Black Sea Journal of Engineering and Science
doi: 10.34248/bsengineering.1749516

Open Access Journal
e-ISSN: 2619 – 8991

Research Article
Volume 8 - Issue 5: 1634-1644 / September 2025

MOLECULAR IDENTIFICATION, ENZYMATIC ACTIVITY AND ANTIBIOTIC RESISTANCE PROFILES OF BACTERIA ISOLATED FROM *Merlangius merlangus*

Huseyin Sezgin CAGLAR[1], Semra SAYGIN[1], Hayrettin SAYGIN[2]*

[1]*Ondokuz Mayis University, Faculty of Science, Department of Biology, 55139, Samsun, Türkiye*
[2]*Ondokuz Mayis University, Faculty of Science, Department of Molecular Biology and Genetics, 55139, Samsun, Türkiye*

Abstract: The present study intended to examine the cultivable bacterial microbiota of *Merlangius merlangus*, an ecologically valuable fish species from the Black Sea. Serial dilution and pre-enrichment steps were undertaken before isolation. Ten different isolates were isolated from muscle, gill, and intestinal tissues of fresh specimens. Molecular identification by 16S rRNA gene sequence showed all isolates to be members of *Enterobacteriaceae*, comprised of the genera *Morganella*, *Providencia*, *Proteus*, *Klebsiella*, and *Escherichia*. As one of the isolates (S09) was found to exhibit the highest similarity with a validly published species within the genus *Morganella*, with a relatively low value of 99.11%, it could be a new taxon. Extracellular enzyme tests showed variable presence of lipase and urease activities among isolates but no detectable amylase, caseinase, lecithinase, or DNase activities. Antimicrobial susceptibility testing was consistent with high rates of multidrug resistance, with eight isolates being resistant to three or more antibiotic groups. Resistance was greatest against amoxicillin/clavulanic acid, tetracycline, and sulfamethoxazole/trimethoprim, with all isolates showing susceptibility to imipenem. The observations point to the occurrence of metabolically active, environmentally adapted, and resilient bacterial forms in *M. merlangus*, with food safety implications and concern for antimicrobial resistance dissemination in aquatic ecosystems. Further, the uniqueness of the phylogenetic status of isolate S09 suggests an additional polyphasic taxonomic study. Future research involving metagenomes will be required to characterize host-associated bacterioplankton communities comprehensively, including their ecological functions.

Keywords: Fish microbiota, *Merlangius merlangus*, *Enterobacteriaceae*, Extracellular enzymatic activity, Antibiotic resistance, 16S rRNA gene phylogeny

***Corresponding author:** Ondokuz Mayis University, Faculty of Science, Department of Molecular Biology and Genetics, 55139, Samsun, Türkiye
E mail: hayrettin.saygin@omu.edu.tr (H. SAYGIN)
Huseyin Sezgin CAGLAR https://orcid.org/0009-0002-2713-3316
Semra SAYGIN https://orcid.org/0000-0002-3249-5074
Hayrettin SAYGIN https://orcid.org/0000-0002-8642-5872

Received: July 23, 2025
Accepted: September 09, 2025
Published: September 15, 2025

Cite as: Caglar HS, Saygin S, Saygin H. 2025. Molecular identification, enzymatic activity and antibiotic resistance profiles of bacteria isolated from *Merlangius merlangus*. BSJ Eng Sci, 8(5): 1634-1644.

1. Introduction

Merlangius merlangus (also referred to as whiting) is a demersal fish species (Asciutto et al., 2024) that is found widely in the northeastern Atlantic Ocean, as well as the Black and Mediterranean Seas (Muus and Nielsen, 1999; Yildiz et al., 2021; Calì et al., 2023; Froese and Pauly, 2024). It is ecologically significant due to its role as a mid-level predator in marine food webs (Nissar et al., 2023) and has significant economic value due to local commercial fishing (FAO, 2020; ICES, 2024). Since it is a species that is often consumed at many coasts, knowledge of microbes present on/in *M. merlangus* is relevant not just for fish health management but also for food safety issues and human health (Köker et al., 2021; Brauge et al., 2024).

Marine fish carry diverse microbial communities on their gills, skin, and in their gastrointestinal tracts (Chiarello et al., 2015; Pratte et al., 2018; Huang et al., 2020). These microorganisms perform vital functions in host metabolism, immune modulation, and inhibition of pathogen growth (Banerjee and Ray, 2017; de Bruijn et al., 2018). Fish can also be reservoirs or vectors for harmful or antibiotic-resistant bacteria (Cabello 2006; Hatosy and Martiny, 2015; Hossain et al., 2022) as well. Examining the fish microbiota from species like *M. merlangus* serves to advance knowledge of host-microbe interactions, marine disease ecology, and microbial transmission in food chains (Romero et al., 2014; Pękala-Safińska, 2018; Egerton et al., 2018).

Isolation of bacteria from fish species is an essential process in determining the microbial ecology of aquatic environments and assessing possible threats to fish health and human health (Sheng and Wang, 2021). Although traditional microbiological procedures are useful in providing early information on colony shape and general biochemical properties, these tend to fail to produce accurate species-level identification due to taxonomic overlap between taxa (Jackman, 2012). Molecular approaches, especially 16S rRNA gene

sequencing, represent a consistent and high-resolution option for determining bacterial taxonomy (Ntushelo, 2013). It enables accurate identification of both cultivable and non-cultivable bacteria, revealing an extended range of microbial diversity that could go unnoticed by traditional approaches (Justé et al., 2008).

Marine bacteria have been reported to possess the ability to produce diverse extracellular enzymes such as proteases, lipases, amylases, and cellulases (Hoppe et al., 2002; Birolli et al., 2019; Cheng et al., 2020). Besides playing a key role in the cycling of nutrients in marine environments, these enzymes also find immense industrial applications in biotechnology, pharmaceuticals, and food industries (Zhang and Kim, 2010; Trincone, 2017). After characterization of the enzymatic activity of isolates, it can identify promising strains for use on a commercial scale as well as understand their functional properties in fish microbiomes (Ray et al., 2012; Dhayalan et al., 2022).

The growth of antibiotic-resistant bacteria in aquatic ecosystems is an emerging concern worldwide, especially because of antibiotic overuse in aquatic farming and environmental pollution by human-related activities (Cabello, 2006; Hatosy and Martiny, 2015). Fish carry antibiotic-resistant bacteria that can be transferred to human beings by direct contact or ingestion (Gauthier, 2015). Antibiotic susceptibility patterns of fish-associated bacteria need to be monitored to identify the dissemination of resistance elements and design effective antimicrobial interventions in aquatic farming and fisheries management (Preena et al., 2020; Pepi and Focardi, 2021).

Despite the ecological and economic significance of *M. merlangus*, there are no systematic studies on their associated bacteria's molecular identity, enzymatic properties, and antibiotic resistance. Filling this knowledge void, in this study, we isolated bacteria from *M. merlangus*, carried out 16S rRNA gene identification, analyzed their extracellular enzymatic activities, and their resistance to antibiotics. This study offers important information on the microbiological status of *M. merlangus* and contributes to discussions on marine microbial ecology and public health.

2. Materials and Methods

2.1. Fish Sampling and Preparation

A total of ten *M. merlangus* (whiting) specimens during the commercial open season were obtained in 2024-February from local fishermen along the southern coast of the Black Sea, in the Samsun province of Türkiye. Immediately after capture, the fish were placed in sterile polyethylene bags, stored on ice (~4 °C), and transported to the laboratory within 3 hours of collection to minimize postmortem microbial shifts (Egerton et al., 2018). In the laboratory, each specimen was individually processed under aseptic conditions within a Class II biosafety cabinet to prevent external contamination. Fish were rinsed thoroughly with sterile seawater to remove external debris and transient microorganisms. Dissection was carried out using flame-sterilized instruments, and the gill arches, muscle tissues, and entire gastrointestinal tract were carefully excised. To standardize the bacterial isolation procedure and reduce individual variation, tissues from ten *M. merlangus* specimens were pooled by type, resulting in three composite samples: one each for gills, intestine, and muscle. Each composite tissue type was transferred into separate sterile containers containing 225 mL of alkaline peptone water (APW) supplemented with 1% NaCl to favor the enrichment of marine and halotolerant bacteria (Wanja et al., 2019). The APW medium was prepared by dissolving 6.75 g peptone, 6.75 g NaCl, and 1.0125 g potassium dihydrogen phosphate (KH_2PO_4) in 675 mL of distilled water. Tissue-enriched flasks were incubated at 37 °C for 24 hours to promote the proliferation of viable but potentially low-abundance bacterial populations prior to isolation.

2.2. Bacterial Isolation and Pure Culture Preparation

After enrichment, each of these tissue homogenates was diluted with sterile Ringer's solution to a dilution series up to 10^{-6} to decrease microbial content and allow for colony separation. 100 µL aliquots from each tissue homogenate (intestine, gills, muscle) from each dilution were aseptically streaked onto thiosulphate citrate bile salt sucrose (TCBS) agar (HiMedia Laboratories, India), and Nutrient agar (Merck, Germany) media with sterile glass spreaders, and then the dishes were left to incubate in an inverted direction at 37 °C for 24-48 hours for colony growth. TCBS agar was used in the current study to encourage selective isolation of halotolerant and potentially marine-derived bacteria. Whereas the use of TCBS is traditionally recommended to isolate *Vibrio* species, its composition of high salt and bile salts also favors the growth of some Gram-negative enterics that can survive in marine- and brackish-water environments (Alikunhi et al., 2017). While *M. merlangus* is a cold-seawater fish species, the use of an incubation temperature of 37°C was specifically intended to promote the growth of mesophilic bacteria of clinical and food safety interest. This method is routinely used in studies of human-associated and pathogenic bacteria in seafood since the mesophilic strains will be favored to grow in such conditions and may be harmful upon ingestion and handling (Santos et al., 2021; Kyule et al., 2022). Post-incubation, corresponding to each dilution step, bacterial colonies were analyzed for morphologically diverse colonies by differences in size, color, elevation, margin, and surface features. Bacterial colonies that were morphologically diverse were isolated to acquire a representative count of the culturable microbiota (Cantas et al., 2012). Ten such morphologically different colonies were selected in succession with subculture onto Nutrient agar plates for pure culture. Subculture plates were incubated at 37 °C for 24-48 hours. Long-term preservation of each of these pure cultures was carried out in a sterile 25% glycerol solution and stored in cryovials at -20 °C and -80 °C.

These isolates were maintained on Nutrient agar in addition to follow-up enzymatic as well as molecular work.

2.3. Genomic DNA Isolation and 16S rRNA Gene-Based Molecular Identification

Genomic DNA was isolated from fresh pure cultures of bacteria with PureLink Genomic DNA Mini Kit (Invitrogen, USA), according to the manufacturer's procedure with some optimizations. DNA concentration and purity were initially determined by a NanoDrop spectrophotometer (Thermo Scientific), and DNA integrity was checked by electrophoresis on a 1% agarose gel stained with ethidium bromide under UV illumination. Amplification of the 16S rRNA gene from bacteria was carried out with primer pair 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1525R (5′-AAGGAGGTGATCCAGCC-3′), which produces an amplicon of ~1500 bp. Amplification confirmation and fragment length were checked on a 1.5% agarose gel with a DNA ladder (100 bp with marker). Amplification products were cleaned up and sequenced with an ABI PRISM 3730 XL automated sequencer with universal primers 518F (5′-CCAGCAGCCGCGGTAATACG-3′) and 800R (5′-TACCAGGGTATCTAATCC-3′). Chromatogram files in ABI format were checked by Chromas version 1.45, and primers overlapped to get 16S rRNA gene nucleotide sequences in FASTA format for each isolate. 16S rRNA gene sequences resulting from each isolate were deposited in GenBank under accession PV407295 to PV407304. 16S rRNA gene sequences were uploaded to the EzBioCloud server (Yoon et al., 2017) and pairwise sequence similarity of closest phylogenetic neighbours was computed. Phylogenetic trees were reconstructed with MEGA X (Kumar et al., 2018) with the neighbour-joining method (Saitou and Nei, 1987). Topologies of resulting trees were checked by bootstrap resampling of 1000 replicates after Felsenstein (1985).

2.4. Enzymatic Activity Assays

The production of extracellular hydrolytic enzymes by the isolates was tested using substrate-supplemented agar media according to standard microbiological procedures. Six enzymes, namely lipase, amylase, caseinase, lecithinase, DNase, and urease, were screened with previously established methods. Basal medium comprised glucose (0.5 g/L), yeast extract (0.5 g/L), $MgSO_4.7H_2O$ (0.5 g/L), NaCl (0.5 g/L), K_2HPO_4 (1 g/L), and agar (15 g/L), with a final pH adjusted to 7.0-7.5 before autoclaving. Lipase production was tested on the basal medium containing tributyrin (Farooq et al., 2022), and amylase production was tested with starch-supplied agar plates by using an iodine staining method according to Albejo and Hamza (2017). Caseinase activity was determined using the protocol outlined by Stratev et al. (2015). Lecithinase activity was evaluated as described by Bunpa et al. (2016). DNase production was tested with commercial DNase test agar plates (Merck, Germany). Urease activity was tested with Christensen's urea agar (Cui et al., 2022). All media were prepared aseptically and inoculated with isolates by loop or spot seeding methods. Incubation was carried out at 37 °C for 24-48 h. Enzyme production was measured based on visual factors such as hydrolysis zones or changes in color.

2.5. Antibiotic Susceptibility Testing

The antimicrobial sensitivity of the bacterial isolates was tested according to the standard Kirby-Bauer disc diffusion method (Hudzicki, 2009). Overnight cultures on Nutrient agar were suspended in sterile saline to 0.5 McFarland turbidity standards. Each inoculum was distributed equally over plates of Mueller-Hinton Agar. Antibiotic discs impregnated with antibiotics were applied to inoculated plates. Amikacin (AK, 30 µg), gentamicin (CN, 10 µg), streptomycin (S, 10 µg), amoxicillin/clavulanic acid (AMC, 30 µg), ciprofloxacin (CIP, 5 µg), tetracycline (TE, 30 µg), sulfamethoxazole/trimethoprim (SXT, 25 µg), imipenem (IPM, 10 µg), linezolid (LZD, 30 µg), and amphotericin B (AMB, 20 µg) were tested. Antibiotics were selected based on their medical relevance and their reported use in human medicine and aquatic environments. Aerobically at 37 °C for 18-24 hours, plates were incubated. Zone diameters of inhibition around each disc were measured in millimeters, and susceptibility interpretations were determined according to CLSI's breakpoints (CLSI, 2023), with zone diameter breakpoints categorized as Susceptible (S), Intermediate (I), or Resistant (R).

3. Results

3.1. Isolation and Cultivation of Bacterial Strains

It was isolated a total of ten morphologically different bacterial isolates from the internal organs of specimens of *M. merlangus* (Figure 1). They were from three types of tissues: intestine (B01, B02, B03, B04), muscle (K06, K07), and gills (S08, S09, S11, S13). Of these, nine (except B03) were isolated on TCBS agar. All isolates had slightly different colony morphologies based on differences in pigmentation, edge, surface texture, and elevation.

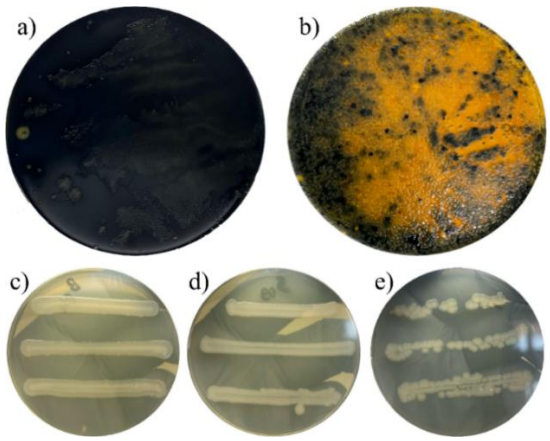

Figure 1. Representative images showing the isolation and pure culture of bacterial strains. (a, b) Growth on TCBS agar at different serial dilution levels, and (c, d, e) pure colonies of isolates B04, S09, and S13 on nutrient agar.

3.2. Molecular Identification via 16S rRNA Gene Sequencing

High-quality genomic DNA was successfully isolated from all of the isolates, and 16S rRNA gene amplification with universal primers produced ~1500 bp products from each one. Sanger sequencing with 518F and 800R internal primers produced clean, high-quality chromatograms amenable to downstream work. Sequence identity was established using the EzBioCloud database, with taxonomic identification based on ≥99% similarity with type strains. Sequences from all isolates demonstrated the highest sequence similarity with known members of the *Enterobacteriaceae*. *Morganella morganii* was most commonly encountered, present in isolates B01, K07, S08, and S09, each with ≥99.11% identity. Sequences also aligned with *Morganella psychrotolerans* at slightly lower similarity. Importantly, strain S09 had a comparatively poor 16S rRNA gene sequence similarity (99,11%) with its nearest validly described *Morganella* species and was positioned in a separate branch in phylogeny, indicating that it could be a putative new taxon of genus *Morganella*. Two isolates (B02 and B04) were found to be members of the genus *Providencia* with closest alignments with *Providencia alcalifaciens* and *Providencia rettgeri*, respectively. Isolate K06, which was obtained from muscle, showed a perfect alignment (100%) with *Proteus terrae*, while isolate S13 aligned best with *Proteus cibi* with 99.93% similarity. Importantly, isolate B03 was not isolated on TCBS agar but was identified as *Klebsiella aerogenes* (99.73%). The last isolate, S11, aligned with *Escherichia whittamii* (99.66%) and *Escherichia ruysiae* (99.52%) with some variation ratios in comparison. These identification findings, presented in Table 1, establish dominance by opportunistic enteric bacteria in *M. merlangus* microbiota. The taxa found are species with established clinical relevance, especially to nosocomial infections and foodborne infections. Further insight was achieved by representing phylogeny between isolates and their respective reference strains with a neighbor-joining tree (Figure 2), which confirmed species-level clusters and community affiliations at intra-genus levels. The distribution of bacterial genera varied according to the tissue type. *Morganella* was isolated from all tissue types, while *Providencia* was exclusively found in the intestine, and *Escherichia* was only detected in the gills. The genus-level distribution of isolates across different tissues is presented in Figure 3.

Table 1. Molecular identification of bacterial isolates based on 16S rRNA gene similarity

Isolate	Closest matching type strain	Similarity (%)	Variation ratio
	Morganella morganii subsp. *morganii* ATCC 25830^T	99.93	1/1463
B01	*Morganella morganii* subsp. *sibonii* DSM 14850^T	99.26	10/1355
	Morganella psychrotolerans U2/3^T	98.38	22/1356
	Providencia alcalifaciens DSM 30120^T	99.86	2/1463
B02	*Providencia rustigianii* DSM 4541^T	99.66	5/1463
	Providencia burhodogranariea DSM 19968^T	99.38	9/1463
	Klebsiella aerogenes KCTC 2190^T	99.73	4/1462
B03	*Raoultella ornithinolytica* JCM 6096^T	99.52	7/1457
	Raoultella terrigena ATCC 33257^T	99.24	11/1452
	Providencia rettgeri DSM 4542^T	99.73	4/1462
B04	*Providencia vermicola* OP1^T	99.59	6/1462
	Providencia huaxiensis WCHPr000369^T	99.59	6/1462
	Proteus terrae subsp. *terrae* N5/687^T	100.00	0/1443
K06	*Proteus terrae* subsp. *cibarius* JS9^T	99.73	4/1464
	Proteus cibi FJ2001126-3^T	99.37	9/1424
	Morganella morganii subsp. *morganii* ATCC 25830^T	99.66	5/1463
K07	*Morganella morganii* subsp. *sibonii* DSM 14850^T	98.97	14/1355
	Morganella psychrotolerans U2/3^T	98.23	24/1356
	Morganella morganii subsp. *morganii* ATCC 25830^T	99.73	4/1463
S08	*Morganella morganii* subsp. *sibonii* DSM 14850^T	99.04	13/1355
	Morganella psychrotolerans U2/3^T	98.16	25/1356
	Morganella morganii subsp. *morganii* ATCC 25830^T	99.11	13/1463
S09	*Morganella morganii* subsp. *sibonii* DSM 14850^T	99.04	13/1355
	Morganella psychrotolerans U2/3^T	98.08	26/1356
	Escherichia whittamii Sa2BVA5^T	99.66	5/1464
S11	*Escherichia ruysiae* OPT1704^T	99.52	7/1464
	Shigella flexneri ATCC 29903^T	99.32	10/1464
	Proteus cibi FJ2001126-3^T	99.93	1/1424
S13	*Proteus alimentorum* 08MAS0041^T	99.86	2/1380
	Proteus vulgaris ATCC 29905^T	99.80	3/1464

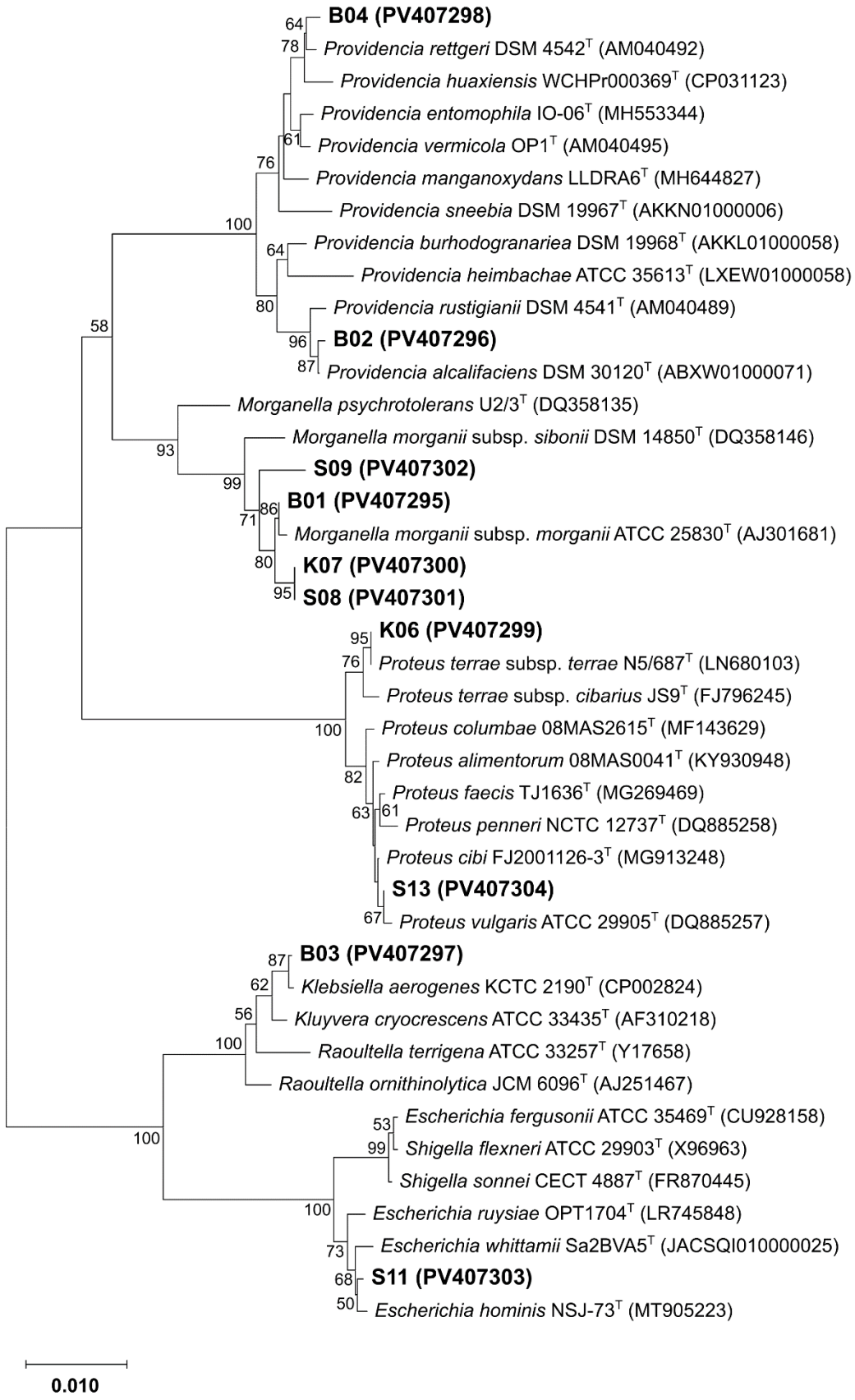

Figure 2. Phylogenetic relationships of bacterial isolates based on 16S rRNA gene sequences. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). This analysis involved 41 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 1308 positions in the final dataset. Bootstrap values (>50%) based on 1,000 replicates are shown at the corresponding nodes.

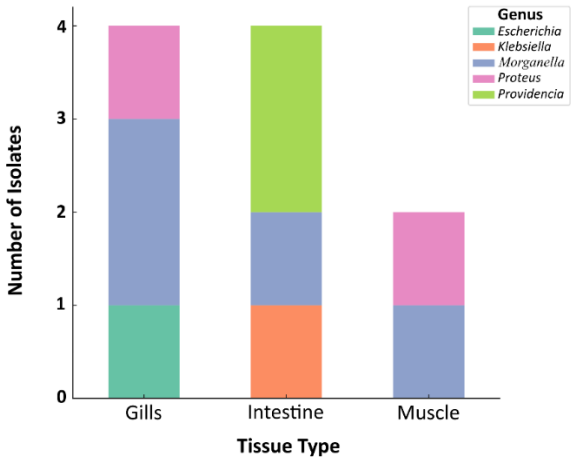

Figure 3. Bar chart showing the distribution of bacterial genera isolated from different tissues of *M. merlangus*. The number of isolates belonging to each genus is represented by colored segments within each tissue type (intestine, muscle, gills). This visual highlights genus-specific tissue localization patterns.

3.3. Extracellular Enzyme Activity Assays

Out of the ten isolates, screening for enzymic activity produced a selective profile based on the presence of lipase and urease activities. None was detected for amylase, caseinase, DNase, or lecithinase under experimental conditions. Lipase was confirmed in three isolates (B04, K06, and S13) by visibly evident hydrolysis zones on lipid-rich agar (Figure 4). Isolate K06 showed extremely high lipolytic activity, with a 30 mm in diameter clearing zone, which was the largest recorded of all. Urease was detected in seven isolates (B01, B04, K06, K07, S08, S09, and S13). Taken together, these findings illustrate that some isolates, especially *Proteus* and *Providencia* strains, possess enzymic functions applicable to lipid and nitrogen cycling with possible effects on tissue breakdown, spoilage, and host interaction (Table 2).

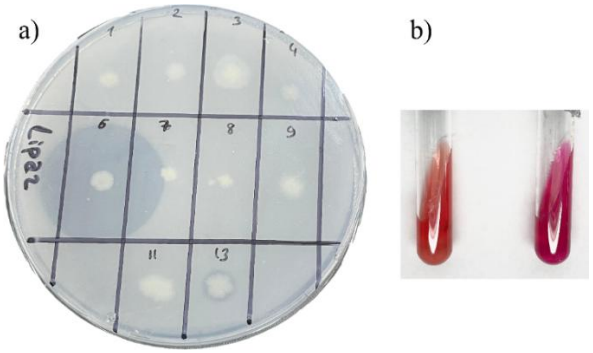

Figure 4. Representative results of lipase and urease activity assays in selected bacterial isolates. (a) Lipase-positive isolates B04, K06, and S13 showing clear zones, and (b) urease activity comparison between isolates B02 (negative, no color change) and K06 (positive, bright pink coloration).

Table 2. Extracellular enzyme activity profiles of isolates

Isolate	Lipase (mm)	Amylase	Caseinase	DNase	Lecithinase	Urease
B01	-	-	-	-	-	+
B02	-	-	-	-	-	-
B03	-	-	-	-	-	-
B04	6	-	-	-	-	+
K06	30	-	-	-	-	+
K07	-	-	-	-	-	+
S08	-	-	-	-	-	+
S09	-	-	-	-	-	+
S11	-	-	-	-	-	-
S13	8	-	-	-	-	+

Symbols: +, Positive activity; -, No activity observed. Zone diameters (in mm) are provided for positive reactions in lipase activity.

3.4. Antibiotic Susceptibility Testing

The antibiotic resistance profiles of the ten isolated bacteria presented varied resistance patterns against the tested agents (Figure 5). The profile presented in Table 3 caught most of the isolates with resistance to one antibiotic, and some with resistance to more than one class. Linezolid and amphotericin B did not show any inhibition zones in all isolates, indicating their minimal activity against Gram-negative bacteria. Resistance to amoxicillin/clavulanic acid was found to be the most prevalent, with eight isolates (B01, B02, B04, K06, K07, S08, S09, S13). Out of all aminoglycosides, amikacin was found with strong activity, with only resistance in B03. Gentamicin was less effective against B02, B03, S11, and S13 (Figure 6). The profile of ciprofloxacin was mostly effective, with some intermediate or resistance in K06 and S11, respectively. Imipenem showed full activity against all isolates, indicating no carbapenem resistance mechanisms. Streptomycin was found with intermediate or resistance profiles in some isolates, such as B04, K06, and S13. Resistance to tetracycline and sulfamethoxazole/trimethoprim was also prevalent. Six isolates showed reduced susceptibility to tetracycline, while three (K06, S11, S13) were resistant to sulfamethoxazole/trimethoprim.

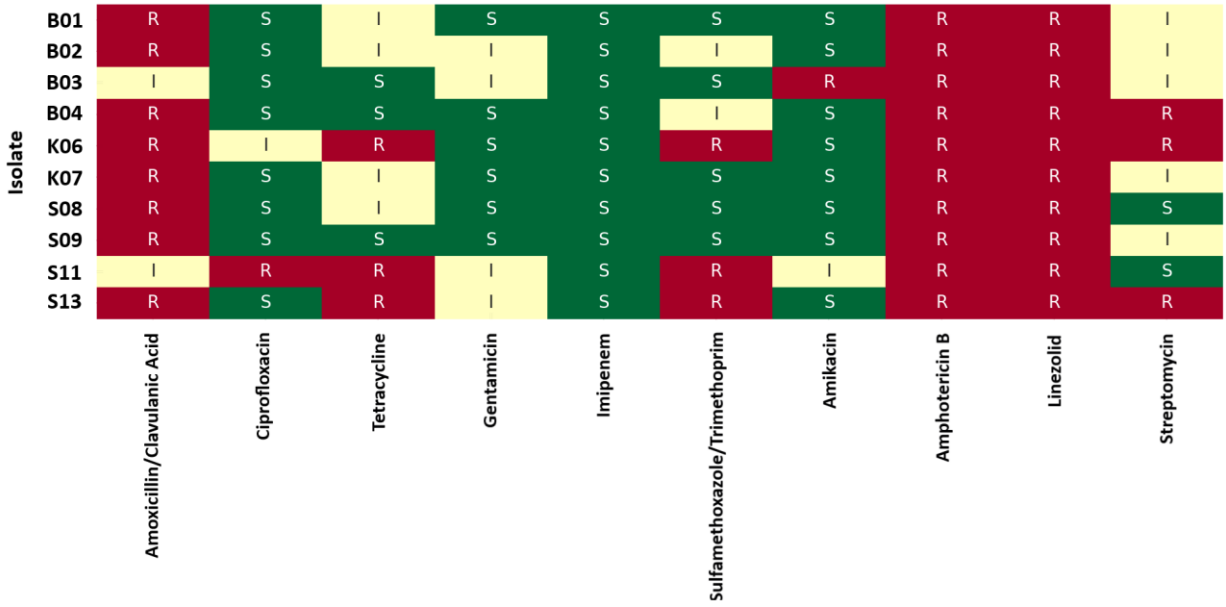

Figure 5. Heatmap representation of antibiotic susceptibility patterns of bacterial isolates. Each cell corresponds to the susceptibility result of an isolate (rows) against a specific antibiotic (columns). The resistance levels are color-coded as follows: green, susceptible (S); yellow, intermediate (I); red, resistant (R).

Table 3. Inhibition zone diameters (mm) of bacterial isolates against selected antibiotics*

Antibiotic	B01	B02	B03	B04	K06	K07	S08	S09	S11	S13
Amoxicillin/Clavulanic Acid	13 (R)	9 (R)	16 (I)	8 (R)	9 (R)	10 (R)	8 (R)	9 (R)	16 (I)	8 (R)
Ciprofloxacin	24 (S)	27 (S)	26 (S)	30 (S)	16 (I)	26 (S)	30 (S)	25 (S)	0 (R)	27 (S)
Tetracycline	14 (I)	14 (I)	26 (S)	18 (S)	0 (R)	12 (I)	13 (I)	9 (S)	10 (R)	0 (R)
Gentamicin	18 (S)	13 (I)	14 (I)	15 (S)	18 (S)	18 (S)	22 (S)	20 (S)	14 (I)	13 (I)
Imipenem	30 (S)	26 (S)	32 (S)	28 (S)	24 (S)	29 (S)	32 (S)	28 (S)	30 (S)	26 (S)
Sulfamethoxazole/Trimethoprim	25 (S)	11 (I)	22 (S)	15 (I)	0 (R)	22 (S)	16 (S)	18 (S)	0 (R)	0 (R)
Amikacin	22 (S)	19 (S)	14 (R)	24 (S)	25 (S)	20 (S)	24 (S)	19 (S)	16 (I)	23 (S)
Amphotericin B	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Linezolid	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Streptomycin	13 (I)	13 (I)	14 (I)	12 (R)	11 (R)	14 (I)	15 (S)	14 (I)	18 (S)	12 (R)

* Resistant values are evaluated according to CLSI (2023), S = Susceptible, I = Intermediate, R = Resistant.

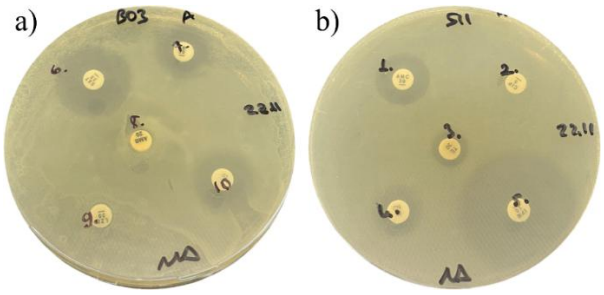

Figure 6. Representative antibiogram results of (a) isolate B03 and (b) isolate S11 for selected antibiotics on Mueller-Hinton agar.

4. Discussion

The present microbiological analysis of *M. merlangus* from the Black Sea presents a multi-faceted view of fish-associated bacteria colonizing a species of ecological and food-related interest. By uniting molecular identification, enzymatic characterization, and antibiotic susceptibility profiling, this study adds to an emerging interest in the overlap between environmental microbiology and human health. Repeated detection of members of *Enterobacteriaceae* in fish tissues highlights both microbial heterogeneity and the potential for opportunistic pathogenicity in marine hosts (Pitout and Laupland, 2008; Yang et al., 2020). Lipolytic and ureolytic capability, together with multidrug resistance, identify adaptive strategies that can facilitate survival under selective pressures whilst threatening at the same time risks associated with spoilage and antimicrobial resistance (AMR) dissemination (Ray et al., 2012; Yang, 2018; Algammal et al., 2022; Dewi et al., 2022). Global concern is understood, with AMR now considered a pressing public health issue. The World Health Organization (O'Neill, 2014) reports 700,000 drug-

resistant infection-related deaths annually, with a predicted rise to surpass cancer-related deaths by 2050 if effective intervention does not occur (WHO, 2019). Within these contexts, marine organisms like *M. merlangus* are potential sentinel species, reflecting not only ecological microbial dynamics but also the silent dissemination of determinants of resistance between interrelated aquatic and terrestrial environments.

In contrast to the selective nature of the TCBS agar that was used for isolation, which supports mainly *Vibrio* spp. preferentially (Uchiyama, 2000), the successful recovery of Gram-negative enteric bacteria reflects the ecological adaptability and salt resistance of some *Enterobacteriales* that enable them to survive in marine habitats (Paarup et al., 1999; Janda and Abbott, 2021). Importantly, that no Gram-positive bacteria or strict aerobes were isolated implies that the approach to isolation, effective for recovering dominant culturable taxa, may have precluded detecting smaller components of the community (Stefani et al., 2015). This is a limitation in all culture-based approaches and highlights the potential benefit of using molecular or metagenomic methods to overcome this in detecting a wider range of fish-associated microbiota (Yukgehnaish et al., 2020). Given the unique phylogenetic location (Figure 2) and low sequence similarity (Table 1) of isolate S09, a future polyphasic study, encompassing genomic, phenotypic, and chemotaxonomic characterization, will be required to clarify the taxonomic status and ascertain if it is a new *Morganella* species.

The enzymatic activities of the isolates exhibited two main extracellular activities: lipase and urease. Whereas these enzymatic activities are often considered to be associated with microbial virulence or spoilage ability (Stead, 1986; Rutherford, 2014), their presence could represent generalist metabolic adaptability or ecological adaptation in the host-associated microbiota (Ikeda-Ohtsubo et al., 2018). Lipase activity, which was detected in isolates B04, K06, and S13, may indicate the capacity to break down lipids in host tissues or in the local environment (Rameshwaram et al., 2018). Although such ability is largely attributed to fish spoilage mechanisms by releasing free fatty acids and volatile compounds, such an ability may also involve mutualistic or commensal functions, such as facilitating lipid digestion in the gastrointestinal tract of hosts (Ghaly et al., 2010). *Proteus* and *Providencia* members that showed this activity have been reported previously among constituents of normal gut microbiota of fish and aquatic animals (Ramkumar et al., 2014; Drzewiecka, 2016). Similarly, urease activity, which was detected in most isolates, may be involved in localized ammonia-mediated pH management and nitrogen cycling. In fish hosts, such an enzymatic capability may support urea metabolism, particularly in species that occupy nitrogenous or brackish habitats (Drzewiecka, 2016). Of interest, a lack of caseinase, amylase, lecithinase, and DNase activity under tested conditions further supports an indication that such isolates may lack strong extracellular virulence determinants. It is, however, possible that such enzymes are induced under certain environmental conditions.

Identification of multidrug-resistant (MDR) bacteria among isolates from *M. merlangus* highlights an emerging concern regarding the dissemination of antibiotic resistance in aquatic environments. Resistance to three or more antibiotic classes was exhibited by eight of every ten isolates, namely β-lactams, sulfonamides, tetracyclines, and aminoglycosides, meeting the definition for MDR status (Rabbani et al., 2017). These resistance patterns, especially widespread insensitivity to amoxicillin/clavulanic acid, tetracycline, and sulfamethoxazole/trimethoprim, reflect long-term environmental exposure to antibiotics (Song et al., 2017; Ng'eno et al., 2024). From a public health point of view, their presence in food fish like *M. merlangus* is alarming, especially with respect to seafood consumption and exposure via food chains. Cross-contamination by improper handling, undercooking, or during food processing can make these bacteria transmittable to humans.

5. Conclusion

This study provides a window into the microbial undercurrents that occupy *M. merlangus*, a species commonly prized for commercial value but seldom analyzed through the lens of resident microbiota. Bacterial genera to which these isolates belong are commonly known to contain opportunistic pathogens, which could pose issues with respect to fish health and food safety. *Enterobacteriaceae* dominance, coupled with selective enzymatic properties and ubiquitous antibiotic resistance, paints a picture of a microbiome influenced by marine ecology and anthropogenic activity. In an era increasingly characterized by interdependent ecosystems and shared microbial territories, such findings hold import outside taxonomic or tissue of origin. They address the silent movement of resistance genes, the effective permeability of boundaries between nature and human activity, and recognition that aquatic organisms are not only food resources but ecological indicators. As we move closer toward an integrated understanding of health between species and environments, such work highlights the need to explore host-associated microbiota from an ecological and health-based paradigm.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	H.S.C.	S.S.	H.S.
C	20	40	40
D	20	40	40
S	-	50	50
DCP	50	25	25
DAI	20	40	40
L	40	30	30
W	10	45	45
CR	10	45	45
SR	10	45	45
PM	30	35	35
FA	50	50	-

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

In accordance with national and institutional guidelines, ethical approval was not required for this study, as the fish specimens (*Merlangius merlangus*) were obtained post-mortem from commercial fishers during routine fishing activities. No experimental procedures were performed on live animals. The collection and use of these samples did not involve any intervention, manipulation, or distress to living organisms, thereby exempting the study from formal ethical review.

Acknowledgements

This research was supported by The Scientific and Technological Research Council of Türkiye (TÜBİTAK), [grant number 1919B012309020].

References

Albejo AL, Hamza TA. 2017. Isolation and characterization of thermostable amylase producing bacteria from hot spring at Arba Minch Nech Sar National Park, Southern Ethiopia. Int J Novel Res Interdiscip Stud, 4: 9-16.

Algammal AM, Mabrok M, Ezzat M, Alfifi KJ, Esawy AM, Elmasry N, El-Tarabili RM. 2022. Prevalence, antimicrobial resistance (AMR) pattern, virulence determinant and AMR genes of emerging multi-drug resistant *Edwardsiella tarda* in Nile tilapia and African catfish. Aquaculture, 548: 737643. https://doi.org/10.1016/j.aquaculture.2021.737643

Alikunhi NM, Batang ZB, AlJahdali HA, Aziz MA, Al-Suwailem AM. 2017. Culture-dependent bacteria in commercial fishes: Qualitative assessment and molecular identification using 16S rRNA gene sequencing. Saudi J Biol Sci, 24: 1105-1116.

Asciutto E, Maioli F, Manfredi C, Anibaldi A, Cimini J, Isailović I, Marčeta B, Casini M. 2024. Spatio-temporal patterns of whiting (*Merlangius merlangus*) in the Adriatic Sea under environmental forcing. PLoS One, 19: e0289999. https://doi.org/10.1371/journal.pone.0289999

Banerjee G, Ray AK. 2017. Bacterial symbiosis in the fish gut and its role in health and metabolism. Symbiosis, 72: 1-11. https://doi.org/10.1007/s13199-016-0441-8

Birolli WG, Lima RN, Porto AL. 2019. Applications of marine-derived microorganisms and their enzymes in biocatalysis and biotransformation, the underexplored potentials. Front Microbiol, 10: 1453. https://doi.org/10.3389/fmicb.2019.01453

Brauge T, Bourdonnais E, Trigueros S, Cresson P, Debuiche S, Granier SA, Midelet G. 2024. Antimicrobial resistance and geographical distribution of *Staphylococcus* sp. isolated from whiting (*Merlangius merlangus*) and seawater in the English Channel and the North Sea. Environ Pollut, 345: 123434. https://doi.org/10.1016/j.envpol.2024.123434

Bunpa S, Sermwittayawong N, Vuddhakul V. 2016. Extracellular enzymes produced by *Vibrio alginolyticus* isolated from environments and diseased aquatic animals. Procedia Chem, 18: 12-17. https://doi.org/10.1016/j.proche.2016.01.002

Cabello FC. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ Microbiol, 8: 1137-1144. https://doi.org/10.1111/j.1462-2920.2006.01054.x

Calì F, Stranci F, La Mesa M, Mazzoldi C, Arneri E, Santojanni A. 2023. Whiting (*Merlangius merlangus*) grows slower and smaller in the Adriatic Sea: New insights from a comparison of two populations with a time interval of 30 years. Fishes, 8: 341. https://doi.org/10.3390/fishes8070341

Cantas L, Sørby JRT, Aleström P, Sørum H. 2012. Culturable gut microbiota diversity in zebrafish. Zebrafish, 9: 26-37. https://doi.org/10.1089/zeb.2011.0712

Cheng TH, Ismail N, Kamaruding N, Saidin J, Danish-Daniel MJB. 2020. Industrial enzymes-producing marine bacteria from marine resources. Biotechnol Rep, 27: e00482.

Chiarello M, Villeger S, Bouvier C, Bettarel Y, Bouvier T. 2015. High diversity of skin-associated bacterial communities of marine fishes is promoted by their high variability among body parts, individuals and species. FEMS Microbiol Ecol, 91: fiv061. https://doi.org/10.1093/femsec/fiv061

Clinical and Laboratory Standards Institute. 2023. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100, Clinical and Laboratory Standards Institute, Wayne, PA, USA, pp: 428.

Cui MJ, Teng A, Chu J, Cao B. 2022. A quantitative, high-throughput urease activity assay for comparison and rapid screening of ureolytic bacteria. Environ Res, 208: 112738. https://doi.org/10.1016/j.envres.2022.112738

de Bruijn I, Liu Y, Wiegertjes GF, Raaijmakers JM. 2018. Exploring fish microbial communities to mitigate emerging diseases in aquaculture. FEMS Microbiol Ecol, 94: fix161. https://doi.org/10.1093/femsec/fix161

Dewi RR, Hassan L, Daud HM, Matori MF, Zakaria Z, Ahmad NI, Aziz SA, Jajere SM. 2022. On-farm practices associated with multi-drug-resistant *Escherichia coli* and *Vibrio parahaemolyticus* derived from cultured fish. Microorganisms, 10: 1520.

Dhayalan A, Velramar B, Govindasamy B, Ramalingam KR, Dilipkumar A, Pachiappan P. 2022. Isolation of a bacterial strain from the gut of the fish, *Systomus sarana*, identification of the isolated strain, optimized production of its protease, the enzyme purification, and partial structural characterization. J Genet Eng Biotechnol, 20: 24.

https://doi.org/10.1186/s43141-022-00299-3

Drzewiecka D. 2016. Significance and roles of *Proteus* spp. bacteria in natural environments. Microb Ecol, 72: 741-758. https://doi.org/10.1007/s00248-015-0720-6

Egerton S, Culloty S, Whooley J, Stanton C, Ross RP. 2018. The gut microbiota of marine fish. Front Microbiol, 9: 873. https://doi.org/10.3389/fmicb.2018.00873

FAO. 2020. The State of Mediterranean and Black Sea Fisheries 2020. General Fisheries Commission for the Mediterranean, Rome, Italy. pp: 129. https://doi.org/10.4060/cb2429en

Farooq S, Ganai SA, Ganai BA, Mohan S, Uqab B, Nazir R. 2022. Molecular characterization of lipase from a psychrotrophic bacterium *Pseudomonas* sp. CRBC14. Curr Genet, 68: 243-251 https://doi.org/10.1007/s00294-021-01224-w

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39: 783-791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Froese R, Pauly D. 2024. FishBase. URL: https://www.fishbase.se (accessed April 01, 2025).

Gauthier DT. 2015. Bacterial zoonoses of fishes: A review and appraisal of evidence for linkages between fish and human infections. Vet J, 203: 27-35.

Ghaly AE, Dave D, Budge S, Brooks MS. 2010. Fish spoilage mechanisms and preservation techniques: Review. Am J Appl Sci, 7: 859-877.

Hatosy SM, Martiny AC. 2015. The ocean as a global reservoir of antibiotic resistance genes. Appl Environ Microbiol, 81: 7593-7599. https://doi.org/10.1128/AEM.00736-15

Hoppe HG, Arnosti C, Herndl GJ. 2002. Ecological significance of bacterial enzymes in the marine environment. In: Burns RG, Dick RP, editors. Enzymes in the Environment: Activity, Ecology, and Applications. Marcel Dekker, New York, USA. pp: 73-107.

Hossain A, Habibullah-Al-Mamun M, Nagano I, Masunaga S, Kitazawa D, Matsuda H. 2022. Antibiotics, antibiotic-resistant bacteria, and resistance genes in aquaculture: risks, current concern, and future thinking. Environ Sci Pollut Res Int, 29: 11054-11075.

Huang Q, Sham RC, Deng Y, Mao Y, Wang C, Zhang T, Leung KM. 2020. Diversity of gut microbiomes in marine fishes is shaped by host-related factors. Mol Ecol, 29: 5019-5034. https://doi.org/10.1111/mec.15699

Hudzicki J. 2009. Kirby-Bauer disk diffusion susceptibility test protocol. Am Soc Microbiol, 15: 1-23.

ICES. 2024. Whiting (*Merlangius merlangus*) in divisions 7.b–c and 7.e–k (southern Celtic Seas and western English Channel). ICES Advice, 2024: whg.27.7b-ce-k. https://doi.org/10.17895/ices.advice.25019729

Ikeda-Ohtsubo W, Brugman S, Warden CH, Rebel JM, Folkerts G, Pieterse CM. 2018. How can we define "optimal microbiota?": A comparative review of structure and functions of microbiota of animals, fish, and plants in agriculture. Front Nutr, 5: 90. https://doi.org/10.3389/fnut.2018.00090

Jackman J. 2012. The microbe: the basics of structure, morphology, and physiology as they relate to microbial characterization and attribution. In: Cliff J, Kreuzer H, Ehrhardt C, Wunschel D, editors. Chemical and Physical Signatures for Microbial Forensics, pp: 13-34.

Janda JM, Abbott SL. 2021. The changing face of the family *Enterobacteriaceae* (Order: "*Enterobacterales*"): New members, taxonomic issues, geographic expansion, and new diseases and disease syndromes. Clin Microbiol Rev, 34: e00021-19. https://doi.org/10.1128/cmr.00174-20

Justé A, Thomma BPHJ, Lievens B. 2008. Recent advances in molecular techniques to study microbial communities in food-associated matrices and processes. Food Microbiol, 25: 745-761. https://doi.org/10.1016/j.fm.2008.04.009

Köker L, Aydın F, Gaygusuz Ö, Akçaalan R, Çamur D, İlter H, Ayoğlu FN, Altın A, Topbaş M, Albay M. 2021. Heavy metal concentrations in *Trachurus mediterraneus* and *Merlangius merlangus* captured from Marmara Sea, Turkey and associated health risks. Environ Manag, 67: 522-531. https://doi.org/10.1007/s00267-020-01352-y

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol, 35: 1547-1549. https://doi.org/10.1093/molbev/msy096

Kyule DN, Maingi JM, Njeru EM, Nyamache AK. 2022. Molecular characterization and diversity of bacteria isolated from fish and fish products retailed in Kenyan markets. Int J Food Sci, 2022: 2379323.

Muus BJ, Nielsen JG. 1999. Sea fish. Scandinavian Fishing Year Book, Hedehusene, Denmark, pp: 340.

Ng'eno E, Cobos ME, Kiplangat S, Mugoh R, Ouma A, Bigogo G, Ouma S, Peterson AT. 2024. Long-term antibiotic exposure landscapes and resistant *Escherichia coli* colonization in a densely populated setting. PLoS One, 19: e0302521. https://doi.org/10.1371/journal.pone.0302521

Nissar S, Bakhtiyar Y, Arafat MY, Andrabi SA, Bhat AA, Yousuf T. 2023. A review of the ecosystem services provided by the marine forage fish. Hydrobiologia, 850: 2871-2902. https://doi.org/10.1007/s10750-022-05033-1

Ntushelo K. 2013. Identifying bacteria and studying bacterial diversity using the 16S ribosomal RNA gene-based sequencing techniques: A review. Afr J Microbiol Res, 7: 5533-5540. https://doi.org/10.5897/AJMR2013.5966

O'Neill J. 2014. Antimicrobial resistance: Tackling a crisis for the health and wealth of nations. Rev Antimicrob Resist, 20: 1-16.

Paarup T, Nieto JC, Peláez C, Reguera JI. 1999. Microbiological and physico-chemical characterisation of deep spoilage in Spanish dry-cured hams and characterisation of isolated *Enterobacteriaceae* with regard to salt and temperature tolerance. Eur Food Res Technol, 209: 366-371. https://doi.org/10.1007/s002170050511

Pękala-Safińska A. 2018. Contemporary threats of bacterial infections in freshwater fish. J Vet Res, 62: 261-267. https://doi.org/10.2478/jvetres-2018-0037

Pepi M, Focardi S. 2021. Antibiotic-resistant bacteria in aquaculture and climate change: A challenge for health in the Mediterranean area. Int J Environ Res Public Health, 18: 5723. https://doi.org/10.3390/ijerph18115723

Pitout JD, Laupland KB. 2008. Extended-spectrum β-lactamase-producing *Enterobacteriaceae*: An emerging public-health concern. Lancet Infect Dis, 8: 159-166. https://doi.org/10.1016/S1473-3099(08)70041-0

Pratte ZA, Besson M, Hollman RD, Stewart FJ. 2018. The gills of reef fish support a distinct microbiome influenced by host-specific factors. Appl Environ Microbiol, 84: e00063-18. https://doi.org/10.1128/AEM.00063-18

Preena PG, Swaminathan TR, Kumar VJR, Singh ISB. 2020. Antimicrobial resistance in aquaculture: A crisis for concern. Biologia, 75: 1497-1517. https://doi.org/10.2478/s11756-020-00456-4

Rabbani MAG, Howlader MZH, Kabir Y. 2017. Detection of multidrug resistant (MDR) bacteria in untreated waste water disposals of hospitals in Dhaka City, Bangladesh. J Glob Antimicrob Resist, 10: 120-125. https://doi.org/10.1016/j.jgar.2017.04.009

Rameshwaram NR, Singh P, Ghosh S, Mukhopadhyay S. 2018.

Lipid metabolism and intracellular bacterial virulence: Key to next-generation therapeutics. Future Microbiol, 13: 1301-1328. https://doi.org/10.2217/fmb-2018-0013

Ramkumar R, Ravi M, Jayaseelan C, Rahuman AA, Anandhi M, Rajthilak C, Perumal P. 2014. Description of *Providencia vermicola* isolated from diseased Indian major carp, *Labeo rohita* (Hamilton, 1822). Aquaculture, 420: 193-197. https://doi.org/10.1016/j.aquaculture.2013.11.013

Ray AK, Ghosh K, Ringø E. 2012. Enzyme-producing bacteria isolated from fish gut: A review. Aquac Nutr, 18: 465-492. https://doi.org/10.1111/j.1365-2095.2012.00943.x

Romero J, Ringø E, Merrifield DL. 2014. The gut microbiota of fish. In: Merrifield D, Ringø E, editors. Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics. Wiley-Blackwell, Oxford, USA, pp: 75-100.

Rutherford JC. 2014. The emerging role of urease as a general microbial virulence factor. PLoS Pathog, 10: e1004062. https://doi.org/10.1371/journal.ppat.1004062

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol, 4: 406-425.

Santos RA, Oliva-Teles A, Pousão-Ferreira P, Jerusik R, Saavedra MJ, Enes P, Serra CR. 2021. Isolation and characterization of fish-gut *Bacillus* spp. as source of natural antimicrobial compounds to fight aquaculture bacterial diseases. Mar Biotechnol, 23: 276-293.

Sheng L, Wang L. 2021. The microbial safety of fish and fish products: Recent advances in understanding its significance, contamination sources, and control strategies. Compr Rev Food Sci Food Saf, 20: 738-786. https://doi.org/10.1111/1541-4337.12671

Song J, Rensing C, Holm PE, Virta M, Brandt KK. 2017. Comparison of metals and tetracycline as selective agents for development of tetracycline resistant bacterial communities in agricultural soil. Environ Sci Technol, 51: 3040-3047. https://doi.org/10.1021/acs.est.6b05342

Stead D. 1986. Microbial lipases: Their characteristics, role in food spoilage and industrial uses. J Dairy Res, 53: 481-505.

Stefani FO, Bell TH, Marchand C, De La Providencia IE, El Yassimi A, St-Arnaud M, Hijri M. 2015. Culture-dependent and -independent methods capture different microbial community fractions in hydrocarbon-contaminated soils. PLoS One, 10: e0128272. https://doi.org/10.1371/journal.pone.0128272

Stratev D, Daskalov H, Vashin I. 2015. Characterisation and determination of antimicrobial resistance of β-haemolytic *Aeromonas* spp. isolated from common carp (*Cyprinus carpio* L.). Rev Med Vet (Toulouse), 166: 54-61.

Trincone A. 2017. Enzymatic processes in marine biotechnology. Mar Drugs, 15: 93. https://doi.org/10.3390/md15040093

Uchiyama H. 2000. Distribution of *Vibrio* species isolated from aquatic environments with TCBS agar. Environ Health Prev Med, 4: 199-204. https://doi.org/10.1007/BF02931258

Wanja DW, Mbuthia PG, Waruiru RM, Mwadime JM, Bebora LC, Nyaga PN, Ngowi HA. 2019. Bacterial pathogens isolated from farmed fish and source pond water in Kirinyaga County, Kenya. Int J Fish Aquat Stud, 7: 295-301.

World Health Organization. 2019. No time to wait: Securing the future from drug-resistant infections. Report to the Secretary-General of the United Nations. World Health Organization, Geneva, Switzerland, pp: 25.

Yang JH, Sheng WH, Hsueh PR. 2020. Antimicrobial susceptibility and distribution of extended-spectrum β-lactamases, AmpC β-lactamases and carbapenemases among *Proteus*, *Providencia* and *Morganella* isolated from global hospitalised patients with intra-abdominal and urinary tract infections: Results of the Study for Monitoring Antimicrobial Resistance Trends (SMART), 2008–2011. J Glob Antimicrob Resist, 22: 398-407. https://doi.org/10.1016/j.jgar.2020.04.011

Yang Y. 2018. Detection, activity measurement and phylogeny of ureolytic bacteria isolated from elasmobranch tissue. Masters Thesis, University of Southern Mississippi, USA, pp: 89.

Yildiz T, Uzer U, Yemişken E, Karakulak FS, Kahraman AE, Çanak Ö. 2021. Conserve immatures and rebound the potential: stock status and reproduction of whiting (*Merlangius merlangus* [Linnaeus, 1758]) in the Western Black Sea. Mar Biol Res, 17: 815-827. https://doi.org/10.1080/17451000.2021.2019787

Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J. 2017. Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol, 67: 1613-1617. https://doi.org/10.1099/ijsem.0.001755

Yukgehnaish K, Kumar P, Sivachandran P, Marimuthu K, Arshad A, Paray BA, Arockiaraj J. 2020. Gut microbiota metagenomics in aquaculture: Factors influencing gut microbiome and its physiological role in fish. Rev Aquac, 12: 1903-1927. https://doi.org/10.1111/raq.12416

Zhang C, Kim SK. 2010. Research and application of marine microbial enzymes: Status and prospects. Mar Drugs, 8: 1920-1934. https://doi.org/10.3390/md8061920