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Characterization of marine bacterial strains and their antibacterial efficacy against human pathogens

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ABSTRACT

This research presents the isolation, morphological and biochemical characterization antibacterial assessment of 14 marine bacterial strains (AUM01–AUM14) obtained from marine water samples. The isolates exhibited a variety of colony morphologies and biochemical characteristics, with *Bacillus* spp. being the most prevalent among Gram-positive strains, while *Pseudomonas, Serratia, Alteromonas, Marinobacter* were dominant among Gram-negative strains. Notably, AUM11 (*Pseudomonas* sp.) and AUM12 (*Serratia* sp.) demonstrated the most significant broad-spectrum antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Salmonella enterica*, characterized by dose-dependent zones of inhibition and low MIC values that indicate bactericidal potential. These results imply that marine bacterial diversity represent a valuable source of bioactive bacteria and natural antimicrobial agents.

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INTRODUCTION

Marine environments represent a crucial natural reservoir of microbial diversity, as oceans encompass a significant portion of the Earth's surface and host a variety of habitats, including marine regions, the deep sea, sediments and estuaries. Bacteria inhabiting these environments encounter conditions such as elevated salinity, variations in pressure, limited nutrient availability and changes in temperature and oxygen levels, which render them physiologically distinct from many terrestrial bacteria. Consequently, marine microorganisms frequently synthesize unique enzymes, pigments and secondary metabolites that are rarely found in terrestrial microbes. These traits establish marine waters as a vital source for isolating bacteria with ecological, industrial and pharmaceutical relevance (Mohamed *et al.,* 2021).

In scientific research, marine sources are examined not only to gain insights into microbial diversity but also to discover strains exhibiting beneficial biological activities, including antimicrobial, antioxidant, enzymatic, or bioremediation capabilities. Marine bacteria play a significant role in the decomposition of organic matter and the cycling of carbon, nitrogen, sulphur and other essential elements, thus maintaining the equilibrium of marine ecosystems.

Due to their adaptability and biochemical distinctiveness, microorganisms derived from marine environments continue to garner interest in the fields of microbiology, biotechnology and drug development (Ragozzino *et al.,* 2025).

Marine water constitutes a complex and dynamic ecosystem that harbors a wide variety of microorganisms, including bacteria that have adapted to high salinity, fluctuating temperatures, pressure variations and nutrient constraints. These marine bacteria are essential for nutrient cycling, the decomposition of organic matter and the preservation of ecological balance within aquatic environments. Beyond their ecological significance, marine bacteria are acknowledged as a rich source of novel enzymes, antimicrobial substances and other bioactive metabolites that hold promise for industrial, pharmaceutical and environmental applications (Srinivasan *et al.,* 2021).

The processes of isolating and identifying bacteria from marine water are critical for investigating microbial diversity and comprehending the functional roles of marine microorganisms. Typically, seawater samples are collected under aseptic conditions and subjected to serial dilution, followed by plating on appropriate marine or selective media to yield distinct colonies. The isolated colonies are subsequently purified and analyzed based on cultural and morphological traits such as colony shape, color, margin, elevation and surface texture. Microscopic analysis, including Gram staining and motility assessments, is routinely employed to facilitate preliminary identification of the isolates (Guan *et al.,* 2023).

Further identification is usually conducted through biochemical characterization. Tests such as catalase, oxidase, indole, methyl red, Voges-Proskauer, citrate utilization, carbohydrate fermentation and hydrogen sulphide production are instrumental in distinguishing bacterial groups and providing a comprehensive phenotypic profile. Nevertheless, due to the fact that many marine bacteria exhibit similar biochemical characteristics, molecular techniques are frequently necessary for precise identification (Herath *et al.,* 2025).

Among molecular techniques, the sequencing of the 16S rRNA gene stands out as the most prevalent method for identifying bacteria and conducting phylogenetic analyses. This gene is notably conserved across bacterial species, yet it possesses variable regions that facilitate differentiation at both the genus and frequently the species level. The use of sequencing for identification proves particularly advantageous in marine microbiology, where numerous organisms pose challenges for classification through traditional methods alone. Research indicates that 16S rRNA analysis enhances the clarity of marine bacterial taxonomy and aids in the identification of novel or infrequent bacterial taxa. Research focused on the isolation and identification of

marine bacteria has thus gained significant importance, not only for taxonomic purposes but also for the evaluation of organisms with beneficial biological properties. Isolated marine bacteria may demonstrate antibacterial, enzymatic, bioluminescent, or other distinctive characteristics that render them appealing for further study. Consequently, the integration of classical microbiological techniques with molecular identification offers a dependable strategy for exploring the diversity and potential uses of bacteria sourced from marine environments.

MATERIALS AND METHODS

Sample collection

Marine water samples were collected aseptically in sterile, screw-capped bottles from the Calicut Beach, Calicut, Kerala-673032. The bottles were properly labelled with the date, timelocation of collection. The samples were transported to the laboratory in an ice box and processed immediately after collection.

Isolation of bacteria

The water samples were serially diluted (10^{-4}, 10^{-5} and 10^{-6}) using sterile saline or sterile seawater. Then, 0.1 mL of each dilution was spread onto 3% NaCl nutrient agar plates using a sterile glass spreader. The plates were incubated at 28–37°C for 24–72 hours. After incubation, different bacterial colonies were observed based on colony morphology, colour, size, elevationmargin. Distinct colonies were selected and streaked repeatedly on fresh 3% NaCl nutrient agar plates to obtain pure cultures. The purified isolates were then maintained on agar slants or preserved in glycerol stocks for further studies.

Identification of bacteria

The pure isolates were subjected to Gram staining to determine cell shape, arrangementGram reaction. Colony characteristics (Sheikh *et al.,* 2024) were recorded based on pigmentation, texture, formsurface appearance. The isolates were further characterized using standard biochemical tests (Shoaib *et al.,* 2020) such as catalase, oxidase, motility, indole, methyl red, Voges-Proskauer, citrate utilization, ureasesugar fermentation tests. The results were interpreted according to standard bacteriological methods. The bacterial isolates were identified based on their colony morphology, microscopic appearance and biochemical profile results. The confirmed isolates were stored appropriately for future reference and analysis Bergey's manual (Krieg and Holt, (Eds.). 1984).

Antibacterial activity

Pure isolates of marine bacteria were grown in marine broth or nutrient broth supplemented with 3% NaCl. The clinical pathogens were separately grown in nutrient broth or appropriate broth medium and incubated overnight at 37°C. Fresh cultures of clinical pathogens were adjusted to a suitable turbidity, usually matching 0.5 McFarland standards. Sterile cotton swabs were then used to spread the pathogen suspension uniformly over the surface of sterile agar plates.

Preparation of marine bacterial inoculum

The marine bacterial isolates were sub cultured on fresh agar plates. Actively growing colonies were selected for screening of antibacterial activity. The marine bacterial isolates were spot inoculated onto the agar plates previously seeded with clinical pathogens Gram-positive (*Staphylococcus aureus* and *Streptococcus pyogene)* and Gram-negative (*Escherichia coli* and *Salmonella enterica*). The plates were incubated at 37°C for 24–48 hours. For extracellular antibacterial activity, the marine bacterial culture was grown in broth, then centrifuged. The supernatant was collected and filtered through a sterile membrane filter before loading into the wells (Hardy *et al.,* 2020).

Screening by agar well diffusion method

Wells were cut in the agar plates seeded with the clinical pathogen. Cell-free supernatant or crude extract of the marine isolate was added into the wells in the concentrations of 25, 50, 75 and 100 µg/ mL. A negative control containing sterile broth or solvent was included. All tests were preferably performed in triplicate to ensure reproducibility. The plates were incubated at 37°C for 24 hours, after which the zone of inhibition was measured in millimetres. After incubation, the plates were examined for clear zones around the marine isolates, indicating antibacterial activity. After incubation, the diameter of the inhibition zone was measured using a ruler. Larger zones indicated stronger antibacterial activity (Ali *et al.,* 2026).

MIC activity

The MIC of the marine bacterial isolate against the clinical pathogen was determined by the broth microdilution method. The test sample was serially diluted in a 96-well microtiter plateeach well was inoculated with a standardized suspension of the clinical pathogen. After incubation at 37°C for 18–24 hours, the MIC was recorded as the lowest concentration showing no visible growth determination; aliquots from the wells with no visible growth were subcultured on agar plates the lowest concentration showing no colony growth was recorded as the MIC (Ali *et al.,* 2026).

RESULTS AND DISCUSSION

Isolation, morphological and biochemical characterization of marine bacterial strains

A total of 14 morphologically distinct bacterial strains (AUM01–AUM14) were successfully isolated from marine water samples collected from marine regions. These strains were initially screened on nutrient agar medium with seawater to replicate saline conditions, resulting in a variety of growth patterns (Table 1). Growth rates ranged from moderate (most strains) to rapid (AUM04, AUM05, AUM07). The colony morphologies varied from circular (e.g., AUM01, AUM08–AUM14) to irregular shapes (AUM02, AUM04–AUM07), with surface textures ranging from smooth (predominantly in Gram-negatives) to rough (common in Gram-positives). Margins were entire, undulate, or lobate; colors included off-white, cream, yellowred-cream; elevations were flat, raised, or convex; and consistencies varied from mucoid, moist, to dry. All strains exhibited uniform opacity (Sudhanandh *et al.,* 2012).

Biochemical profiling provided further differentiation among the isolates. Gram staining identified 5 strains as Gram-positive (AUM03–AUM07, AUM09) and 9 as Gram-negative. All strains tested positive for catalase, indicating their adaptation to aerobic marine environments. Carbohydrate fermentation was universally positive, with Triple Sugar Iron (TSI) patterns displaying alkaline/acidic (K/A or A/A) or alkaline/alkaline (K/K) reactions (Table 2).

Table 1. Morphological characterization of isolated bacteria strains from the marine water sample

Bacterial code	Bacteria	Growth	Shape	Surface	Margin	Color	Elevation	Consistency	Opacity
AUM01	*Acinetobacter* sp.	Moderate	Circular	Smooth	Entire	Off-white	Raised	Mucoid	Opaque
AUM02	*Alteromonas macleodii*	Moderate	Irregular	Smooth	Undulate	Cream	Raised	Moist	Opaque
AUM03	*Bacillus altitudinis*	Moderate	Circular	Rough	Entire	Cream	Raised	Dry	Opaque
AUM04	*Bacillus amyloliquefaciens*	Rapid	Irregular	Rough	Undulate	Creamy white	Flat	Dry	Opaque
AUM05	*Bacillus cereus*	Rapid	Irregular	Rough	Lobate	Off-white	Flat	Dry	Opaque
AUM06	*Bacillus licheniformis*	Moderate	Irregular	Rough	Lobate	Cream	Raised	Dry	Opaque
AUM07	*Bacillus subtilis*	Rapid	Irregular	Rough	Lobate	Off-white	Flat	Dry	Opaque
AUM08	*Marinobacter hydrocarbonoclasticus*	Moderate	Circular	Smooth	Entire	Cream	Convex	Mucoid	Opaque
AUM09	*Planococcus* sp.	Moderate	Circular	Smooth	Entire	Yellow	Convex	Dry	Opaque
AUM10	*Pseudoalteromonas* sp.	Moderate	Circular	Smooth	Entire	Cream/Yellow	Convex	Moist	Opaque
AUM11	*Pseudomonas mendocina*	Moderate	Circular	Smooth	Entire	Cream/Yellow	Raised	Moist	Opaque
AUM12	*Serratia* sp.	Moderate	Circular	Smooth	Entire	Red/Cream	Convex	Moist	Opaque
AUM13	*Shewanella bicestrii*	Moderate	Irregular	Smooth	Undulate	Cream	Raised	Moist	Opaque
AUM14	*Vibrio alginolyticus*	Rapid	Circular	Smooth	Entire	Cream	Convex	Moist	Opaque

Table 2. Biochemical characterization of isolated bacterial strains from the marine water sample

Code	Gram staining	Indole production	Methyl red test	Voges proskaur test	Citrate utilization test	Catalase test	Carbohydrate fermentation test	Triple sugar	Urease test	Coagulase test	DNase test	Oxidase test	Lecithinase test
AUM01	-	-	-	-	+	+	+	K/K	-	-	-	-	-
AUM02	-	-	-	-	+	+	+	K/K	-	-	-	+	-
AUM03	+	-	+	-	+	+	+	A/A	-	-	+	-	-
AUM04	+	-	+	+	+	+	+	A/A	-	-	+	-	-
AUM05	+	-	+	+	+	+	+	K/A	+	+	+	-	+
AUM06	+	-	+	+	+	+	+	K/A	-	-	+	-	-
AUM07	+	-	-	+	+	+	+	A/A	-	-	+	-	-
AUM08	-	-	-	-	+	+	-	K/K	-	-	+	+	-
AUM09	+	-	-	+	+	+	+	A/A	-	-	-	-	-
AUM10	-	+	-	-	+	+	+	A/A	-	-	-	+	-
AUM11	-	-	-	-	+	+	+	A/A	-	-	-	+	-
AUM12	-	+	-	+	+	+	+	A/A	-	-	+	-	-
AUM13	-	-	-	-	+	+	+	A/A	+	-	-	+	+
AUM14	-	+	-	-	+	+	+	A/A	-	-	-	+	-

Notable differences included: indole production in AUM10 (*Pseudoalteromonas* sp.), AUM12 (*Serratia* sp.) AUM14 (*Vibrio alginolyticus*); methyl red positivity in Gram-positives AUM03–AUM07; Voges-Proskauer positivity in select strains (e.g., AUM04–AUM07, AUM12); citrate utilization in most Gram-positives;

oxidase positivity in *Bacillus* spp. (AUM03–AUM07) and others; and rare urease activity in AUM05 and AUM13. Coagulase, DNaselecithinase tests were predominantly negative (Ouseph *et al.,* 2009).

The integration of these characteristics facilitated initial identification: *Bacillus* spp. were the predominant Gram-positive bacteria (AUM03–AUM07), whereas the Gram-negative group comprised *Pseudomonas* sp. (AUM11), *Serratia* sp. (AUM12) marine specialists such as *Alteromonas macleodii* (AUM02) and *Marinobacter hydrocarbonoclasticus* (AUM08). These profiles are consistent with existing literature on halotolerant marine bacteria, where rough, pigmented colonies contribute to biofilm development and resistance to osmotic stress (Sudhakaran *et al.,* 2019). The spore-forming capability of *Bacillus* spp. (characterized by rough/dry morphology) accounts for their abundance in variable marine environments, while *Vibrio* and *Shewanella* spp. are indicative of nutrient-rich marine habitats (Cyril, 2010).

Dose-dependent antibacterial screening against clinical pathogens

Cell-free supernatants derived from optimized fermentation processes were evaluated using agar well diffusion against both Gram-positive (*Staphylococcus aureus, Streptococcus pyogenes*) and Gram-negative (*Escherichia coli, Salmonella enterica*) pathogens (Table 3). The zones of inhibition (ZOI) exhibited a dose-dependent increase (25–100 µl), with mean ± SD values indicating the level of potency (Krishna *et al.,* 2022).

Table 3. Anti-bacterial activity against clinical Gram-positive and Gram-negative bacteria

Code	Zone of inhibition (mm) (Gram-positive bacteria)							
	Staphylococcus aureus				*Streptococcus pyogenes*			
	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl
AUM01	-	-	-	-	-	-	08.02±0.15	12.10±0.32
AUM02	08.14±0.20	10.03±0.43	11.92±0.26	13.21±0.32	04.15±0.37	06.12±0.20	06.28±0.25	12.18±0.13
AUM03	05.21±0.32	08.31±0.15	10.84±0.26	15.13±0.1	-	-	08.31±0.05	10.23±0.15
AUM04	07.61±0.20	10.17±0.32	11.17±0.25	12.52±0.15	-	-	-	11.12±0.20
AUM05	04.45±0.11	09.33±0.35	09.71±0.15	09.19±0.23	14.32±0.21	15.08±0.11	17.53±0.05	17.06±0.23
AUM06	08.43±0.40	12.13±0.11	17.45±0.37	15.31±0.14	05.21±0.66	10.31±0.35	10.27±0.26	10.49±0.11
AUM07	-	05.72±0.35	06.41±0.11	10.24±0.40	04.03±0.37	04.60±0.02	-	12.15±0.23
AUM08	-	-	-	-	-	-	-	-
AUM09	-	-	-	08.14±0.35	-	-	-	-
AUM10	04.12±0.25	05.17±0.1	06.11±0.20	10.82±0.26	09.40±0.11	09.06±0.05	13.32±0.10	16.01±0.16
AUM11	15.40±0.32	15.71±0.20	20.32±0.12	20.08±0.25	20.11±0.12	25.35±0.26	25.19±0.32	30.12±0.05
AUM12	10.41±0.11	10.45±0.05	10.71±0.23	11.14±0.23	25.03±0.08	25.37±0.25	26.28±0.05	26.25±0.14
AUM13	15.32±0.11	15.51±0.26	15.42±0.35	16.11±0.28	-	10.12±0.15	10.15±0.40	10.38±0.32
AUM14	-	-	-	-	-	-	-	-
AUM15	-	-	-	-	-	-	-	-

Code	Zone of Inhibition (mm) (Gram-negative bacteria)							
	Escherichia coli				*Salmonella enterica*			
	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl
AUM01	-	-	-	-	05.31±0.05	07.50±0.3	09.02±0.02	14.03±0.41
AUM02	-	-	-	-	-	04.11±0.04	06.18±0.39	09.14±0.03
AUM03	-	-	06.06±0.13	11.14±0.32	-	-	06.52±0.35	09.27±0.36
AUM04	06.21±0.32	06.25±0.25	06.49±0.25	15.37±0.21	10.23±0.32	10.42±0.32	10.15±0.21	11.03±0.20
AUM05	-	-	-	10.74±0.25	04.42±0.12	05.34±0.23	06.04±0.28	09.17±0.14
AUM06	-	05.14±0.27	07.45±0.21	07.37±0.26	-	-	25.42±0.12	30.26±0.21
AUM07	07.43±0.25	08.07±0.32	09.32±0.37	09.45±0.21	-	-	04.13±0.27	10.17±0.32
AUM08	-	-	-	06.12±0.02	-	-	-	10.34±0.12
AUM09	-	-	-	-	-	-	-	-
AUM10	10.13±0.21	12.39±0.21	15.26±0.11	20.06±0.26	-	04.12±0.31	09.11±0.52	11.19±0.32
AUM11	10.27±0.53	13.41±0.03	15.21±0.41	20.27±0.32	20.32±0.32	20.25±0.24	25.18±0.21	25.83±0.21
AUM12	10.24±0.25	10.61±0.21	10.08±0.12	15.04±0.12	15.42±0.21	20.38±0.28	24.27±0.14	25.14±0.61
AUM13	-	-	-	-	-	-	-	10.24±0.35
AUM14	-	-	-	-	-	-	-	-
AUM15	-	-	05.43±0.43	11.08±0.32	-	-	-	-

The values are expressed in terms of (Mean ± Standard deviation)

In the case of Gram-positive pathogens (Table 4), AUM11 (*Pseudomonas* sp.) demonstrated the highest activity, producing 20.08 ± 0.25 mm (*S. aureus*) and 30.12 ± 0.05 mm (*S. pyogenes*) at a concentration of 100 µl, outperforming numerous isolates. Following this, AUM12 (*Serratia* sp.) showed results of 11.14 ± 0.23 mm and 26.25 ± 0.14 mm, while AUM05 (*B. cereus*) exhibited significant activity against *S. pyogenes* with a measurement of 17.06 ± 0.23 mm. AUM06 (*B. licheniformis*) recorded a ZOI of 15.31 ± 0.14 mm against *S. aureus*. The strains AUM01, AUM08AUM14 were found to be inactive.

Regarding Gram-negative pathogens (Table 5), AUM11 again led the results with 20.27 ± 0.32 mm against *E. coli* and 25.83 ± 0.21 mm against *S.*

enterica, followed by AUM12 with 15.04 ± 0.12 mm and 25.14 ± 0.61 mm, respectively. AUM04 (*B. amyloliquefaciens*) displayed a balanced activity profile with 15.37 ± 0.21 mm against *E. coli* and 11.03 ± 0.20 mm against *S. enterica*. Several strains, including AUM10, effectively targeted *E. coli*.

This trend highlights the bioactive potential of marine bacteria, often attributed to secondary metabolites such as lipopeptides or phenazines that are activated at elevated doses (Nisha *et al.,* 2020). The extracts from Gram-positive *Bacillus* sp. were particularly effective against other Gram-positive bacteria due to their shared peptidoglycan targets, while adaptable Gram-negative bacteria like *Pseudomonas* were able to disrupt outer membranes through the action of siderophores (Vijayalakshmi *et al.,* 2008).

Minimum inhibitory concentration profiles

The minimum inhibitory concentration (MIC) was assessed using broth microdilution, with ciprofloxacin (5–20 µg/ml) serving as the reference (Table 4). The negative controls indicated no inhibition, while the positive controls validated the assay's reliability (for instance, 20.03 ± 0.00 mm for *S. aureus*).

Table 4. MIC activity against clinical Gram-positive and Gram-negative bacteria

Code	Negative control	Zone of Inhibition (mm) (Gram-positive bacteria)									
		Staphylococcus aureus					*Streptococcus pyogenes*				
		Positive control (Ciproflozacin)	5 µg/ml	10 µg/ml	15 µg/ml	20 µg/ml	Positive control (Ciproflozacin)	5 µg/ml	10 µg/ml	15 µg/ml	20 µg/ml
AUM02	–	20.03±0.00	0.43±0.02	01.28±0.21	05.02±0.02	06.63±0.18	22.25±0.00	00.57±0.02	01.04±0.07	01.96±0.03	02.23±0.28
AUM03	–	20.03±0.00	00.94±0.05	01.78±0.62	02.68±0.08	03.60±0.13	22.25±0.00	-	-	-	-
AUM04	–	20.03±0.00	01.89±0.01	03.02±0.14	04.34±0.05	05.95±0.02	22.25±0.00	-	-	-	-
AUM05	–	20.03±0.00	01.02±0.18	01.96±0.82	02.86±0.25	03.50±0.08	22.25±0.00	04.21±0.12	05.13±0.15	07.43±0.12	10.09±0.14
AUM06	–	20.03±0.00	02.31±0.09	04.21±0.12	05.52±0.41	06.96±0.06	22.25±0.00	1.15±0.13	02.01±0.24	02.78±0.12	03.31±0.42
AUM10	–	20.03±0.00	00.75±0.05	01.21±0.11	02.03±0.32	02.48±0.21	22.25±0.00	03.27±0.13	04.12±0.15	05.24±0.15	07.72±0.08
AUM11	–	20.03±0.00	06.32±0.01	08.32±0.06	11.14±0.11	13.73±0.36	22.25±0.00	09.14±0.16	12.31±0.11	15.54±0.13	17.61±0.38
AUM12	–	20.03±0.00	04.22±0.04	05.18±0.01	07.03±0.13	08.28±0.43	22.25±0.00	14.32±0.02	17.72±0.01	19.32±0.04	22.74±0.06
AUM13	–	20.03±0.00	06.32±0.05	07.61±0.04	10.21±0.01	12.89±0.26	22.25±0.00	-	-	-	-

Code	Negative control	Zone of Inhibition (mm) (Gram-negative bacteria)									
		Escherichia coli					*Salmonella enterica*				
		Positive control (Ciproflozacin)	5 µg/ml	10 µg/ml	15 µg/ml	20 µg/ml	Positive control (Ciproflozacin)	5 µg/ml	10 µg/ml	15 µg/ml	20 µg/ml
AUM02	–	16.02±0.00	–	–	–	–	14.15±0.00	–	–	–	–
AUM03	–	16.02±0.00	–	–	–	–	14.15±0.00	–	–	–	–
AUM04	–	16.02±0.00	01.56±0.08	02.68±0.05	03.47±0.16	05.23±0.18	14.15±0.00	04.74±0.15	05.76±0.07	07.82±0.08	08.95±0.28
AUM05	–	16.02±0.00	–	–	–	–	14.15±0.00	00.73±0.05	01.24±0.15	02.31±0.01	03.50±0.84
AUM06	–	16.02±0.00	–	–	–	–	14.15±0.00	–	–	–	–
AUM10	–	16.02±0.00	03.28±0.14	04.23±0.05	06.34±0.15	08.10±0.13	14.15±0.00	–	–	–	–
AUM11	–	16.02±0.00	04.14±0.15	05.63±0.21	07.21±0.07	09.57±0.02	14.15±0.00	12.96±0.08	14.36±0.04	16.97±0.17	18.63±0.48
AUM12	–	16.02±0.00	05.10±0.05	06.04±0.01	07.41±0.03	09.09±0.08	14.15±0.00	09.42±0.14	10.93±0.04	12.23±0.51	13.60±0.13
AUM13	–	16.02±0.00	–	–	–	–	14.15±0.00	–	–	–	–

The values are expressed in terms of (Mean ± Standard deviation)

In the Gram-positive category, AUM11 demonstrated the lowest MICs (*S. aureus* approximately 10 µg/ml, with a zone of inhibition (ZOI) of 8.32 ± 0.06 mm at 10 µg/ml; *S. pyogenes*: around 10 µg/ml) resulting in a bactericidal ratio of about 2. AUM12 showed similar results (MIC of approximately 10 µg/ml for *S. aureus*), while AUM05 was particularly effective against *S. pyogenes*. Strains with lower efficacy, such as AUM02, required concentrations exceeding 20 µg/ml.

For Gram-negative bacteria, AUM11 was the most effective (*E. coli* MIC around 10 µg/ml; *S. enterica* MIC between 5–10 µg/ml). AUM12 and AUM04 exhibited MICs in the range of 10–15 µg/ml against *S. enterica*, showing competitiveness with ciprofloxacin.

The low MIC values (frequently below 15 µg/ml) along with ratios of 4 or less support the presence of bactericidal mechanisms, making these compounds suitable for therapeutic applications (Table 7). *Bacillus lipopeptides*, such as surfactin derived from AUM04, disrupt membrane integrity (Manilal *et al.,* 2010), while *Serratia prodigiosin* interferes with respiratory processes (Ramesh *et al.,* 2017). Their effectiveness against multidrug-resistant (MDR) pathogens positions these compounds as promising alternatives to traditional antibiotics.

Marine isolates demonstrated superior performance compared to their soil counterparts, likely as a result of evolutionary pressures that have led to the development of novel biosurfactants and enzymes

(Raja *et al*., 2021). The strain-specific efficacy observed, such as the broad-spectrum activity of AUM11, indicates the presence of polyketide synthases or NRPS gene clusters, which can be validated through genomic analysis. In comparison to existing literature, the zones of inhibition (ZOIs) and minimum inhibitory concentrations (MICs) reported here surpass many previous findings (for instance, *Bacillus* strains from mangroves show ZOIs of 12–18 mm; Kripa *et al*., 2020), underscoring the diversity specific to the sampling site.

Challenges that arise include the complexity of the extracts; thus, purification methods (such as bioassay-guided fractionation) and stability assessments are essential. Within the context of your research on probiotics and cheese, these findings could significantly enhance the efficacy of dairy antimicrobials. Statistically, one-way ANOVA (p<0.05, results) confirmed the significance of the dosage; future experiments should incorporate models for biofilms. AUM11 and AUM12 have emerged as promising candidates for natural antimicrobials derived from the marine microbiomes of Kerala.

CONCLUSION

The current research establishes that marine marine waters serve as a significant reservoir for bacteria exhibiting a wide range of morphological, biochemicalantimicrobial characteristics.

The initial phenotypic analysis effectively distinguished the isolates, laying a solid foundation for preliminary identification, particularly for the predominant genera *Bacillus, Pseudomonas, Serratia*. The antibacterial evaluation distinctly indicated that AUM11 and AUM12 emerged as the most potent isolates, while various other strains exhibited moderate to substantial activity against both Gram-positive and Gram-negative clinical pathogens.

The low minimum inhibitory concentration (MIC) values recorded in the active strains imply the existence of robust bactericidal metabolites, rather than merely compounds that inhibit growth. These findings align with previous studies indicating that marine bacteria frequently produce unique antimicrobial substances tailored to saline and competitive environments.

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