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Antibacterial activity of marine-derived *Sutcliffiella horikoshii* KSV4 isolated from Rushikonda water source, Vishakhapatnam

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Original Article

Abstract

Marine bacteria have emerged as prominent sources of novel pharmaceutical agents produced through fermentation processes to meet global demands for antibiotics in recent years. Marine bacteria *Sutcliffiella horikoshii*, found in marine and soil ecosystems, has been found to produce a potent neurotoxin, Tetrodotoxin (TTX). This study demonstrates the isolate KSV4 of *S. horikoshii*, from Rushikonda Beach, exhibiting remarkable antibacterial properties against clinically relevant test bacteria using the Kirby-Bauer agar-well diffusion method. Thin-layer chromatography-mediated bioautography associated with Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed bioactive compounds in the crude extract of KSV4. Additionally, molecular identification confirmed KSV4, suggesting applications in environmental remediation and pharmaceuticals. Overall, this study underscores the potential of maritime ecosystems as a promising source of novel antibiotics, contributing to combating antibiotic resistance. Further research is required to corroborate these findings against a broader range of pathogens and elucidate the bacterial isolate's therapeutic potential, mechanism of action and biotechnological applications.

Keywords: *Antibacterial efficacy, kirby-Bauer disc diffusion method, Sutcliffiella horikoshii, Thin-layer chromatography (TLC)-bioautography, marine bacteria, Dodecane*

Introduction

Microorganisms are prolific antibiotic reservoirs, essential to combat bacterial infections in humans and animals. These antibiotics are critical for treating a broad spectrum of infections. However, their misuse and overuse have led to the emergence of antibiotic-resistant strains, posing a growing public health problem, and necessitating alternative therapeutic modalities (Sahar *et al*., 2021).

Oceans, encompassing over 70% of the Earth's surface, harbour diverse microbial life that produces novel antibiotics (Kasanah and Hamann, 2004; Santos *et al*., 2020). Marine environments represent a promising source of bioactive compounds due to the unique ecological conditions that foster microbial diversity and metabolic potential. Certain bacteria like *Bacillus*, *Pseudomonas*, and marine fungi, have shown promise in combating multidrug-resistant bacterial strains (Bultel-Poncé *et al*., 1998; Nweze *et al*., 2020), producing antibiotics and bioactive compounds. For instance, marine-derived compounds such as Macrolactin A from *Bacillus* species have shown significant antimicrobial properties (Mondol *et al*., 2013a). Additionally, Salinosporamide A, derived from marine actinomycetes, has entered clinical trials as a potential treatment for cancer (Giordano *et al*., 2015). Other bioactive compounds, isolated from marine-derived *Bacillus* species, include polyketides and bacteriocins with significant antimicrobial activity and antialgal and anticancer activities (Xiao *et al*., 2022). The ability of *Bacillus* species to produce diverse classes of antibiotics has been evidenced by several genomic studies, indicating that about 8% of the genome is devoted to synthesising antibiotics (Mondol *et al*., 2013b). The potential applications of marine-derived antibiotics extend beyond human medicine; they can also be utilised in aquaculture to prevent bacterial infections in fish, in agriculture as biopesticides, and in environmental remediation to mitigate pollution (Desbois and Smith, 2010). This study aims to explore the antibacterial activity of *Sutcliffiella horikoshii* KSV4 isolated from Rushikonda Beach and its potential applications in various fields.

Material and methods

Sample collection

Water samples were collected at a depth of approximately 1 m from Rushikonda Beach (17.7825° N, 83.3851° E) in Vishakhapatnam, Andhra Pradesh, using 500 mL of sterile and autoclaved bottles. The samples were then stored at 4°C until further examination (Kumari *et al*., 2020). Environmental parameters such as temperature, pH and salinity were recorded at the time of collection.

Isolation and purification

The collected water sample (100 µL) was inoculated into plates containing Zobell Marine Agar media (Hi-Media, India) and incubated at 37°C for 4 days. Distinct pigmented colonies were selected and transferred to sterile Zobell agar plates for subculturing. For further analysis, these isolates were preserved as slants at 4°C in a refrigerator and as glycerol stocks at -80°C (Jayadev and Lekshmi, 2016).

Collection of test strains

Five bacterial strains, namely *Bacillus subtilis*, *Bacillus cereus*, *Proteus vulgaris*, *Proteus mirabilis*, and Methicillin-resistant *Staphylococcus aureus* used in this study were isolated on selective media from urine and pus collected from local hospitals and biochemical tests were performed to confirm the identity of these strains. The rationale for choosing the aforementioned bacteria is that they are all considered potential opportunistic pathogens commonly associated with bacterial infections (Sandhya *et al*., 2014).

Morphological and biochemical assessment

The morphological aspects of the marine bacterial isolates were documented. These aspects were determined using the standard Gram-staining procedure and morphological characters such as size, shape, colour, texture, opacity, elevation, and margin. The bacterial isolates further underwent biochemical activity assays as stated in Bergey's manual and compared with known type strains for characterization which includes Indole, Methyl Red, Voges Proskauer, and Citrate *etc.* to assist in their taxonomic classification (Bartholomew and Mittwer, 1952; Cappuccino and Sherman, 2007).

Effect of temperature and pH on growth

To maximize secondary metabolite synthesis, it is necessary to determine the ideal pH and temperature ranges. In our study, we adjusted the pH of the Zobell medium to 7.5-8.5 and tested incubation temperatures from 35 to 39°C to identify the optimal conditions for maximal secondary metabolite production. A sample of 200µL was added to a 96-well microtiter plate, and the optical density was recorded at 600 nm using an ELISA reader (Epoch, United States). Absorbance readings were taken bi-hourly over 24 hours relative to a blank control by use of a microtiter plate with a final volume of 200 µL in each well and performed in duplicates. The collected data were used to plot a graph using MS Excel software (Aparna *et al*., 2020).

Extraction of crude compounds

The aforementioned isolates were cultured in Erlenmeyer flasks and incubated at 37°C for 5-7 days. The cultures were subjected to centrifugation at 5000 rpm for 15 minutes at a temperature of 4°C. The pellet containing the pigment was washed thoroughly with distilled water to lyse the cells, and the supernatant was removed. Subsequently, the crude compound was extracted by suspending the pellet in 1000 ml of Ethanol and placing the falcon tube in a water bath for 60 minutes. Following the cooling of the tube, it was centrifuged again for 15 minutes at 5000 rpm until the pellet turned colourless. The resulting supernatant with diffused pigment was filtered through Whatman's No. 1 filter paper to extract the crude compound of the isolate (Shanthi Kumari *et al*., 2024).

Antibacterial efficacy

The antibacterial properties of the crude compound extracted from the bacterial isolates were evaluated against the test bacterial strains *B. subtilis*, *B. cereus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *P. mirabilis*, and *P. vulgaris* using the Kirby-Bauer agar-well diffusion method. The nutrient agar plates were inoculated with 100 µl of the test bacterial cultures and wells were created using a sterile well borer. Under sterile conditions, (1000 µl) crude compound was added to the wells. The plates were then incubated upright for 24 hrs at 37°C with a negative control of ethanol and a positive control of Ampicillin. After incubation, the inhibition zones were meticulously measured to evaluate the antibacterial efficacy of the compound. This experiment was performed in triplicates (Nikita *et al*., 2020).

GC-MS analysis of the crude extract of KSV4

The present study employed a TLC-mediated bioautography to investigate the antibacterial activity of an isolate. The crude compound extract (5 µl) was applied to TLC plates (Merck, India), developed using different solvent mixtures, and visualized under UV light. The mobile phases included

Chloroform/ethanol (2:1 and 3:1), acetone/water (5:3), and petroleum ether/ethyl acetate (1:9 and 9:1). Subsequently, the developed TLC plate was used to perform bioautography and the zones of inhibition were measured. GC-MS analysis was performed on the scrapped silica from the plates using a GC-MS (GC-MS-QP2020 NX, Shimadzu, Japan) equipped with a SH-Rxi-5Sil MS (30 X 0.25 X 0.25 mm) column. The carrier gas was helium, the components were separated at a 1 mL/minute flow rate, under a temperature program starting at an initial temperature of 50°C for 4 minutes followed by an increase to 260°C at a rate of 10°C/min up to 300°C and sustained for 12 minutes. GC-MS analysis determined the components of the unidentified mass spectra with recognised components found in the NIST library. Names, structures, and molecular weights of the test material components were accurately identified (Yogitha Bala, 2024).

Molecular identification and phylogenetic analysis of KSV4

The results showed that KSV4 is a potential bacterial isolate with antibacterial activity against all the test bacteria. Thus, the genomic DNA of this isolate is subjected to 16S rRNA gene amplification using 16S rRNA-F and 16S rRNA-R primers for species identification. PCR product of length 1400 bp purified and sequenced on ABI 3730 X 1 Genetic Analyser using the BDT v3.1 Cycle sequencing kit. The 16S rRNA sequences were subjected to BLAST analysis using the mega blast tool of GenBank (http://www.ncbi.nlm.nih.gov/). Among different species comprising of closest neighbouring strains in NCBI-BLAST analysis used in the phylogenetic analysis. A phylogenetic tree was constructed based on the neighbour-joining method and percentage differences in the genetic relationships between the neighbouring strains (Imran *et al.*, 2019; Nisha *et al.*, 2019).

Results and discussion

Isolation and characterisation

Five distinct bacterial isolates were obtained from water samples collected at Rushikonda Beach. Bacterial isolates were labelled as KSV1, KSV2, KSV3, KSV4, and KSV5. KSV4 was studied in detail as it showed significant antibacterial efficacy. This study identified *S. horikoshii* KSV4, marking its first identification from this specific marine environment compared to previous studies that reported this species from soil habitats and other marine locations. Morphological and biochemical characteristics of the isolate KSV4 were reported in Tables 1 and 2. Colony morphology is given in Fig. 1. In a similar study conducted by Nikita et. al. (2020) a comparable bacterial isolation method was employed to isolate pigmented

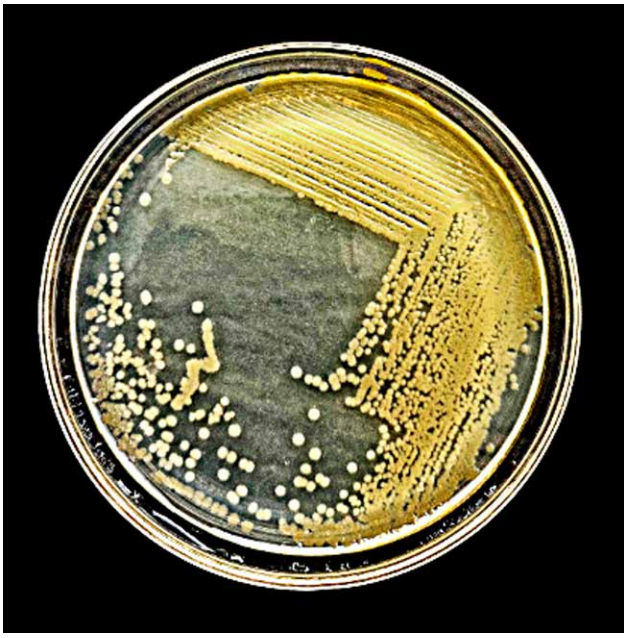

Fig. 1. *Sutcliffiella horikoshii* KSV4, gram-positive bacilli with small, circular, opaque yellow-brown colour colonies with entire margins

Table 1. Morphological characteristics of KSV4

Morphological characteristics	KSV4 isolate
Colony shape	Circular
Colony size	Small
Margin	Entire
Pigmentation	Yellow-brown
Elevation	Raised
Texture	Smooth
Optical property	Opaque
Gram nature	Positive
Shape	Bacilli

Table 2. Results of biochemical characterization of KSV4

Biochemical tests	KSV4 isolate
Indole test	Negative
Methyl red test	Negative
Voges-Proskauer test	Negative
Citrate utilisation test	Negative
Catalase test	Positive
Oxidase test	Negative
Motility test	Motile
Carbohydrate fermentation	
1. Glucose	Positive
2. Fructose	Positive
3. Galactose	Positive
4. Xylose	Positive
5. Ribose	Positive
6. Sucrose	Positive

colonies from water samples of the Arabian Sea. The authors chose standard microbiological techniques to isolate and characterize bacterial colonies based on morphological and biochemical traits. In another study by Nisha *et al.* (2019), a bacterial isolation method was employed to isolate bacterial colonies from soil samples collected in Alfalfa plant fields, in Coimbatore. These colonies were further characterized morphologically and biochemically.

Molecular identification and phylogenetic analysis of KSV4

In the present study, isolate KSV4 was found to exhibit noteworthy antibacterial efficacy. Molecular analysis revealed it to be *S. horikoshii* (as depicted in Fig. 5). This molecular identification and analysis are crucial in understanding bacterial isolates' genetic relatedness and evolutionary history. The 16S rRNA gene sequence was submitted to the Gene Bank (NCBI) and accession number PP733937 was received. The evolutionary history was inferred using the Maximum Likelihood method and the Tamura-Nei model. A phylogenetic tree was constructed using MEGA X software (Nisha *et al.*, 2019). The identification of the isolate underscores the importance of molecular techniques in bacterial classification. This method has proven effective in assessing bacterial diversity, as shown by (Carlos *et al.*, 2013), who noted significant proteobacterial abundance in coral ecosystems. The antibacterial properties align with those documented for *Bacillus horikoshii*, which possesses genes coding for bacteriocins, enhancing its ecological fitness (Zarza *et al.*, 2017). Furthermore, understanding the evolutionary relationships within the bacillus genus of these strains (Alcaraz *et al.*, 2010).

Effect of pH and temperature on the growth of the isolates

All bacterial isolates showed increased growth with changes in pH and temperature. Among all the isolates, KSV4 showed enhanced growth at 37 °C and pH 8 (Fig. 2). This ability to flourish under these conditions suggests its potential utility in various biotechnological applications, including bioremediation and agricultural practices. It supports the notion that this strain is well-suited for alkaline environments. Previous studies have documented that certain *Bacillus* strains can produce plant growth-promoting substances under alkaline conditions, which may contribute to their overall growth and metabolic efficacy (Radhakrishnan *et al.*, 2017).

Bacterial crude compound extraction

Crude compounds were extracted using solvents, such as ethanol, chloroform, ethyl acetate, methanol and acetone. However, Ethanol was used to effectively extract crude

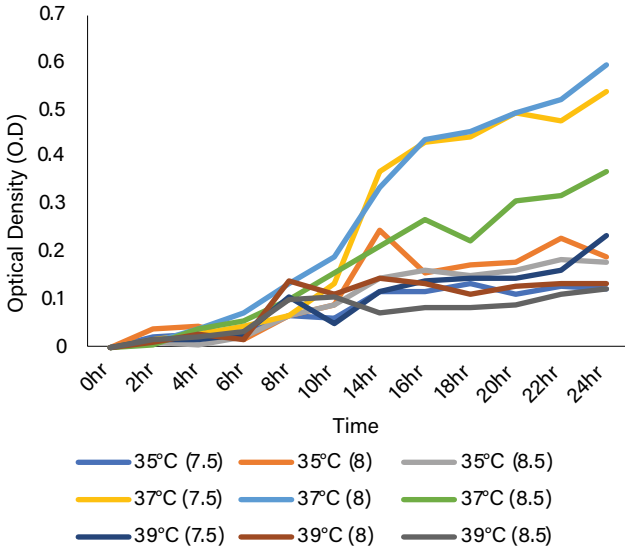

Fig. 2. Investigating the effect of pH and temperature on the growth of bacterial isolate KSV4

compounds from the culture broth of the bacterial isolates. In a study conducted by Shanthi Kumari *et al.* (2024) the crude extract of the isolate, *Pseudomonas aeruginosa* VSB3 was extracted using ethanol.

Antibacterial activity of crude compounds

The extracted crude compounds displayed antibacterial efficacy against different test bacteria (as shown in Table 3). Among the five bacterial isolates, KSV1, KSV4 and KSV5 demonstrated inhibitory activity against the test bacteria. The inhibition zones measured were presented in Fig. 3. This suppressive effect was more noticeable against Gram-positive species like *B. cereus* and MRSA compared to Gram-negative species such as *P. mirabilis*, and *P. vulgaris*. These findings highlight the potential of marine bacteria as a source of novel bioactive compounds. Further research is required to elucidate the mechanisms of action of these compounds and their potential therapeutic applications. *Bacillus horikoshii* isolated from the rhizosphere region of Alfalfa plant fields produces antimicrobial compounds against clinical pathogens

Table 3. Antibacterial efficacy of crude compound extracted from KSV4 against different test bacteria

Test organism	Diameter of zone of inhibition (mm)
Bacillus subtilis	17±0.5
Bacillus cereus	14±1
Proteus mirabilis	13±0.5
Proteus vulgaris	15
Methicillin-resistant (MRSA)	14±1

Values are representatives of mean ± S.D., (n=3).

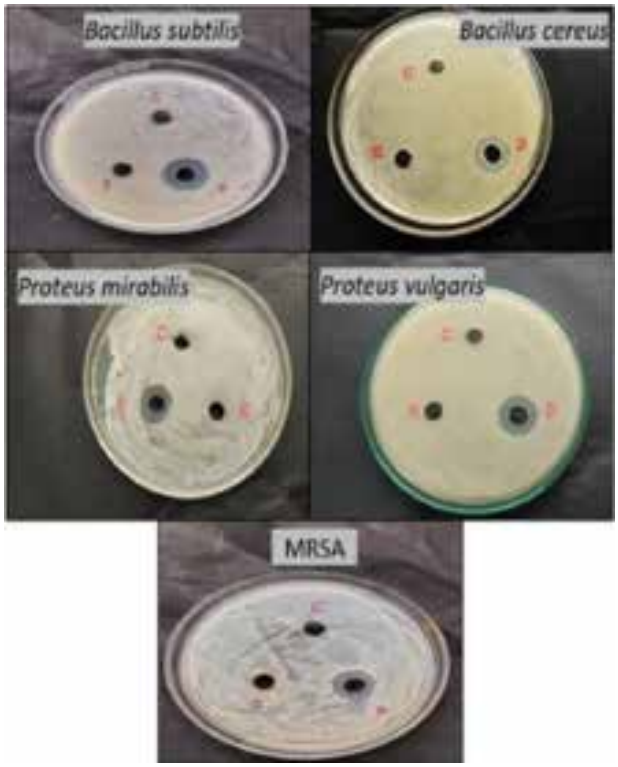

Fig. 3. Antibacterial efficacy of *Sutcliffiella horikoshii* KSV4 against different test bacteria. C-Control; P-Pellet; S-Supernatant

such as *P. aeruginosa*, *Klebsiella* species, *Staphylococcus aureus*, *P. vulgaris*, *Streptococcus pneumoniae*, *Escherichia coli* and *Bacillus cereus* (Nisha *et al*., 2019). Meanwhile, a study in Brazil found that a *Bacillus altitudinis* isolate from wetland sediment did not show antibacterial activity against Gram-negative bacteria (Abednego and Silago, 2023).

GC-MS analysis

The present study used thin-layer chromatography (TLC) and bioautography to identify bioactive compounds in the crude extract of KSV4. Notably, the fraction with Rf 0.9 was found to strongly inhibit the growth of *B. subtilis*. GC-MS analysis revealed the presence of ten major compounds (Fig. 4 and Table 4). These findings demonstrate the potential of TLC-Bioautography and GC-MS analysis for identifying bioactive compounds responsible for antibacterial activity. Stearic acid having an intensity of 1.75% was one of the compounds responsible for the antibacterial activity of KSV4. A study evaluated the antibacterial activities of various seed oils containing stearic acid against clinical isolates, demonstrating their potential as natural antimicrobial agents when used in mixtures. This study highlights the importance of fatty acid profiles in enhancing antibacterial activity (Joujou *et al*., 2024). Butyric acid and Valeric acid were also reported for their antimicrobial activity against Gram-positive and Gram-negative bacteria. The minimum inhibitory concentration (MIC) for butyric acid against *E. coli* and *Salmonella enterica* was 2300-2500 mg/L, while it exhibited lower MIC values against *Campylobacter jejuni* (500-800 mg/L). These results suggest that butyric acid has significant potential as an antimicrobial agent (Kovanda *et al*., 2019). Additionally, a survey revealed that butyrate exerts direct antimicrobial effects against distinct strains, including *Bacillus*, *E. coli*, and *Acinetobacter baumannii*. This study highlights both direct antimicrobial actions and

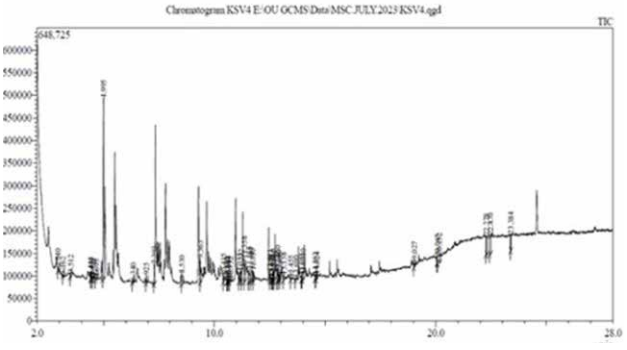

Fig. 4. Chromatogram of crude compound extracted from KSV4

Table 4. Tabulated representation of the major compounds identified through GC-MS analysis

Compound	R. Time	Molecular formula	Compound nature
Dodecane, 4,6-dimethyl-	4.995	$C_{14}H_{30}$	Alkane hydrocarbon
Caprolactone oxime, (NB)-O-[(diethylboryloxy)(ethyl)boryl]	22.470	$C_{12}H_{25}B_2NO_2$	Oxime ester
Oxalic acid	22.278	$C_{15}H_{21}NO_4$	Dicarboxylic acid
Eicosane	11.358	$C_{20}H_{42}$	Alkane
Pentanoic acid, 3-mercaptohexyl ester	20.152	$C_{11}H_{22}O_2S$	Alkyl carboxylic acid
Heneicosane	9.363	$C_{21}H_{44}$	Alkane
Triacontane, 1-bromo-	14.010	$C_{30}H_{61}Br$	Alkane
Steric acid hydrazide	2.940	$C_{18}H_{38}N_2O$	Saturated fatty acid
Butyric acid hydrazide	4.414	$C_4H_{10}N_2O$	Fatty acid
Valeric acid hydrazide	3.162	$C_5H_{12}N_2O$	Fatty acid

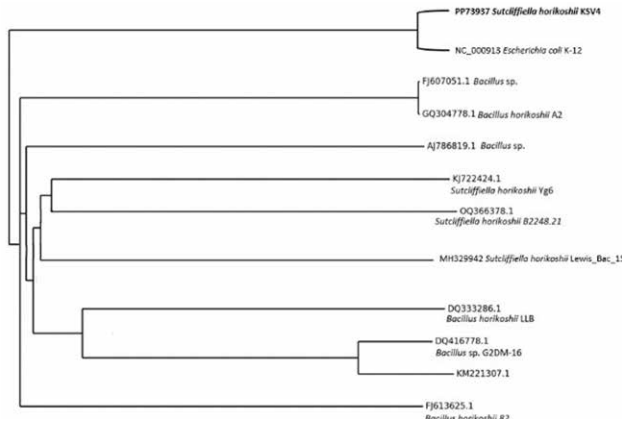

Fig. 5. Phylogenetic tree deciphering evolutionary lineages suggests the isolate KSV4 as *Sutcliffiella horikoshii*

indirect effects through the induction of host immune responses, making it a promising candidate for treating bacterial infections (Du *et al*., 2021). Dodecane has been reported at an intensity of 32.83% and can also be found in essential oils of *Zingiber officinalis*, *Camellia sinensis*, *Aristolochia triangularis* and others showing various biological activities (Fujisawa, 2010). The next major constituent of *S. horikoshii* crude compound is Caprolactone oxime, which can be utilised for synthesising pH nanoparticles that can be used for drug delivery, especially in cancer therapy (Xiaowei Yang *et al*., 2013). Triacontane is a natural product found in *Vanilla madagascariensis*, *Echinacea angustifolia*, and other organisms (Béatrice *et al*., 1997). The increasing prevalence of antibiotic resistance necessitates the exploration of alternative therapeutic strategies. Marine-derived bacteria, such as those from the *Bacillus* genus, are known for producing a variety of antimicrobial compounds that can effectively combat pathogenic bacteria in aquatic environments (Nweze *et al*., 2020). Utilising natural products derived from marine bacteria aligns with sustainable aquaculture practices. These compounds can reduce reliance on synthetic antibiotics, minimising environmental impact and promoting healthier fish stocks (Adnan *et al*., 2018). The incorporation of such biocontrol agents can enhance fish health while addressing the challenges posed by antibiotic resistance. These studies collectively accentuate the antibacterial capabilities of *S. horikoshii* and its potential applications in fish health and environmental remediation.

Conclusion

This study identified KSV4, isolated from Rushikonda Beach, as a promising source of novel antibacterial agents with strong efficacy against both Gram-positive and Gram-negative bacteria, including MRSA. The unique bioactive profile of KSV4, likely influenced by Rushikonda's ecological conditions, underscores its potential as an alternative to conventional antibiotics. GC-MS analysis revealed bioactive compounds with applications in environmental remediation and pharmaceuticals, while molecular identification confirmed KSV4 as *Sutcliffiella horikoshii*, showcasing the role of molecular techniques in bacterial classification. Future research should investigate these compounds' mechanisms and therapeutic potential and focus on optimizing extraction and production to advance marine-derived antimicrobials.

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Author contributions

Conceptualization: DYB, BKG; Methodology: DYB, BKG; Data Collection: DYB, BKG; Writing Original Draft: DYB; Writing Review and Editing: DYB; Supervision: KSK

Data availability

The data supporting this study are publicly available at the NCBI (https://www.ncbi.nlm.nih.gov/), accession no. PP733937.

Conflict of interest

The authors declare no conflicts of financial or non-financial interests that could have influenced the outcome or interpretation of the results.

Ethical statement

No ethical approval is required as the study does not include activities that require ethical approval or involve protected organisms/ human subjects/ collection of samples/ protected environments.

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References

Abednego, R. N. and V. Silago. 2023. Antibacterial Activity of Soil-isolated *Bacillus Altitudinis*/*Pumilus* Complex against Methicillin-resistant *Staphylococcus Aureus* from Mwanza, Tanzania. *Afr. J. Lab. Med.*, 12 (1): a2167.

Adnan, M., E. Alshammari, P. Mitesh, S. Amir Asharaf, S. Khan and S. Hadi. 2018. Significance and Potential of Marine Microbial Natural Bioactive Compounds against Biofilms/ Biofouling: Necessity for Green Chemistry. *Peer. J.*, 6: e5049.

Alcaraz, L. D., G. Moreno-Hagelsieb, L. E. Eguiarte, V. Souza, L. Herrera-Estrella and G. Olmedo. 2010. Understanding the evolutionary relationships and major traits of Bacillus through comparative genomics. *BMC Genomics*, 11: 332.

Aparna, S., A. Parvathi and A. Kaniyassery. 2020. Isolation and Characterization of a Moderately Halophilic *Marinobacter* Phage-host System from the Arabian Sea. *Springer Science and Business Media LLC, Environ. Monit. Assess.*, 192: 1-9.

Bartholomew, J. W. and T. Mittwer. 1952. THE GRAM STAIN. *Bacteriol. Rev.,* 16 (1): 1-29.

Béatrice, R. R., Émile M. Gaydou and I. Bombarda. 1997. Hydrocarbons from Three Vanilla Bean Species: *V. fragrans, V. madagascariensis*, and *V. tahitensis. J. Agric. Food. Chem.*, 45 (7): 2542–2545.

Bultel-Poncé, C. D., J. P. Berge, C. Cerceau and M. Guyot. 1998. 'Metabolites from the Sponge-Associated Bacterium *Micrococcus Luteus'. J. Mar. Biotechnol.*, 6 (4): 233-236.

Cappuccino, J. G. and N. Sherman. 2007. Microbiology A Laboratory Manual, *Dorling Kindersley* Pvt. Ltd, License of Pearson Education, New Delhi, India, p. 143-193.

Carlos, C., T. T. Torres and L. M. M. Ottoboni. 2013. Bacterial communities and species-specific associations with the mucus of Brazilian coral species. *Sci. Rep.,* 3 (1): 1-7.

Desbois, A. P. and V. J. Smith. 2010. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. *Appl. Microbiol. Biotechnol.,* 85: 1629-1642.

Du, K., S. Bereswill and M. M. Heimesaat. 2021. A literature survey on antimicrobial and immune-modulatory effects of butyrate reveals non-antibiotic approaches to tackle bacterial infections. *Eur. J. Microbiol. Immunol.,* 11 (1): 1-9.

Fujisawa, M., H. Harada, H. Kenmoku, S. Mizutani and N. Misawa. 2010. Cloning and characterization of a novel gene that encodes (S)-β-bisabolene synthase from ginger, Zingiber officinale. *Planta,* 232: 121-130.

Giordano, D., D. Coppola, R. Russo, R. Denaro, L. Giuliano, F. M. Lauro, G. Di Prisco and C. Verde. 2015. Chapter 4-Marine Microbial secondary metabolitesl: Pathways, Evolution and Physiological Roles, *Adv. Microb. Physiol.,* 66: 357-428.

Imran, A. K., P. Jahan, Q. Hasan and P. Rao. 2019. Genetic Confirmation of T2DM Meta-analysis Variants Studied in Gestational Diabetes Mellitus in an Indian Population. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, *Elsevier BV,* 13 (1): 688–694.

Jayadev, A. and M. Lekshmi. 2016. Screening and Isolation of Protease Producing Marine Bacteria. *Emergent Life Sci. Res.,* 2: 73-76.

Joujou, F. M., N. E. Darra, H. N. Rajha, E. S. Sokhn and N. Alwan. 2024. Evaluation of synergistic/antagonistic antibacterial activities of fatty oils from apricot, date, grape, and black seeds. *Sci. Rep.,* 14 (1): 1-12.

Kasanah, N. and M. T. Hamann. 2004. Development of Antibiotics and the future of marine microorganisms to stem the tide of antibiotic resistance. *Curr. Opin. Investig. Drugs.,* (London, England: 2000), 5 (8): 827-837.

Kovanda, L., W. Zhang, X. Wei, J. Luo, X. Wu, E. R. Atwill, S. Vaessen, X. Li and Y. Liu. 2019. In Vitro Antimicrobial Activities of Organic Acids and Their Derivatives on Several Species of Gram-Negative and Gram-Positive Bacteria. *Molecules*, 24 (20). 3770.

Kumari, K. S., P. Shivakrishna, Atef M. Al-attar and V. S. Rama Krishna Ganduri. 2020. Antibacterial and cytotoxicity activities of bioactive compounds from Micrococcus species OUS9 isolated from seawater. *J. King Saud Univ. Sci.,* 32 (6): 2818-2825.

Mondol, M. A. M., F. S. Tareq, J. H. Kim, M. A. Lee, H. S. Lee, J. S. Lee, Y. J. Lee, H. J. Shin. 2013a. New Antimicrobial Compounds from a Marine-derived *Bacillus* Sp. *J. Antibiot.*, 66 (2): 89-95.

Mondol, M. A. M., H. J. Shin and M. T. Islam. 2013b. Diversity of Secondary Metabolites from Marine *Bacillus* Species: Chemistry and Biological Activity. *Mar. Drugs.,* 11 (8): 2846-2872.

Nisha, N. M., R. Kanthasamy, R. Mahesh and S. Iruthaya Kalai Selvam. 2019. Identification of Antibacterial Compound from *Bacillus Horikoshii*, Isolated from Rhizosphere Region of Alfalfa Plant. *J. Appl. Sci.*, 19 (2): 140-47.

Nweze, J. A., N. M. Florence, G. Huang, Y. Li, L. Yang, Y. Zhang, S. Huang, L. Pan and D. Yang. 2020. Antibiotics Development and the Potentials of Marine-Derived Compounds to Stem the Tide of Multidrug-Resistant Pathogenic Bacteria, Fungi, and Protozoa. *Mar. Drugs.,* 18 (3): 145.

Nikita, P., M. Dwivedi, S. Jadeja and R. Begum. 2020. Antibacterial Activity of Marine Bacterial Pigments Obtained from Arabian Sea Water Samples. *J. Pure Appl. Microbiol.,* 14 (1): 517-26.

Radhakrishnan, R., A. Hashem, E. F. Abd Allah. 2017. Bacillus: A Biological Tool for Crop Improvement through Bio-Molecular Changes in Adverse Environments. *Front. Physiol.,* 8: 1-14.

Sahar, S. M., S. A. Abdelhamid and R. H. Ali. 2021. Isolation and Identification of Marine Microbial Products. *J. Genet. Eng. Biotechnol.*, 19 (1): 1-10.

Sandhya, Mvs,, E. Ramyakrishna, P. Divya, P. Kumar, K. Rajkumar, E. Yazein, and S. Burgula. 2014. Isolation of antibiotic producing bacteria from soil. *Research Gate*. https://www.researchgate.net/publication/334824592_isolation_of_antibiotic_producing_bacteria_from_soil

Santos, J. D., I. Vitorino, F. Reyes, F. Vicente and O. M. Lage. 2020. From Ocean to Medicine: Pharmaceutical Applications of Metabolites from Marine Bacteria. *Antibiotics*, 9 (8): 455.

Shanthi Kumari, V. Angeri and Y. B. Dugyala. 2024. Isolation and Molecular Characterization of a Bacteria VSB3 from the Marine Environment and its Antibacterial Activities. *Acta Scientific Biotechnology,* 5.3: 03-12.

Xiao, S., N. Chen, Z. Chai, M. Zhou, C. Xiao, S. Zhao and X. Yang. 2022. Secondary Metabolites from Marine-Derived Bacillus: A Comprehensive Review of Origins, Structures, and Bioactivities. *Mar. Drugs.,* 20 (9): 567.

Xiaowei, Y., C. Cui, Z. Tong, R. Chandran and S. X. Jia. 2013. Poly (ε-Caprolactone)-Based Copolymers Bearing Pendant Cyclic Ketals and Reactive Acrylates for the Fabrication of Photocrosslinked Elastomers. *Acta Biomaterialia*, *Elsevier*, 9 (9): 8232-8244.

Yan, Lu and Y. Ruizao. 2009. *Bacillus Horikoshii,* a Tetrodotoxin-producing Bacterium Isolated from the Liver of Puffer Fish. *Ann. Microbiol.* 59 (3): 453-58.

Yogitha, B. D. and S. K. Kuthadi. 2024. Antibacterial Activity of Micrococcus luteus YSD1 Isolated from Velankanni Water Source, Tamil Nadu. *Acta Scientific Biotechnology,* 5 (2): 26-33.

Zarza, E., L. D. Alcaraz, B. Aguilar-Salinas, A. Islas and G. Olmedo-Álvarez. 2017. Complete Genome Sequence of Bacillus horikoshii Strain 20a from Cuatro Cienegas, Coahuila, Mexico. *Genome Announcements*, 5 (30): e00592-17.