S., Ijaz, F., Zahid, M. U., Mushtaq, M., Khan, M., Rahman, A., & Rahman, M. A. U. (2025). Dietary supplementation of *Bacillus subtilis* as probiotic influenced the growth performance, hematological parameters, immune function, antioxidant status, and digestive enzyme activity of Nile tilapia fingerlings (*Oreochromis niloticus*). *Animals*, 15(9), 1256. <https://doi.org/10.3390/ani15091256>
- Zhang, D. X., Kang, Y. H., Zhan, S., Zhao, Z. L., Jin, S. N., Chen, C., ... & Qian, A. D. (2019). Effect of *Bacillus velezensis* on *Aeromonas veronii*-induced intestinal mucosal barrier function damage and inflammation in crucian carp (*Carassius auratus*). *Frontiers in microbiology*, 10, 2663. <https://doi.org/10.3389/fmicb.2019.02663>
- Zhang, M., Xue, M., Xiao, Z., Liu, W., Jiang, N., Meng, Y., Fan, Y., Liu, X., & Zhou, Y. (2022). *Staphylococcus sciuri* causes disease and pathological changes in hybrid sturgeon *Acipenser baerii* × *Acipenser schrencki*. *Frontiers in Cellular and Infection Microbiology*, 12, 1029692. <https://doi.org/10.3389/fcimb.2022.1029692>
- Zhou, Y., Chen, X., & Wang, H. (2023). Global prevalence, antimicrobial resistance, and virulence of *Aeromonas hydrophila* in aquatic food animals: A systematic review and meta-analysis. *Frontiers in Microbiology*, 14, 12322169.
- Zhu, M., Zhu, Q., Yang, Z., & Liang, Z. (2021). Clinical characteristics of patients with *Micrococcus luteus* bloodstream infection in a Chinese tertiary-care hospital. *Polish Journal of Microbiology*, 70(3), 321–326. <https://doi.org/10.33073/pjm-2021-030>

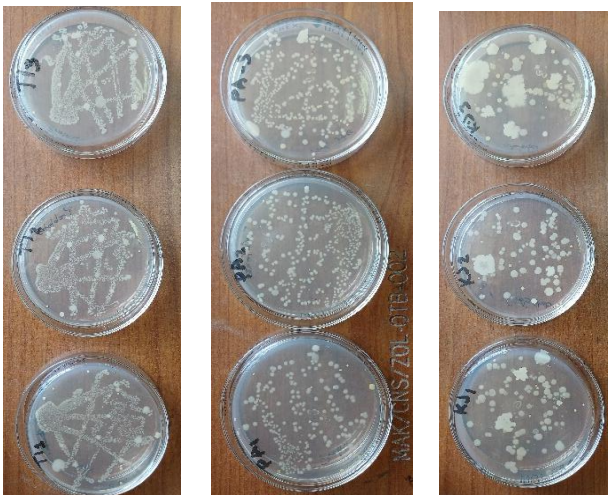
## APPENDICES

### *Appendix 1. Gut extraction.*



From left to right: dissected sample; laid out gut on dissection

### *Appendix 2. Primary culture plates growth.*



Triplicate primary cultures of TI (left), PA (center) and KJ (right) samples

### *Appendix 3. Subcultured isolations.*



Pure isolates subcultures based on colony morphologies.

*Appendix 4. CFS inhibition assay setup inoculation.*



100 $\mu$ l of the CFS supernatant inoculated in agar wells for inhibition assay.

*Appendix 5. Table showing inhibition assay results.*

| isolate ID                  | Isolate code         | Satellitism (mm) |    |    |    | CFS (mm) |    |    |    | mean        |     | SD          |           |
|-----------------------------|----------------------|------------------|----|----|----|----------|----|----|----|-------------|-----|-------------|-----------|
|                             |                      |                  |    |    |    |          |    |    |    | satellitism | CFS | satellitism | CFS       |
| <b><i>S. gallinarum</i></b> | I <sub>1</sub>       | 0                | 0  | 0  | 0  | 0        | 0  | 0  | 0  | 0           | 0   | 0           | 0         |
| <b><i>M. luteus</i></b>     | I <sub>2</sub>       | 0                | 0  | 0  | 0  | 0        | 0  | 0  | 0  | 0           | 0   | 0           | 0         |
| <b><i>K. kristinae</i></b>  | I <sub>3</sub>       | 0                | 0  | 0  | 0  | 0        | 0  | 0  | 0  | 0           | 0   | 0           | 0         |
| <b><i>B. subtilis</i></b>   | I <sub>4</sub>       | 0                | 0  | 0  | 0  | 0        | 0  | 0  | 0  | 0           | 0   | 0           | 0         |
| <b><i>S. sciuri</i></b>     | I <sub>5</sub>       | 0                | 0  | 0  | 0  | 0        | 0  | 0  | 0  | 0           | 0   | 0           | 0         |
|                             |                      |                  |    |    |    |          |    |    |    |             |     | <b>Mean</b> | <b>SD</b> |
|                             | <b>control (+ve)</b> | 21               | 24 | 21 | 21 | 23       | 21 | 22 | 21 | 22          | 21  | 21.7        | 1.1       |
|                             | <b>control (-ve)</b> | 0                | 0  | 0  | 0  | 0        | 0  | 0  | 0  | 0           | 0   | 0           | 0         |