Research Article

Isolation, Screening and Identification of Marine bacteria for their Potential Bioactivity

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Submission Date: 21-05-2024; Revision Date: 14-06-2024; Accepted Date: 01-08-2024.

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ABSTRACT

Bacteria in marine environment are the most versatile organisms capable of producing a wide spectrum of potential bioactive secondary metabolites, some with novel structures. Many of these secondary metabolites of marine bacterial origin have been explored to develop new antimicrobial compounds. **Aim:** To study the inhibitory activity of metabolites produced by isolated marine bacteria water samples from marine environment were collected from ten random sites of Bheemli shore, Visakhapatnam, Bay of Bengal Coast, Andhra Pradesh. The samples were brought to the lab in sterile containers, made into composite sample which was utilized for the isolation of bacteria employing Zobell agar medium. **Results:** 23 representative isolates of bacterial strains were obtained from the water sample after inoculation and incubation on Zobell agar medium. The isolates were designated as C1, C2, C3, C4 and so on. Colony characteristics of all the pure isolates, Gram staining nature and shape of the cells were recorded. The obtained bacterial isolates were screened for their antibacterial activity against selected Gram- positive and Gram- negative groups of test pathogens. Of the 23 bacterial isolates screened, only 12 isolates showed growth inhibitory activity against the test bacteria. Among the 12 isolates positive for antibacterial activity, isolates viz., C1, C6, C7, C8 and C9 were found to have potential with relatively high inhibitory activity. **Conclusion:** The five potential isolates were investigated by 16S rRNA gene sequencing for the species identification and revealing the C1 identified as *Bacillus paramycoides*, C6 as *Lysinibacillus fusiformis*, C7 as *Bacillus oshimensis*, C8 as *Bacillus velezensis*, and C9 as *Bacillus paranthracis*. Further work on isolation, purification and identification of metabolite compounds from the most potential bacterial species is in progress.

Keywords: Antibacterial activity, Bioactive compounds, Marine bacteria, Secondary metabolites.

INTRODUCTION

Addressing the infections caused by pathogenic bacteria is a global concern. Prolonged use of traditional antibiotics has been associated with the development of tolerant bacterial species. In order to address the issues caused by pathogen resistance, a number of different approaches have been developed that employ chemically and biologically synthesized chemicals as antibacterial compounds. One of the best possible sources for isolating and identifying bioactive chemicals is marine organisms.[1]

In recent years, more attention is being given to marine habitat than terrestrial habitat as biological resources to tap the microorganisms for antimicrobial compounds. Oceans comprise different habitats distinguished by various degrees of salinity, water pressure and temperature. To thrive in such distinct environments, marine microbes have evolved a variety of adaptation strategies, such as the synthesis of particular protein biomolecules.[2] Marine biological resources are extremely rich in amino polysaccharide chitin, ulvans, structural proteins like spongin, collagens, gelatin, keratin and biominerals etc. The environment creates the possibility for marine organisms to produce different natural active substances.[3] Marine natural products play a vital role

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DOI: 10.5530/ajbls.2024.13.68

in scientific research and development of medicines, either directly as pharmaceuticals or as principal components for manufacture of bio inspired chemical based products.[4] Over 15,000 novel metabolites have been uncovered from marine organisms since 1985, several of these exhibit notable bioactivities.[5] Marine microorganisms possess the capability to synthesize a wide array of biologically active substances exhibiting various therapeutic characteristics such as antibiotic, anti-inflammatory, hypoglycemic and cancer preventing activity.[6] There has been increasing interest in exploring marine fungi and bacteria as potential sources of bioactive substances that can be used in cosmetic and cosmeceutical uses.[7]

Chemicals such as didemnin B (AplidineTM) and thiocoraline are produced from marine microorganisms that are being studied in clinical and pre-clinical trials of cancer.[8] Antimicrobial activity of *Bacillus sps.* isolated from marine habitats combat the bacterial strains causing foodborne illness, such as *Staphylococcus aureus* and *Vibrio parahaemolyticus* was reported in Mexico.[9] The present study was aimed to examine the inhibitory ability of metabolites of the isolated heterotrophic marine bacteria against some selected test bacteria.

MATERIALS AND METHODS

Collection of samples

Water samples from the marine environment were gathered from ten different sites along the coast of Bheemli Beach, Visakhapatnam, Bay of Bengal Coast, Andhra Pradesh at a depth between 30 and 500cm. The samples were brought to the lab in sterile containers and stored at 4ºC in the refrigerator until further work.

Isolation of marine bacteria

Serially diluted the collected marine water samples to 10^{-6} and 10^{-7} and dispersed over the entire surface of Zobell agar plates by spread plate method, composed of Peptone-5 g/L; Yeast extract 1g/L; Sodium chloride -10 g/L; Magnesium chloride-5 g/L; Calcium chloride-1 g/L; Potassium chloride-5 g/L; Ammonium nitrate-16 g/L; Disodium phosphate-8 g/L; Sodium sulphate-32 g/L; Glucose-5 g/L, Agar: 15.0 g/L with a pH of 7.2. After incubation at 30ºC for 48-72 hr, Zobell agar plates were examined for the colonies. The bacteria that differ in colony morphology and pigmentation were selected, named as C1 to C23 and subcultured to obtain in pure form. All the isolates have been stored at 4ºC and as glycerol stocks.[10]

Macroscopic and microscopic features

Colony characteristics viz., Shape, Size, Colour, Margin, Elevation, Surface and Texture of the pure isolates obtained were recorded based on Bergey's Manual of Determinative Bacteriology.[11] A range of biochemical activities were carried for those isolates that showed the high antibacterial activity for the tentative identification of organism up to genus level that encompassing the capacity to metabolize carbon sources, produce indole, methyl red and Voges-Proskauer test, utilization of citrate, catalyze the breakdown of hydrogen peroxide, presence of oxidase, generation of hydrogen sulfide and urease activity.

Antibacterial activity

Test Bacteria

Test bacteria obtained from Microbial Type Culture Collection were utilized such as viz., *Micrococcus luteus* MTCC 106, *Arthrobacter protophormiae* MTCC 2682, *Rhodococcus rhodochrous* MTCC 265, *Alkaligens faecalis* MTCC 126, *Lactobacillus acidophilus* MTCC 10307, *Enterobacter aerogenes* MTCC 10208, *Proteus vulgaris* MTCC 426, *Bacillus megaterium* MTCC 428, *Enterococcus faecalis* MTCC 439, *Salmonella enterica* MTCC 3858, *Staphylococcus aureus* MTCC 737, *Escherichia coli* MTCC 1687, *Pseudomonas aeruginosa* MTCC 1688, *Bacillus subtilis* MTCC 441 and *Streptococcus mutans* MTCC 497, in the study. The antimicrobial effectiveness of the bacterial strains obtained from marine water was evaluated through the utilization of the agar well diffusion technique.

Agar well method

The bacterial isolates which showed the antimicrobial activity in preliminary screening were tested for their extracellular metabolite production capabilities using the agar well diffusion method. The selected active isolates were introduced into Zobell liquid medium and subjected to incubation at a temperature of 30ºC for duration of 3 days. Following incubation, the broth was centrifuged at a rotational speed of 4000 rpm for 10 min. The resulting supernatant containing the secreted bioactive compounds was collected for the analysis by agar well method.[12] The collected supernatant of 50µl was added to the wells made, using 6 mm cork borer, in the agar plates that were seeded with target microorganisms. The inoculated plates were incubated at a temperature of 37ºC for 24 hr and the antibacterial activity was recorded in terms of inhibition zones formed surrounding the wells of marine isolates.[13]

Molecular characterization

The bacterial isolates that exhibited the most promising inhibitory activity against the selected test bacteria were further investigated through molecular identification techniques using 16S rRNA sequencing. The DNA from each of the selected bacterial isolates was extracted and amplified using specialized primers designed to target the highly conserved 16S rRNA gene. Subsequently, the amplified DNA fragments were subjected to sequencing, which determined the nucleotide sequence of the 16S rRNA gene for each isolate. These obtained sequences were then analyzed using the BLAST (Basic Local Alignment Search Tool) similarity search program. This program compared the query sequences against the nucleotide database of GenBank, maintained by the National Center for Biotechnology Information (NCBI). By identifying the closest matches in the database, the taxonomic identities of the bacterial isolates were inferred. Furthermore, a phylogenetic tree was constructed using advanced computational methods, depicting the evolutionary relationships among the selected sequences based on their genetic similarities and differences. The phylogenetic tree provided a visual representation of the evolutionary connections between the identified bacterial isolates and their closest relatives, aiding in their classification and understanding of their positioning.[14]

RESULTS

From the collected marine water samples, a total of 23 marine bacterial isolates (C1 to C23) were obtained. The pure colony characteristics such as size, surface, texture, colour, elevation and margin of all the 23 isolates were noted and presented in Table 1. In shape, 9 isolates were irregular and 8 were circular. Considerable variation was found among the isolates in all the other parameters of the colonies. Gram staining nature and cell shape observations (Table 2) indicated that only two isolates (C11 and C22) were found Gram -ve and the remaining isolates were Gram +ve in nature. Regarding the cell shape, isolate C4 was found to be Staphylococci, C11, C15 and C21 as Cocci in shape and the rest 19 isolates were of bacilli nature.

Table 1: Colony characteristics of the isolated bacterial cultures.

Isolate	Shape	Size	Surface	Texture	Colour	Elevation	Margin
C1	Irregular	Small	Smooth	Dry	Cream	Raised	Curled
C2	Irregular	Medium	Smooth	Hard	Light yellow	Raised	Undulate
C3	Circular	Very small	Shiny	Mucous	Cream	Flat	Curled
C4	Circular	Large	Shiny	Viscous	Opaque	Flat	Lobate
C5	Irregular	Small	Smooth	Moist	Opaque	Raised	Curled
C6	Filamentous	Large	Rough	Dry	White	Umbonate	Filamentous
C7	Irregular	Small	Rough	Viscous	Yellow	Umbonate	Undulate
C8	Rhizoid	Medium	Wrinkled	Dry	White	Convex	Undulate
C9	Circular	Medium	Smooth	Butyrous	Orange	Raised	Curled
C10	Irregular	Large	Rough	Viscous	Brown	Umbonate	Lobate
C11	Irregular	Large	Shiny	Dry	Brown	Raised	Undulate
C12	Circular	Very small	Shiny	Moist	White	Flat	Entire
C13	Filamentous	Medium	Wrinkled	Dry	White	Umbonate	Undulate
C14	Filamentous	Medium	Wrinkled	Viscous	Brown	Raised	Lobate
C15	Irregular	Very small	Dull	Hard	Cream	Raised	Lobate
C16	Rhizoid	Medium	Wrinkled	Dry	Orange	Convex	Undulate
C17	Rhizoid	Large	Smooth	Viscous	Yellow	Umbonate	Undulate
C18	Circular	Small	Shiny	Viscous	White	Raised	Entire
C19	Irregular	Small	Shiny	mucous	Cream	Raised	Undulate
C20	Circular	Very small	Rough	Dry	White	Flat	Entire
C21	Circular	Very small	Smooth	Mucous	Opaque	Umbonate	Lobate
C22	Circular	Small	Wrinkled	Hard	Yellow	Convex	Lobate
C23	Irregular	Large	Smooth	Viscous	Brown	Convex	Lobate

Table 2: Microscopic features.

Bacterial isolate	Gram's nature	Cell Shape
C1	+ ve	*Bacilli*
C2	+ ve	*Bacilli*
C3	+ ve	*Bacilli*
C4	+ ve	*Staphylococci*
C5	+ ve	*Bacilli*
C6	+ ve	*Bacilli*
C7	+ ve	*Bacilli*
C8	+ ve	*Bacilli*
C9	+ ve	*Bacilli*
C10	+ ve	*Bacilli*
C11	- ve	*Cocci*
C12	+ ve	*Bacilli*
C13	+ ve	*Bacilli*
C14	+ ve	*Bacilli*
C15	+ ve	*Cocci*
C16	+ ve	*Bacilli*
C17	+ ve	*Bacilli*
C18	+ ve	*Bacilli*
C19	+ ve	*Bacilli*
C20	+ ve	*Bacilli*
C21	+ ve	*Cocci*
C22	- ve	*Bacilli*
C23	+ ve	*Bacilli*

Among the 23 isolates, only 12 isolates showed inhibition activity against test bacteria on preliminary screening. These positive 12 isolates were selected for further antibacterial activity testing by the agar well method. All these 12 isolates showed moderate to good inhibition against the test pathogens (Table 3). Of these 12 isolates, five isolates, denoted as C1, C6, C7, C8 and C9 exhibited relatively greater growth inhibitory activity against the majority of the tested bacteria with an inhibition zone between 10 and 22.5 mm. The classification of antibacterial activity based on the area of Zone of Inhibition (ZOI) was accounted as strong or excellent (more than 16 mm ZOI), moderate or good (11- 16 mm ZOI), weak (7-11 mm ZOI) and inactive (<7 mm ZOI) according to Selvakumar *et al.*[15]

The biochemical characters of the five potential isolates that showed good inhibition are detailed in Table 4. Towards the IMViC tests, the isolates showed varied positive and negative results. The isolates are catalase-positive and urease-negative, except C8 isolate. All five isolates were found negative for H2S production and gelatin hydrolysis, but positive to starch hydrolysis, except the isolate C7. The bacteria showed varying degrees of utilization towards carbon such as glucose, sucrose and lactose with acid and/or gas production or not.

The 16S rRNA sequencing of these 5 isolates revealed the identification of C1, C6, C7, C8 and C9 as *Bacillus paramycoides, Lysinibacillus fusiformis*, *Bacillus oshimensis*, *Bacillus velezensis* and *Bacillus paranthracis*, respectively. The phylogenetic tree structures of the five identified isolates are presented in Figures 1-5.

Bacillus paramycoides showed strong inhibition against *Enterobacter aerogenes* (21.5 mm±0.7*)*, *Streptococcus mutans* (18.5 mm±0.5), *Lactobacillus acidophilus* (17 mm±0.5), *Micrococcus luteus* (16.5 mm±0.7*)*, *Arthrobacter protophormiae* (16 mm±0.4), and moderate activity against *Rhodococcus rhodochrous* (14.5 mm±0.8), *Alkaligens faecalis* (13.5mm±0.5) and *Enterococcus faecalis* (13.5 mm±0.5).

Lysinibacillus fusiformis displayed strong inhibition towards *Enterobacter aerogenes* (22 mm±0.3), *Bacillus subtilis* (21.8 mm±0.2), *Streptococcus mutans* (21.3 mm±0.5), *Rhodococcus rhodochrous* (17.1 mm±0.2), *Alkaligens faecalis* (16.3 mm±0.5), *Arthrobacter protophormiae* (16.2 mm±0.5), but moderate activity towards *Micrococcus luteus* (12.7 mm±0.2).

Bacillus oshimensis exhibited strong activity towards three organisms *Enterobacter aerogenes*, *Lactobacillus acidophilus* and *Pseudomonas aeruginosa* with inhibition zones of 21.6 mm, 21.5 mm and 17.5 mm, respectively and showed moderate inhibition zones ranging against six test bacteria viz., *Alkaligens faecalis* (15.5 mm), *Arthrobacter protophormiae* (14.5 mm), *Rhodococcus rhodochrous* (14.1 mm), *Streptococcus mutans* (12.1 mm), *Escherichia coli* (11.5 mm), and *Staphylococcus aureus* (11.3 mm).

Bacillus velezensis displayed strong activity against five test cultures namely *Arthrobacter protophormiae* (18.5 mm), *Rhodococcus rhodochrous* (19.6 mm), *Alkaligens faecalis* (16.3 mm), *Enterobacter aerogenes* (23.1 mm), *Streptococcus mutans* (17.3 mm) and moderate activity against five cultures of *Micrococcus luteus* (15.8 mm), *Lactobacillus acidophilus* (14.6 mm), *Staphylococcus aureus* (12.5 mm) and *Bacillus subtilis* (11.8 mm).

Bacillus paranthracis showed strong inhibition towards four test cultures, *Enterobacter aerogenes* (22.5 mm), *Lactobacillus acidophilus* (20.6 mm, *Streptococcus mutans* (18.5 mm) and *Rhodococcus rhodochrous* (16.0 mm), whereas moderate inhibition against six cultures viz., *Enterococcus faecalis* (15 mm), *Pseudomonas aeruginosa* (14.6 mm), *Micrococcus luteus* (14.5 mm), *Arthrobacter protophormiae* (14 mm), *Alkaligens faecalis* (14 mm) and *Bacillus megaterium* (12.9 mm).

Table 3: Antimicrobial activity (zone of inhibition in mm) of the isolates against testbacteria.

Isolate Name	Test Bacteria														
	T1	T2	T3	T5	T7	T8	T9	T10	T11	T12	T14	T16	T17	T18	T19
C1	16.5 ±0.7	16.0 ±0.4	14.5 ±0.8	13.5 ±0.5	21.5 ±0.7	8.5 ±0.5	--	17.0 ±0.5	13.5 ±0.5	18.5 ±0.5	7.0 ±0.6	5.0 ±0.8	9.5 ±0.5	7.0 ±0.6	12.5 ±0.7
C3	13.0 ±0.3	16.5 ±0.6	16.5 ±0.6	17.0 ±0.3	16.5 ±0.6	8.0 ±0.4	--	6.0 ±0.3	10.5 ±0.6	7.5 ±0.6	6.0 ±0.3	7.0 ±0.2	10.5 ±0.6	20.0 ±0.5	7.0 ±0.2
C6	12.2 ±0.2	16.2 ±0.5	17.1 ±0.2	16.3 ±0.5	22.0 ±0.3	12.8 ±0.7	--	9.3 ±0.5	15.6 ±0.5	21.3 ±0.5	5.0 ±0.1	15.0 ±0.8	12.6 ±0.5	21.8 ±0.2	6.0 ±0.5
C7	10.8 ±0.7	14.5 ±0.8	14.1 ±0.2	15.5 ±0.5	21.6 ±0.5	7.5 ±0.5	--	21.5 ±0.5	8.5 ±0.5	12.1 ±0.2	11.3 ±0.5	11.5 ±0.5	17.5 ±0.5	7.8 ±0.7	5.5 ±0.5
C8	15.8 ±0.7	18.5 ±0.5	19.6 ±0.5	16.3 ±0.5	23.1 ±0.7	7.1 ±0.7	--	14.6 ±0.7	7.5 ±0.5	17.3 ±0.5	12.5 ±0.8	--	8.5 ±0.5	11.8 ±0.7	5.8 ±0.2
C9	14.5 ±0.8	14.0 ±1.0	16.0 ±0.4	14.0 ±1.0	22.5 ±0.8	5.3 ±0.5	12.1 ±0.7	20.6 ±0.5	15.0 ±0.5	18.5 ±0.5	5.3 ±0.5	7.3 ±0.5	14.6 ±0.7	5.3 ±0.5	5.6 ±0.5
C10	13.0 ±0.7	13.0 ±0.7	10.8 ±0.7	8.8 ±0.7	14.5 ±0.8	6.5 ±0.5	14.3 ±0.7	18.5 ±0.5	15.8 ±0.7	11.6 ±0.7	6.4 ±0.4	9.06 ±0.7	5.3 ±0.4	5.6 ±0.3	4.1 ±0.3
C11	14.4 ±0.7	12.5 ±0.5	16.2 ±0.7	16.0 ±0.2	16.0 ±0.2	6.4 ±0.5	16.7 ±0.2	14.5 ±0.8	12.2 ±0.5	--	9.2 ±0.2	9.2 ±0.5	6.2 ±0.5	11.5 ±0.5	5.6 ±0.5
C16	16.2 ±0.5	12.4 ±0.7	13.2 ±0.5	15.4 ±0.7	20.3 ±0.7	6.0 ±0.5	--	--	11.1 ±0.3	16.0 ±0.2	7.1 ±0.7	18.0 ±0.2	7.9 ±0.5	7.06 ±0.3	6.0 ±0.5
C18	16.7 ±0.3	12.3 ±0.7	7.1 ±0.3	18.1 ±0.4	12.2 ±0.5	5.8 ±0.2	--	9.4 ±0.4	--	18.3 ±0.6	5.5 ±0.4	8.6 ±0.9	13.4 ±0.5	4.5 ±0.7	5.8 ±0.3
C19	18.5 ±0.6	15.0 ±0.5	16.1 ±0.3	6.2 ±0.5	11.5 ±0.6	4.5 ±0.6	--	14.3 ±0.7	13.3 ±0.6	21.1 ±0.5	6.0 ±0.6	6.5 ±0.5	7.0 ±0.7	12.1 ±0.6	9.1 ±0.6
C20	11.6 ±0.7	9.4 ±0.4	12.3 ±0.3	8.4 ±0.4	16.4 ±0.8	4.5 ±0.7	--	12.5 ±0.5	12.0 ±0.3	--	8.1 ±0.5	10.6 ±0.5	--	6.5 ±0.4	6.5 ±0.4

T1-*Micrococcus luteus*
T2-*Arthrobacter protophormiae*
T3-*Rhodococcus rhodochrous*
T5-*Alkaligens faecalis*
T7-*Enterobacter aerogenes*
T8-*Proteus vulgaris*
T9-*Bacillus megaterium*
T10-*Lactobacillus acidophilus*
T11-*Enterococcus faecalis*
T12-*Streptococcus mutans*
T14-*Staphylococcus aureus*
T16-*Escherichia coli*
T17-*Pseudomonas aeruginosa*
T18-*Bacillus subtilis*
T19-*Salmonella enterica*
-- No zone of inhibition
--±SD values
All the values are mean of triplicates.

Table 4: Biochemical characterization of isolates.

Name of the reaction	C1	C6	C7	C8	C9
Indole production	- ve	+ve	-ve	+ve	-ve
Methyl Red	-ve	-ve	+ve	+ve	-ve
Vogues Proskauer test	+ve	+ve	-ve	-ve	+ve
Citrate utilization	+ve	+ve	-ve	+ve	+ve
Catalase	+ve	+ve	+ve	-ve	+ve
Urease	-ve	-ve	-ve	+ve	-ve
Nitrate reduction test	-ve	-ve	+ve	-ve	+ve
H_2S production	-ve	-ve	-ve	-ve	-ve
Gelatin liquefaction	-ve	-ve	-ve	-ve	-ve
Starch hydrolysis	+ve	+ve	- ve	+ve	+ve
Carbon utilization					
Glucose	A and GNA	A and G	A and GA	A and GA	A NA and G
Sucrose	NA and NG	NA and G	NA and NG	A	A
Lactose		NA and NG		A	
Motility	Non-Motile	Non-Motile	Motile	Motile	Motile

+ ve=positive;- ve=Negative; A=Acid; NA-No acid; G=Gas; NG-No gas.

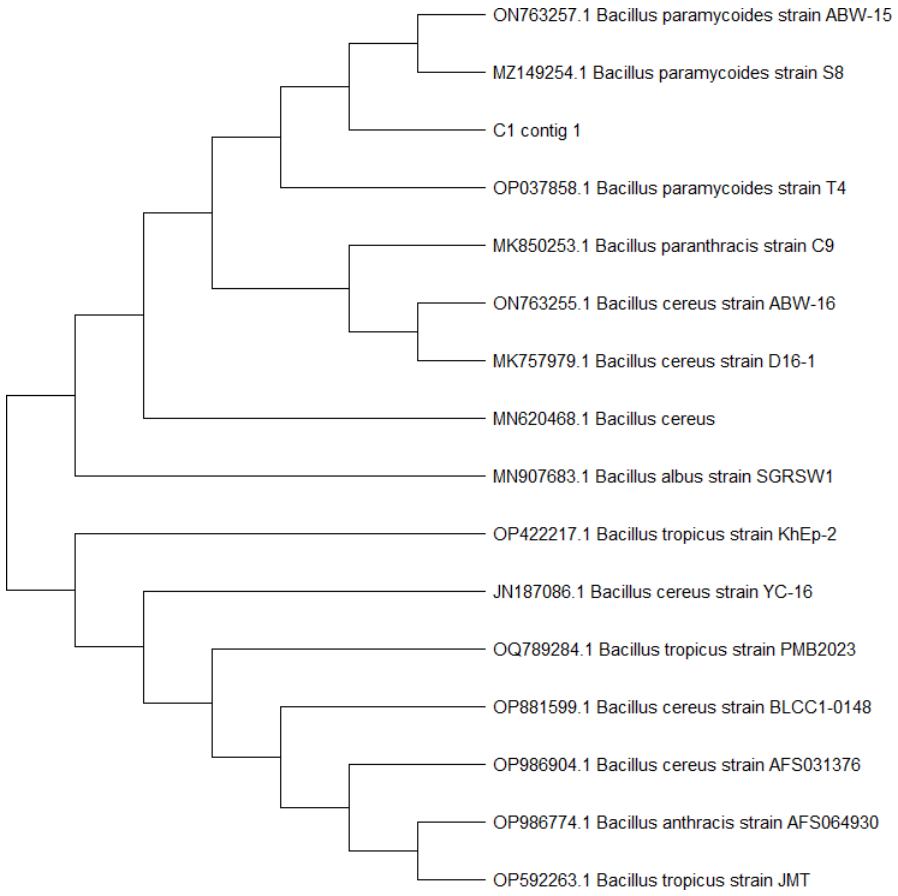

Figure 1: Molecular Phylogenetic Reconstruction of Bacterial Isolate C1 Using Maximum Likelihood Approach.

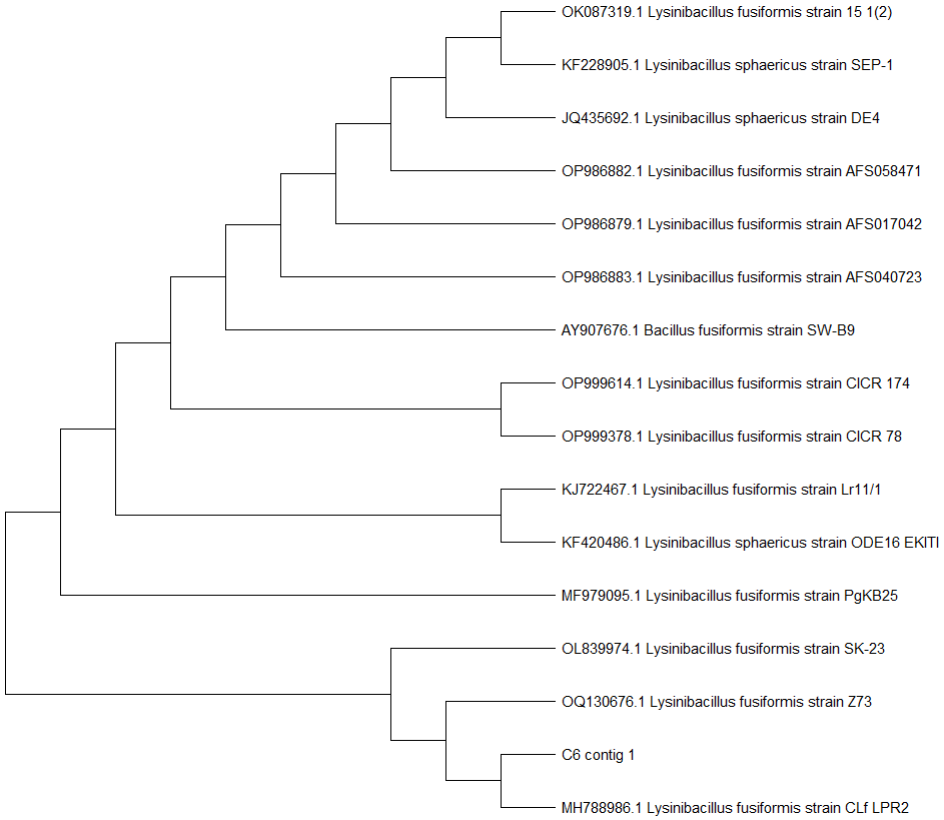

Figure 2: Molecular Phylogenetic Reconstruction of Bacterial Isolate C6 Using Maximum Likelihood Approach.

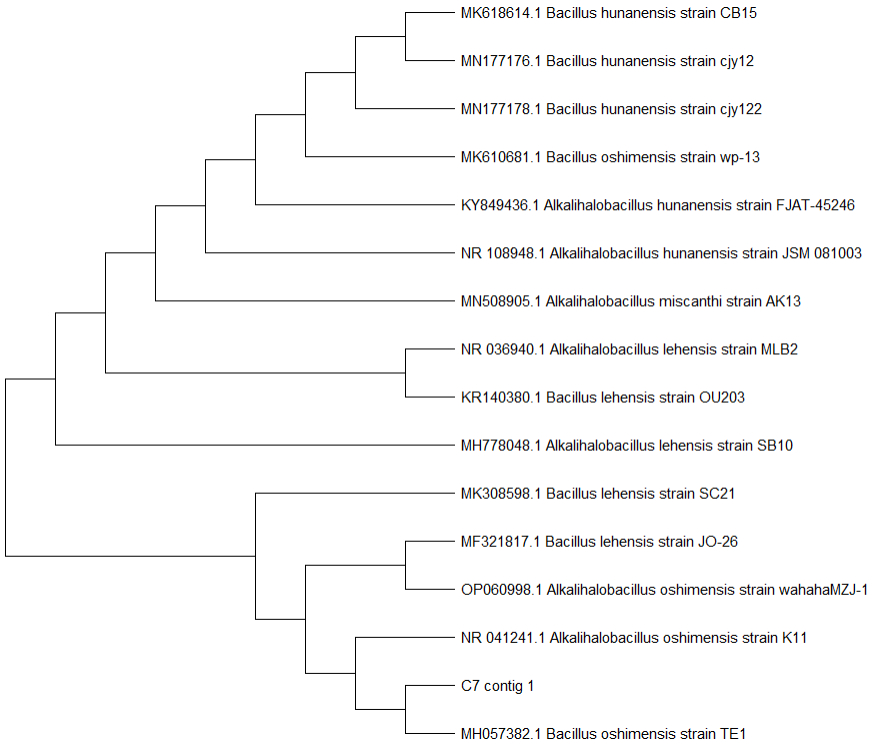

Figure 3: Molecular Phylogenetic Reconstruction of Bacterial Isolate C7 Using Maximum Likelihood Approach.

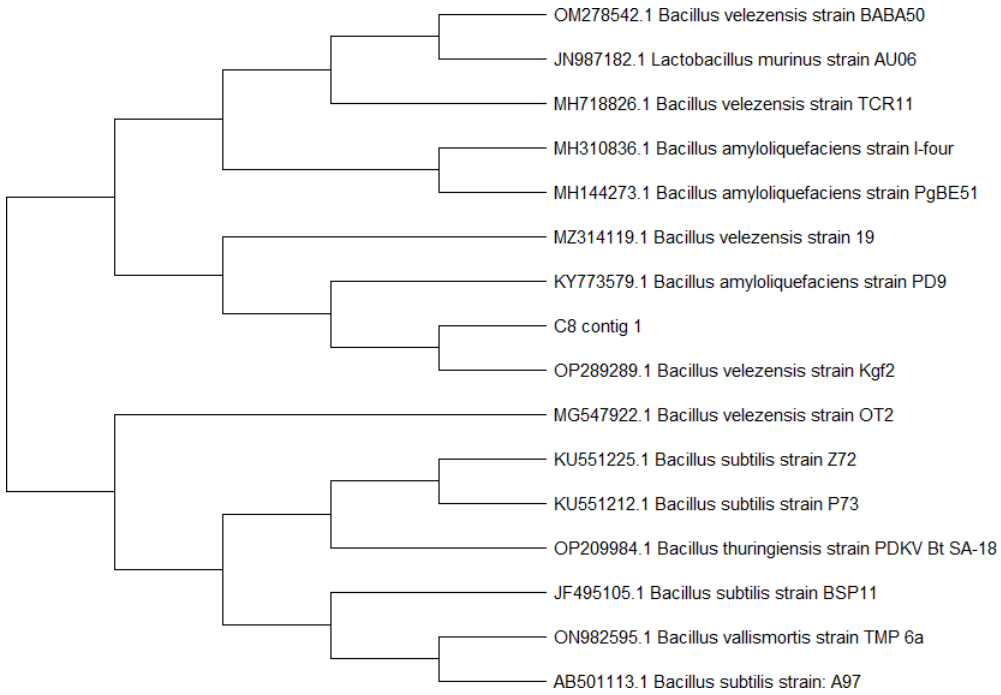

Figure 4: Molecular Phylogenetic Reconstruction of Bacterial Isolate C8 Using Maximum Likelihood Approach.

DISCUSSION

Marine sources are familiar for their potent resources of antibacterial, antifungal, anticancer and anti-inflammatory metabolites. Among 23 isolates from our study 12 were found to be antagonistic against bacterial pathogens. Sankari *et al.*[16] isolated *Pseudomonas* and *Actinomycetes* from Bay of Bengal that exhibited both antibacterial and antifungal properties. Marine actinomycetes belonging to the species *Gordomia terrae* have exhibited promising antimicrobial properties against various bacterial strains that are pathogenic to humans.[17] Antagonistic marine bacteria such as *Bacillus,*

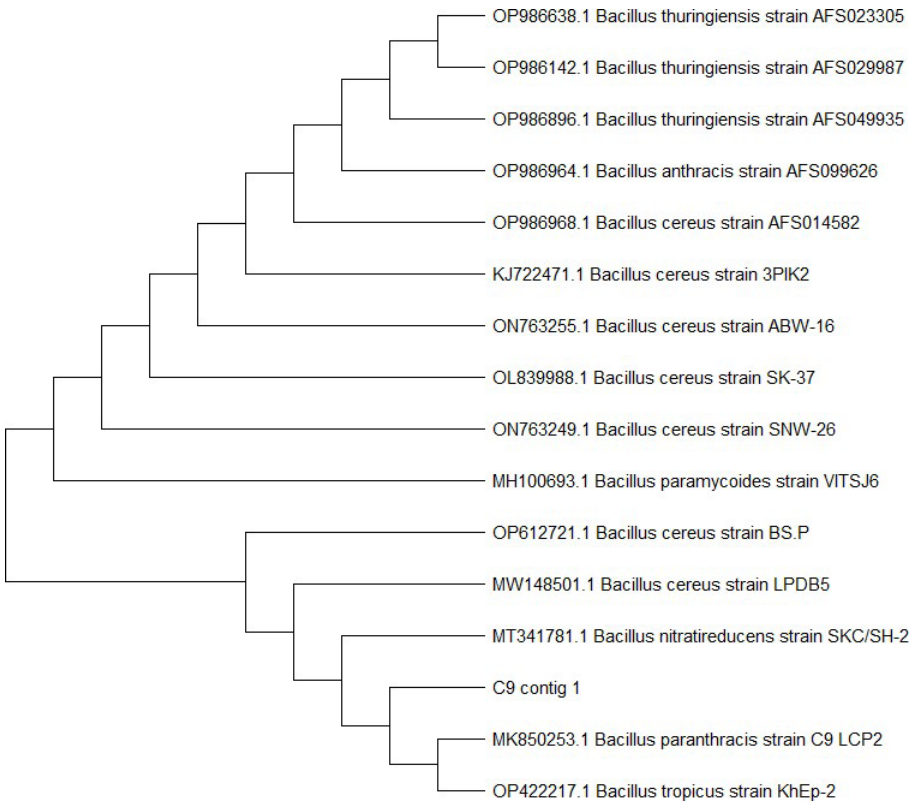

Figure 5: Molecular Phylogenetic Reconstruction of Bacterial Isolate C9 Using Maximum Likelihood Approach.

Staphylococcus, Halobacillus and *Marinobacter* from deep sea sediment collected from the Bay of Bengal was reported in the study conducted by Natarajan and colleagues.[18]

In concurrence with earlier studies, the present study showed that 5 strains are more antagonistic towards test pathogens belonging to both Gm+ve and Gm-ve organisms. 16s rRNA homology of these strains, subsequent phylogeny analyses confirmed these bacteria belong to *Bacillus* group. *Bacillus* species is one of the predominant microflorae widely distributed in marine ecosystems.[19] The metabolites produced by marine *Bacillus* species include a wide range of chemical structures and classes belonging to lipopeptides, polyketides, non-ribosomal peptides, macrolides and glycopeptides. The exploration of these compounds has unveiled the remarkable biosynthetic capabilities of marine *Bacillus* strains and their potential applications in various fields.

Bacillus secondary metabolites exhibited antibacterial activity against both Gm+ve and Gm-ve pathogens such as *Vibrio parahaemolyticus, Vibrio vulnificus, Aeromonas hydrophila*, *Escherichia coli, Vibrio cholerae, Vibrio harveyi, Pseudomonas aeruginosa, Staphylococcus aureus, B.subtilis, Salmonella typhi* and *Proteus species* demonstrating their broad-spectrum bacteriostatic characters.[20]

Bacillus sp. YB1701 isolated from sediment samples from coastal region was identified with antibacterial, cell-to-cell communication disruption ability and suggested as its application as fish probiotic.[21] *Bacillus paramycoides PBG9D and BCS10* demonstrated the ability to inhibit the growth of *Aeromonas hydrophila*, a pathogen that cause infections in fish species.[22]

Lysinibacillus possess the capacity to exhibit antimicrobial properties which is attributed to their production of various bioactive compounds, including bacteriocins, peptide-based antibiotics, and other therapeutic molecules. In line with study of *Lysinibacillus fusiformis* isolated from marine fish exhibited antibacterial activity against pathogens that cause urinary tract infections and eye infections which are *Staphyloccoccus aureus, Klebsiella, Streptococcus, Pseudomonas, Proteus, Acinetobacter sps,*[23] the present work also reports the antibacterial activity against *Enterobacter, Bacillus subtilis, Streptococcus mutans, Alkaligens faecalis, Rhodococcus* and *Arthrobacter sps.*

Bacillus oshimensis was named from the region oshima, Japan was halophilic and halotolerant organism require NaCl for PH homeostasis or for energy production contains isoprenoid quinone which is menaquinone-7.[24] *Bacillus oshimensis* strain FJ3 showed antibacterial activity against various bacterial pathogens, including Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The researchers attributed the antibacterial activity to the production of lipopeptide compounds, such as surfactin and

fengycin.[25] Expanding on the results, our study reports the inhibitory activity against other pathogens such as *Alkaligens faecalis, Arthrobacter protophormiae, Rhodococcus rhodochrous* and *Streptococcus mutans.*

Bacillus velezensis is endowed with a remarkably diverse and dynamic genetic makeup, enabling it to synthesize a wide array of valuable secondary metabolites. One notable characteristic of the HNA3 strain is its ability to emit Volatile Organic Compounds (VOCs) that possess the capacity to inhibit the growth of phytopathogenic microorganisms affecting various fruits and vegetables, even in the absence of direct contact. Researchers have identified a total of 14 major VOCs produced by this strain, with phenol,2,4-bis(1,1-dimethylethyl) and 1,2-benzenedicarboxylic acid being the most abundant semi-volatile compounds. Interestingly, both of these compounds are known for their antifungal properties and their ability to promote plant growth.[26]

The antimicrobial activity exhibited by our isolate is comparable to the findings reported earlier work wherein the antibacterial activity of *Bacillus paranthracis* strain BCMU 116 against various bacterial pathogens, including *Staphylococcus aureus, Escherichia co*li, and *Bacillus cereus.* Lipopeptide compound was isolated and characterized, named paranthrenolide, which possess potent antibacterial properties.[27] Other novel lipopeptide, named paranthramide B, from *B. paranthracis* BCRC 17929, was reported having antibacterial activity against Gram-positive bacteria.[28]

CONCLUSION

The present study reports that the bacteria isolated from marine water exhibited antimicrobial activity. Further studies on the optimization of environmental conditions, nutrient requirements and extraction of the bioactive compounds produced by these organisms could open up new avenues for the discovery of unique metabolites with potential applications in drug development.

ACKNOWLEDGEMENT

First author is thankful to Government Degree College for Women (Autonomous), Nalgonda for providing facilities in the Department of Microbiology to carry out the research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The findings of this study highlight the promising nature of these bacterial strains as potential sources of potent antimicrobial agents with a broad spectrum of activity against both Gram-positive and Gram-negative pathogens. Notably, the study encourages the exploration of bacterial extracts as potential alternatives to conventional modern medicine in combating pathogenic microorganisms. The results emphasize the need to harness the antimicrobial potential of these bacterial sources for the development of effective therapeutic interventions against infectious diseases.

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Cite this article: Devavani K, Rao VU. Isolation, Screening and Identification of Marine bacteria for their Potential Bioactivity. Asian J Biol Life Sci. 2024;13(2):551-60.