



**INTERNATIONAL JOURNAL OF
PHARMACEUTICAL SCIENCES**
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: <https://www.ijpsjournal.com>



Review Paper

Isolation, Identification and Genetic Quantification of Bacteria from Sea-Bed Sand – A Comprehensive Review

Kapil Raj PK*, Kenneth N, Manokaran R, Azeena TS, Edwin John JS, Priya S, Sree Devika SJ

Immanuel Arasar college of pharmacy, Nattalam, Tamil Nadu, India 629165.

ARTICLE INFO

Published: 16 Sept 2026

Keywords:

Marine sediment bacteria;
bacterial isolation; 16S
rRNA gene sequencing;
qPCR; bioremediation;
marine biotechnology

DOI:

10.5281/zenodo.22793431

ABSTRACT

Seabed sand is a vital and ecologically significant microbial habitat, consisting of diverse bacterial and archaeal communities that play crucial roles in biogeochemical cycling, organic matter decomposition, and the overall functioning of marine ecosystems. These communities, found in the subsurface seabed, face strong energy limitations and exhibit growth rates that are significantly slower than those in lab environments. This poses a challenge for scientists attempting to isolate and characterize these microorganisms. This review discusses traditional and modern techniques for isolating, identifying, and quantifying these marine bacteria. Traditional methods like serial dilution, streak plate technique, and heat shock, alongside modern techniques such as the sandwich agar plate method and spent culture medium, have enhanced the recovery of previously unculturable marine bacteria. Morphological characterization, biochemical testing, MALDI-TOF mass spectrometry, and 16S rRNA gene sequencing are utilized for identification, with 16S rRNA gene sequencing being a widely used molecular marker. Genetic quantification through quantitative PCR (qPCR) that targets the 16S rRNA gene facilitates an accurate assessment of bacterial abundance in marine sediments. Further, amplicon sequencing of the 16S rRNA gene V4 region corroborates qPCR results and uncovers novel bacterial diversity. Comparative genomics and metagenomics studies reveal a wide range of bacterial taxa with unique functional properties essential for seabed ecosystem functions, including degradation and recycling of DNA and organic material. The review underscores the significant biotechnological potential of these bacterial communities in enzyme production, bioremediation of hydrocarbons and heavy metals, and the synthesis of biosurfactants, along with the discovery of novel bioactive compounds relevant to clinical and pharmaceutical applications.

***Corresponding Author:** Kapil Raj PK

Address: M Pharm, Assistant Professor, Department of Pharmacology, Immanuel Arasar College of Pharmacy, Nattalam, kanniyakumari, Tamil Nadu, 629165.

Email ✉: pkkapil74@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



INTRODUCTION

Marine environments constitute the largest and most diverse ecosystems globally, encompassing over 70% of Earth's surface. Within these ecosystems, seabed sediments present a complex habitat that support rich diversity of microbial life. These microbes are integral to global biogeochemical cycling, influencing carbon, nitrogen, and oxygen dynamics ⁽¹⁾. Rather than being passive, these microbial communities actively drive nutrient transformation and energy flow in the oceans. Physical, chemical, and biological processes—including currents, sedimentation rates, and interactions with marine organisms—shape marine sediment environments. Benthic microbial communities create a boundary layer interfacing the water column and seafloor, where they contribute significantly to organic matter decomposition, biogeochemical cycling, and contaminant remediation ⁽¹⁾. The diversity found in deep marine sediments possess metabolic capabilities that can influence global biogeochemical cycles. Importantly, only about 1% of microbial species are cultivable, with the vast majority still unexplored regarding their roles in biogeochemical processes ⁽²⁾, indicating a significant area for further study in seabed sediment microbiology. Research focused on isolating and cultivating bacteria from marine sediments yields essential insights into their physiology characteristics, metabolic functions, and ecological roles. Cultivation-based methods shed light on the abundance of specific taxa, including *Bacillus*, *Proteobacteria*, *Actinobacteria*, and *Firmicutes*, in these environments ⁽³⁾. The isolation of microorganisms enhances understanding of their genomic properties and ecology, facilitating the characterization of

novel species ⁽⁴⁾. The recent advancement of molecular techniques, such as 16S rRNA gene sequencing, has become a pivotal methodology for bacterial identification, surpassing traditional morphological and biochemical methods in phylogenetic classification precision ⁽⁴⁾. Furthermore, quantitative PCR (qPCR) methods targeting the 16S rRNA gene allow accurate quantification of bacterial populations in marine sediments. Coupled with amplicon sequencing, qPCR also reveals previously unnoticed bacterial diversity ⁽⁵⁾. Together, these genetic tools enable comprehensive analyses of both qualitative and quantitative aspects of microbial populations in intricate environmental contexts. While microbial communities are crucial for biogeochemical cycling, their functional distributions across various geographic marine features remain inadequately studied ⁽⁶⁾. Considering this, a thorough investigation focused on the isolation, identification, and genetic quantification of bacteria from seabed sand is vital for unravelling the microbial ecology of these habitats and their contributions to marine ecosystem functioning ⁽⁷⁾. This review aims to synthesize evidence on the isolation and characterization of bacterial communities from seabed sand samples employing cultural, biochemical, and molecular methodologies, further quantifying the bacterial populations through qPCR and 16S rRNA gene analysis.

REVIEW SCOPE AND LITERATURE COVERAGE

This narrative review synthesizes literature on the isolation, identification, and genetic quantification of bacteria associated with marine and seabed sediments, with emphasis on culture-dependent methods, molecular identification, quantitative PCR, high-throughput sequencing, and emerging cultivation strategies. The literature set used for



this review includes peer-reviewed studies and methodological sources covering marine microbiology, microbial cultivation, bacterial identification, qPCR, sequencing, bioremediation, and pharmaceutical or biotechnological applications. qPCR, Bioremediation, Seabed microbiology, MALDI-TOF, Marine biotechnology.

HISTORY OF BACTERIAL ISOLATION

The history of bacterial isolation originates in the 17th century with Antonie van Leeuwenhoek's first observations of microorganisms in 1676, laying the groundwork for microbiology. However, systematic methods for bacterial isolation developed in the 19th century, particularly within bacteriology and parasitology. A pivotal advancement occurred in 1860 with Louis Pasteur's introduction of liquid media, enhancing the visualization of bacterial growth patterns ⁽⁸⁾. In the subsequent period from 1876 to 1883, Robert Koch significantly advanced these concepts, perfecting pure culture techniques. Koch pioneered methods that allowed disease organisms to be cultured outside the host, demonstrating the complete life cycle of pathogens through meticulous experiments. He introduced methods such as the hanging drop technique to assess bacterial motility, staining techniques with aniline dyes, and essential sterilization methods including the hot air oven and steam sterilizer. The introduction of agar for solid cultures in 1881 by Koch, along with Fanny Eilshemius's contributions in 1882, revolutionized microbial isolation by enabling the culture of a broader range of Microorganisms ⁽⁸⁾.

Koch's methods established pure culture techniques as foundational for research in infectious

diseases, with the streak plate method emerging as the standard for isolating individual microbes. This method involves using an inoculating loop to spread a microbial population over solid agar plates, leading to isolated colonies from which pure cultures can be obtained ⁽⁹⁾.

Despite traditional microbial techniques' substantial contributions, their inability to cultivate

the majority of microorganisms in natural environments has hindered the discovery of novel microbial resources. This limitation prompted the development of molecular-based identification techniques, integrating advanced technologies that have vastly improved precision in microbial isolation. Microfluidic technologies, which manipulate tiny fluid volumes with high accuracy, mark an essential transformation in this field ^(10,11).

In the current era, the rise of artificial intelligence introduces new computational tools to address the challenges in microbial resource discovery. A five-stage framework illustrates this evolution: the germination period (1997–2008), early exploration (2008–2015), rapid development (2015–2019), a deep learning explosion (2020–2022), and ongoing integration (2023–present) ⁽¹²⁾.

IMPORTANCE OF BACTERIAL ISOLATION

The identification and isolation of bacterial species are pivotal in understanding their role in disease processes. Traditionally, the techniques utilized for bacterial identification emphasize morphological, biochemical, and metabolic properties, necessitating the need for precise identification to manage bacterial pathogens effectively. The foundation of microbiome research relies on pure bacterial cultures, which allow for detailed experimental studies,



although traditional isolation methods are often labour-intensive and lack integration between phenotypic and genotypic data. Consequently, the development of efficient isolation techniques has emerged as a primary focus within microbiology ^(13,14).

The economic significance of microbiology extends beyond mere taxonomy, with projections suggesting that the global microbial market's value will surpass USD 675.2 billion by 2024.

Bacterial isolation is crucial for producing a variety of bioactive compounds including

enzymes, antibiotics, and probiotics, thereby reinforcing its fundamental role in the industry.

The cultivation of bacteria remains the gold standard for diagnosing bacterial and fungal pathogens and understanding antimicrobial resistance ⁽¹⁵⁾. The discovery of novel bacteria in unexplored environments, such as marine sediments, have unveiled new antibiotics critical for

combating antimicrobial resistance ⁽¹⁶⁾.

Furthermore, bacteria play a vital role in environmental monitoring by detecting pollutants, heavy metal ions, and other hazardous substances in ecosystems. Engineered bacterial strains can elicit specific biological responses to pollutants, allowing for the quantifiable detection of

various organic compounds and heavy metals, enhancing environmental safety measures ⁽¹⁷⁾.

Innovative approaches, driven by artificial intelligence, have facilitated the exploration of microbial interactions and the identification of potential microbial biomarkers for applications in environmental monitoring and disease detection. A refined understanding of bacterial taxonomy is essential for effective operations in medical microbiology laboratories and communication with key stakeholders ⁽¹⁴⁾.

Recent advancements in high-throughput isolation and cultivation methodologies, including

culturomics, droplet microfluidics, and genomic selection techniques, have dramatically improved the cultivability of previously non-culturable bacteria. This technological progress signifies a substantial leap in bacterial isolation capabilities, thereby enhancing our understanding and control of microbial communities in various settings, from clinical to Environmental Realms ^(18,19).

ISOLATION METHODS REPORTED IN MARINE SEDIMENT STUDIES

PART A: METHODS OF BACTERIAL ISOLATION

The methods of bacterial isolation describe various methodologies for isolating bacteria from marine sediment samples.

A1. SAMPLE COLLECTION

Sample Collection involves using sterile corers or SCUBA diving to obtain sediment samples, specifically from the upper 1-5 cm of seabed sand, with careful recording of GPS coordinates and subsequent storage at 4°C ⁽²⁰⁾.

A2. SERIAL DILUTION METHOD

Serial Dilution Method entails serially diluting 0.2 g of sediment in sterile seawater and spreading aliquots onto culture media plates, incubating them at 28–30°C for up to 30 days to cultivate colonies, which are then streaked onto Marine Broth agar for pure isolations ⁽²⁰⁾.

A3. HEAT SHOCK METHOD

Heat Shock Method mixes wet sediment with sterile seawater and heats it to 55°C to efficiently isolate spore-forming bacteria like *Bacillus* spp. by subsequent dilution and inoculation onto agar ⁽²⁰⁾.



A4. STAMPING METHOD

Stamping Method involves transferring wet sediment to a sterile dish, drying it, grinding it, and then stamping it onto agar to achieve a serial dilution effect, effectively preparing the media with filtered seawater ⁽²⁰⁾.

A5. CULTURE MEDIA

Culture Media Used includes Marine Agar 2216 and Marine Broth 2216, among others like Water Extracted Matter (WEM), which is noted for yielding a broader diversity of actinobacteria, especially *Micromonospora* and *Streptomyces* species ⁽²¹⁾.

A6. SANDWICH AGAR PLATE METHOD

Sandwich Agar Plate Method employs a coculture technique to enhance microbial interactions, which significantly increases the recovery of previously uncultured species by nearly tenfold ⁽²²⁾.

A7. SPENT CULTURE MEDIUM

Spent Culture Medium (SCM) Method dilutes sediment samples in artificial seawater, enriching them with modified media to promote recovery of visible colonies through aerobic incubation ⁽²³⁾.

PART B: IDENTIFICATION OF BACTERIA

The identification of bacteria outlines procedures for characterizing bacteria after isolation.

B1. MORPHOLOGICAL CHARACTERIZATION

Morphological Characterization examines colony characteristics and employs Gram staining for microscopic observation ⁽²⁰⁾.

B2. BIOCHEMICAL TESTS

Biochemical Tests use systems such as API 20E for isolates showing low -confidence identification, thereby enhancing identification accuracy for various species ⁽²⁴⁾.

B3. MALDI-TOF MASS SPECTROMETRY

MALDI-TOF Mass Spectrometry is highlighted for its rapid and cost-effective bacteria identification capabilities in microbiological laboratories ^(24,25).

B4. 16S rRNA GENE SEQUENCING

16S rRNA Gene Sequencing is widely used as a molecular marker for bacterial identification, leveraging platforms like EzBioCloud to ensure accurate classification of major bacterial phyla ⁽²⁴⁾.

B5. COMPARISON OF IDENTIFICATION METHODS

Comparison of Identification Methods indicates that each of biochemical tests, MALDI-TOF MS, and 16S rRNA sequencing has specific advantages, with the former being widespread and the latter providing the quickest results ^(24,25).

PART C: GENETIC QUANTIFICATION METHODS

The genetic quantification details techniques for quantifying bacterial populations.

C1. DNA EXTRACTION

DNA Extraction uses the **DNeasy Power Lyzer Power Soil Kit** for genomic DNA extraction and quantification of 16S rRNA gene abundance relative to wet sediment weight ⁽²⁶⁾.

C2. QUANTITATIVE PCR

Quantitative PCR (qPCR) is utilized to measure total bacterial abundance by assessing 16S



rRNA gene copy numbers, applying specific primer sets, with negative controls incorporated for accuracy ^(26,27).

C3. PRIMER SETS TARGET

Primer Sets target the bacterial 16S rRNA gene in qPCR analysis, with V4 region amplicon sequencing confirming quantification accuracy and revealing additional microbial diversity ⁽²⁶⁾.

C4. ABSOLUTE QUANTIFICATION

Absolute Quantification Using Spike -in Standards employs synthetic DNA standards for normalization purposes in qPCR, which ensures precise bacterial abundance calculations per gram of sediment ⁽²⁸⁾.

C5. MULTIPLEX RT-PCR

Multiplex RT-qPCR facilitates rapid quantification of various bacterial groups, showing higher gene copy numbers in sediments compared to seawater and documenting seasonal bacterial abundance variations ⁽²⁹⁾.

PHARMACEUTICAL AND CLINICAL RELEVANCE

1. ANTIBIOTIC DISCOVERY

Marine environments are increasingly recognized as valuable resources for pharmaceutical applications, particularly antibiotic discovery, due to the diverse bioactive compounds produced by marine bacteria. Members of the genus *Streptomyces* possess remarkable biosynthetic capabilities that yield numerous antimicrobial secondary metabolites.

Salinosporamide A, derived from the marine actinomycete *Salinispora tropica*, has entered clinical trials for hematological malignancies, demonstrating the clinical importance of marine sediment bacteria ⁽³⁰⁾.

2. ANTICANCER DRUG DEVELOPMENT

Advances in marine microbiology, genomics, metabolomics, and bioinformatics have accelerated the identification of previously uncultured marine bacteria and their biosynthetic gene clusters. These studies have revealed metabolites exhibiting potent cytotoxic activity against various cancer cell lines, including drug-resistant forms. Natural products continue to play a crucial role in anticancer drug development ⁽³¹⁾.

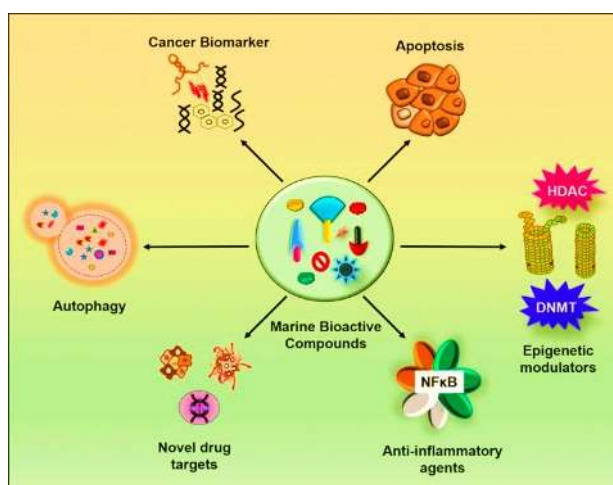


FIG.NO :1 (ANTICANCER DRUG DEVELOPMENT)

3. BACTERIAL BIOSENSORS IN CLINICAL DIAGNOSTICS

The increasing demand for rapid and efficient diagnostic tools has stimulated development

of bacterial biosensors and nano -biosensors. These technologies facilitate point-of-care detection of bacterial and viral pathogens and have significant applications in diseases such as HIV, Ebola, and SARS-CoV-2 (32,33).

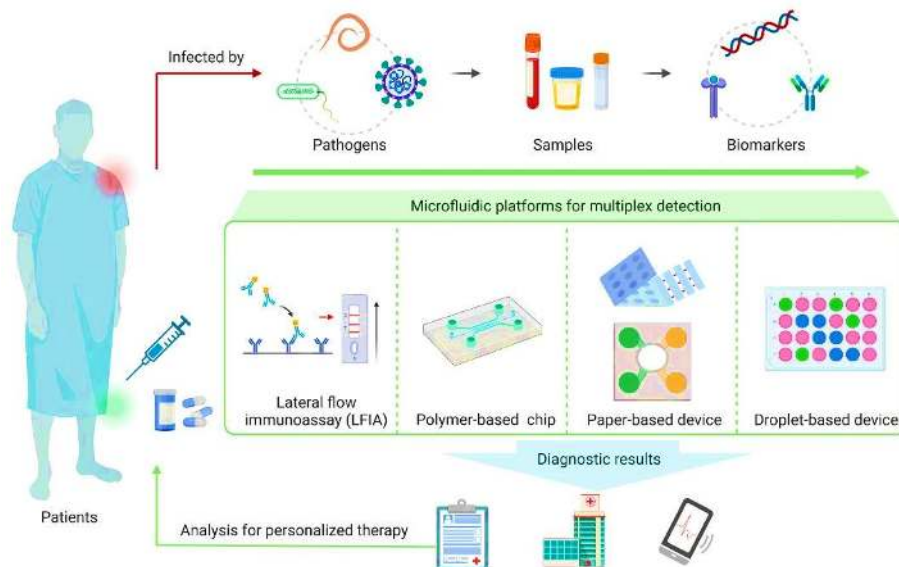


FIG NO:2(BACTERIAL BIOSENSORS IN CLINICAL DIAGNOSTICS)

4. ANTIMICROBIAL RESISTANCE (AMR) STUDIES

Marine-derived bacteria, especially members of the genus *Streptomyces*, produce structurally unique metabolites with promising activity against multidrug -resistant pathogens. Genome mining approaches have identified novel biosynthetic gene clusters and compounds with therapeutic potential against antimicrobial resistance (34).

5. GENOME MINING FOR DRUG DISCOVERY

Genome mining strategies have revealed several biosynthetic gene clusters responsible for the synthesis of antibacterial and antifungal compounds. These findings highlight marine ACTi mycetoma as promising sources of novel drug candidates (34).

6. ENVIRONMENTAL PATHOGEN DETECTION

Modern molecular techniques, including quantitative PCR and next -generation sequencing, have significantly improved pathogen detection in environmental samples. Biosensor -based approaches provide highly sensitive and specific detection of waterborne pathogens (35).

7. BIOACTIVE COMPOUNDS – ACTINOBACTERIA FROM SEA BED

Intertidal marine sediments harbor diverse Actinobacteria with significant bioactive and biosynthetic potential. These microorganisms represent valuable resources for drug discovery and infectious disease management (35).

APPLICATION IN BIOTECHNOLOGY

1. INDUSTRIAL ENZYME PRODUCTION



Marine microbial enzymes play a crucial role in various bioindustries due to their stability and adaptability to harsh conditions. Enzymes such as amylase, caseins, lipase, gelatinase, and DNA obtained from marine microorganisms exhibits remarkable thermostability and pH tolerance, making them suitable for industrial applications ^(36,37).

2. PROTEASE, LIPASE & AMYLASE PRODUCTION

A large proportion of bacterial isolates have been reported to produce industrially important enzymes such as amylase, lipase, and protease, demonstrating their considerable biotechnological potential. The expanding lipase market further highlights the importance of these enzymes in biodiesel production, food processing, textiles, and pharmaceutical industries ⁽³⁷⁾.

3. HEAVY METAL BIOREMEDIATION

Marine bacteria have demonstrated significant potential in the biodegradation of polycyclic aromatic hydrocarbons and heavy metals. Species such as *Halo monas* and *Alcanivorax* have shown remarkable efficiency in removing toxic contaminants from polluted marine sediments ^(38,39).

4. BIOSURFACTANT PRODUCTION

Marine bacteria, particularly *Bacillus* and *Pseudomonas* species, are capable of producing biosurfactants and secondary metabolites that have applications in wastewater treatment, oil spill remediation, cosmetics, pharmaceuticals, and food industries ⁽⁴⁰⁾.

5. GENOME MINING FOR NOVEL BIOACTIVE COMPOUNDS

Recent advances in genome mining of marine-derived *Streptomyces* and *Micromonospora* species have enabled the identification of biosynthetic gene clusters associated with antibiotic and other bioactive compound production. These discoveries have accelerated the search for novel therapeutic molecules and industrial enzymes ⁽⁴¹⁾.

6. BIOFUEL & BIODIESEL PRODUCTION

Marine microbial enzymes have also found applications in biofuel and biodiesel production. Their exceptional catalytic properties under extreme environmental conditions make them valuable tools for sustainable biotechnological processes and human health -related applications ⁽⁴²⁾.

CHALLENGES AND LIMITATIONS

1. THE UNCULTURABLE PROBLEM

The challenge of bacterial isolation in microbiology is significantly hindered by the inability to culture the majority of microorganisms present in natural environments. This limitation arises mainly from the disruption of symbiotic interactions during pure culture techniques, which are essential for microbial communication and cooperation. In their natural habitats, microorganisms exchange metabolites, growth factors, chelating agents, and signaling molecules that are difficult to reproduce under laboratory monoculture conditions ⁽⁴³⁾.

2. DEEP SEA AND MARINE SEDIMENT SPECIFIC CHALLENGES

Isolation of bacteria from deep -sea and marine sediment environments is particularly



challenging because of low nutrient availability and the recalcitrant nature of organic matter.

Conventional isolation methods often disturb microbial interactions, there by limiting the cultivation of microorganisms that depend on cooperative relationships for growth ⁽⁴⁴⁾.

3. NUTRIENT AND GROWTH LIMITATIONS

The inability to culture many bacteria is also associated with the limited availability of essential nutrients and growth factors in laboratory media. Certain nutrients are supplied by neighbouring microorganisms through mechanisms such as satellitism, whereas excessive nutrient concentrations may inhibit oligotrophic bacteria that thrive under nutrient -poor conditions commonly found in marine sediments ⁽⁴³⁾.

4. VIABLE BUT NON-CULTURABLE (VBNC) STATE

Another major challenge is the viable but non - culturable (VBNC) state, in which bacteria remain metabolically active but fail to grow on conventional media. This phenomenon results in an underestimation of microbial diversity and restricts the recovery of microorganisms with potential biotechnological importance ⁽⁴⁵⁾.

5. LOW RECOVERY RATE

Traditional cultivation methods have a relatively low success rate for isolating novel bacterial strains. Advanced approaches such as the spent culture medium (SCM) method have considerably improved recovery rates and enabled the cultivation of previously overlooked taxa including Planctomycetes, Deinococcota, and Balneolota ⁽⁴⁶⁾.

6. OVERCOMING LIMITATIONS

Recent developments such as microencapsulation, targeted isolation, and resuscitation techniques have enhanced the recovery of bacterial diversity from marine sediments.

Encapsulation methods have demonstrated higher operational taxonomic unit (OTU) recovery than conventional resuspension methods. Furthermore, diffusion chambers and growth - Promoting factors have shown considerable promise for recovering uncultured bacteria with potential applications in bioremediation and environmental biotechnology ⁽⁴⁷⁾.

RECENT ADVANCES

1. SANDWICH AGAR PLATE METHOD (2024)

The sandwich agar plate method is an innovative coculture technique designed to enhance microbial interactions during bacterial isolation and cultivation. This approach resulted in an almost tenfold increase in the recovery of previously uncultured species compared with conventional methods. Several isolates exhibited commensal lifestyles, and heme was identified as an important growth -promoting factor for certain marine bacteria, thereby facilitating the cultivation of previously unculturable marine sediment microorganisms

2. HIGH-THROUGHPUT SEQUENCING FOR MARINE BACTERIA

Despite remarkable improvements in microbial culture techniques, hi gh-throughput sequencing (HTS) studies have revealed that a substantial proportion of marine microorganisms remain uncultured. HTS analyses showed the predominance of Gammaproteobacteria, whereas culture-dependent methods mainly recovered Actinobacteria.



Only a small percentage of operational taxonomic units (OTUs) identified by sequencing were represented in culture collections, emphasizing the need for multiple culture media to recover broader microbial diversity

3. MALDI-TOF MASS SPECTROMETRY

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry has emerged as a rapid and cost-effective approach for bacterial identification, providing results within minutes. In addition, nanopore sequencing technologies enable rapid pathogen detection and antimicrobial resistance profiling, offering improved sensitivity and broader pathogen coverage compared with conventional culture-based methods

4. NANOPORE SEQUENCING (OXFORD NANOPORE)

Long-read metagenomic sequencing based on Oxford Nanopore technology has accelerated the accurate identification of pathogens and antimicrobial resistance determinants without the need for cultivation. This culture-independent shotgun metagenomic approach has significantly improved the understanding of complex microbial communities across different microbiological environments

5. MICROENCAPSULATION AND IN SITU INCUBATION

Recent developments involving gel-filled microwell arrays and microencapsulation techniques have enhanced the recovery of bacterial diversity from marine sediments. Encapsulation approaches have demonstrated superior diversity recovery compared with conventional resuspension methods, providing access to previously hidden microbial resources

with environmental and biotechnological significance

6. BALTIC SEA AND TARA OCEAN EXPEDITION

Advances in DNA sequencing technologies and bioinformatics, particularly through the Tara Ocean expedition and studies of Baltic Sea microbial communities, have revealed extensive taxonomic and functional diversity within the global ocean microbiome

7. AI-INTEGRATION (2023–PRESENT)

The integration of artificial intelligence with physical and chemical pretreatment strategies has further improved microbial isolation and recovery. AI-assisted approaches facilitate high-throughput screening of novel bacterial taxa while minimizing contamination and enhancing the efficiency of microbial resource discovery

CONCLUSION

1. SUMMARY OF THE REVIEW

This review on the isolation, identification, and genetic quantification of bacteria from seabed sand highlights a pivotal intersection between marine microbiology and applied biotechnology. Despite significant advances in microbiology, much of bacterial taxonomy remains unexplored. The research emphasizes the richness and diversity of bacterial communities within seabed sediments, and the efficacy of merging traditional culturing methods with molecular techniques for comprehensive characterization.

2. ISOLATION METHODS

Regarding isolation methods, a combination of various media and modern co-culture techniques markedly improved bacterial recovery from sediment samples. Utilizing multiple



media yielded more taxa than single media, while nutrient-rich and specific-carbon/nitrogen-source media limited taxa growth. Notably, nitrogen quality proved crucial in influencing bacterial isolation outcomes, indicating the need for optimized culture conditions tailored to marine sediment environments.

3. IDENTIFICATION METHODS

For identification methods, molecular techniques, particularly 16S rRNA gene sequencing, emerged as powerful tools, enabling precise bacterial identification that surpasses morphological and biochemical methods. The application of DNA sequencing to phylogenetic characterization facilitated accurate taxonomic placement of novel strains isolated from marine sediments. Furthermore, the integration of MALDI-TOF and nanopore sequencing enhanced the speed and accuracy of identifications.

4. GENETIC QUANTIFICATION METHODS

In terms of genetic quantification, a quantitative PCR (qPCR) method was employed to assess bacterial populations within marine sediments, leveraging primer sets targeting the total bacterial community. This approach, validated by amplicon sequencing of the 16S rRNA gene V4 region, demonstrated not only the accuracy of bacterial enumeration via qPCR but also suggested previously unrecognized diversity among the communities.

5. BIOTECHNOLOGICAL SIGNIFICANCE

Examining the biotechnological implications, genome analyses of isolates indicated the presence of genes associated with bacterial secondary metabolism and various biodegradation pathways. This finding underscores the potential of marine sediment bacteria in bioremediation

efforts, contributing to the broader understanding of microbial adaptations in extreme environments.

FUTURE PROSPECTS

Looking ahead, metagenomic next-generation sequencing promises higher detection sensitivity and broader pathogen coverage compared to traditional methods. Future research endeavours are encouraged to incorporate metagenomics, transcriptomics, and sophisticated bioinformatics to unlock the functional capabilities of seabed bacterial communities. The adoption of AI-driven tools and advanced sequencing technologies like Oxford Nanopore could significantly expedite the discovery of novel bacterial taxa and bioactive compounds.

CLOSING STATEMENT

In summary, sea bed sand represents a vital reservoir of microbial diversity with considerable ecological, biotechnological, and pharmaceutical potential. Highly contaminated marine sediments could harbour rare bacterial taxa beneficial for bioremediation, underscoring the necessity for continued exploration and characterization of bacterial life. Comprehensive investigations on isolation, identification, and genetic quantification are critical to fully understand marine microbial ecology and its applications.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FUNDING

No specific funding was received for the preparation of this review.



ETHICAL APPROVAL

Not applicable. This article is a literature review and does not involve human participants, animals, or new experimental samples.

AUTHOR CONTRIBUTIONS

All listed authors contributed to the conception, literature review, drafting, critical revision, and approval of the final manuscript.

REFERENCES

- Hicks N, Liu X, Gregory R, Kenny J, Lucaci A, Lenzi L, et al. Temperature driven changes in benthic bacterial diversity influences biogeochemical cycling in coastal sediments. *Front Microbiol.* 2018;9:1730.
- Zhang X, Wu K, Han Z, Chen Z, Liu Z, Sun Z, et al. Microbial diversity and biogeochemical cycling potential in deep-sea sediments associated with seamount, trench, and cold seep ecosystems. *Front Microbiol.* 2022;13:1029564.
- da Silva MAC, Cavalett A, Spinner A, Rosa DC, Jasper RB, Quecine MC, et al. Phylogenetic identification of marine bacteria isolated from deep-sea sediments of the eastern South Atlantic Ocean. *Springerplus.* 2013;2(1):127.
- Rey-Velasco X, Deulofeu-Capo O, Sanz-Sáez I, Cardelús C, Ferrera I, Gasol JM, et al. Manipulation in grazing, viral pressure and resource availability leads to success in the isolation of abundant marine bacteria. *mBio.* 2023;14(5):e00890-23.
- Ramljak A, Jurburg S, Chatzinotas A, Lučić M, Žižek M, Babić I, et al. Identifying the drivers of microbial community changes and interactions in polluted coastal sediments. *Environ Microbiome.* 2025;20(1):117.
- Geelhoed JS, van de Velde SJ, Meysman FJR. Quantification of cable bacteria in marine sediments via qPCR. *Front Microbiol.* 2020;11:1506.
- Mootapally C, Sharma P, Dash S, Kumar M, Sharma S, Kothari RK, et al. Microbial drivers of biogeochemical cycles in deep sediments of the Kathiawar Peninsula Gulfs of India. *Sci Total Environ.* 2025;965:178609.
- Blevins SM, Bronze MS. Robert Koch and the golden age of bacteriology. *Int J Infect Dis.* 2010;14(9):e744-e751.
- Adedeji D. Isolation techniques in microbiology. *AZoLifeSciences.* 2023.
- Lagier JC, Edouard S, Pagnier I, Mediannikov O, Drancourt M, Raoult D. Current and past strategies for bacterial culture in clinical microbiology. *Clin Microbiol Rev.* 2015;28(1):208-236.
- Cheptsov VS, Tsykina SI, Minaev NV, Yusupov VI. New microorganism isolation techniques with emphasis on laser printing. *Int J Bioprint.* 2019;5(1):165.
- Xu MQ, Pan F, Peng LH, Yang YS. Advances in the isolation, cultivation, and identification of gut microbes. *Mil Med Res.* 2024;11(1):34.
- da Silva MRF, Souza K, Bezerra T, Silva T, et al. Molecular identification of bacterial isolates. *Braz J Med Biol Res.* 2023;56:e12894.
- Kapinusova G, Lopez Marin MA, Uhlik O. Reaching unreachable: obstacles and successes of microbial cultivation and their reasons. *Front Microbiol.* 2023;14:1089630.
- Wang M, Noor S, Huan R, Liu C, Li J, Shi Q, et al. Comparison of the diversity of cultured and total bacterial communities in marine sediment using culture-dependent and sequencing methods. *PeerJ.* 2020;8:e10060.
- Zhang J, Liang QY, Mu DS, Lian F, Gong Y, Ye M, et al. Cultivating the uncultured: harnessing the sandwich agar plate approach to isolate heme-dependent bacteria from marine sediment. *mLife.* 2024;3(1):143-155.



17. Carella A, Carroll KC, Munson E, Ledebore NA. An update on novel taxa and revised taxonomic status of bacteria isolated from human clinical specimens described in 2024. *J Clin Microbiol.* 2025;63(12):e01068-25.
18. Velez-Cortes F, Blazejewski T, Kaufman A, Ronda C, et al. High-throughput microbial culturomics using automation and machine learning. *Nat Biotechnol.* 2023;41:1424-1433.
19. Liu M, Yang W, Zhu W, Yu D. Innovative applications and research advances of bacterial biosensors in medicine. *Front Microbiol.* 2025;16:1507491.
20. Huang Z, Mo S, Yan L, Wei X, Huang Y, Zhang L, et al. A simple culture method enhances the recovery of culturable Actinobacteria from coastal sediments. *Front Microbiol.* 2021;12:675048.
21. Buongiorno J, Turner S, Webster G, Asai M, Shumaker AK, Roy T, et al. Interlaboratory quantification of Bacteria and Archaea in deeply buried sediments of the Baltic Sea (IODP Expedition 347). *FEMS Microbiol Ecol.* 2017;93(3):fix007.
22. Zemb O, Achard CS, Hamelin J, De Almeida ML, et al. Absolute quantitation of microbes using 16S rRNA gene metabarcoding: a rapid normalization of relative abundances by quantitative PCR targeting a 16S rRNA gene spike-in standard. *MicrobiologyOpen.* 2020;9(3):e977.
23. Castillo-Henríquez L, Brenes-Acuña M, Castro-Rojas A, Vega-Baudrit JR, et al. Biosensors for the detection of bacterial and viral clinical pathogens. *Sensors (Basel).* 2020;20(23):6926.
24. Ishaq SE, Ahmad T, Liang L, Xie R, Yu T, Wang Y, et al. Cultivation of diverse novel marine bacteria from deep ocean sediment using spent culture supernatant of *Ca. Bathyarchaea* enrichment. *J Microbiol.* 2024;62(8):611-625.
25. Hassan SS, Jin H. Harnessing marine bacteria for next-gen antibiotics: potent inhibition of *S. aureus* and *Riemerella anatipestifer* through in vitro, omics, and chemoinformatics approach with enhanced production of secondary metabolites through zinc sulfate. *Front Mar Sci.* 2024;11:1461607.
26. Strejcek M, Smrhova T, Junkova P, Uhlik O. Whole-cell MALDI-TOF MS versus 16S rRNA gene analysis for identification and dereplication of recurrent bacterial isolates. *Front Microbiol.* 2018;9:1294.
27. Topić Popović N, Kepec S, Kazazić SP, Strunjak-Perović I, Bojanić K, Čož-Rakovac R. Identification of environmental aquatic bacteria by mass spectrometry supported by biochemical differentiation. *PLoS One.* 2022;17(6):e0269423.
28. Carrasco-Acosta M, Garcia-Jimenez P, et al. Development of multiplex RT-qPCR assays for simultaneous detection and quantification of faecal indicator bacteria in bathing recreational waters. *Microorganisms.* 2024;12(6):1223.
29. Theivendren P, Panneerselvam S, Swaminathan J, Murugesan V, Pradeep K, Bhat MJ, et al. Marine bacterial metabolites in anticancer drug discovery: ecological insights, mechanisms of action, and clinical translation. *Reg Stud Mar Sci.* 2026;96:104935.
30. Narsing Rao MP, Quadri SR, Sathish M, Quach NT, Li WJ, Thamchaipenet A. Exploring omics strategies for drug discovery from Actinomycetota isolated from the marine ecosystem. *Front Pharmacol.* 2025;16:1634207.
31. Jose PA, Jha B. Intertidal marine sediment harbours Actinobacteria with promising



- bioactive and biosynthetic potential. *Sci Rep*. 2017;7:10041.
32. Oon YL, Oon YS, Ayaz M, Deng M, Li L, Song K. Waterborne pathogen detection technologies: advances, challenges, and future perspectives. *Front Microbiol*. 2023;14:1286923.
33. Dell'Anno F, Brunet C, van Zyl LJ, Tuffin M, Golyshin PN, Sansone C, et al. Degradation of hydrocarbons and heavy metal reduction by marine bacteria in highly contaminated sediments. *Microorganisms*. 2020;8(9):1402.
34. Zhang C, Kim SK. Research and application of marine microbial enzymes: status and prospects. *Mar Drugs*. 2010;8(6):1920-1934.
35. Dell'Anno F, Rastelli E, Sansone C, Brunet C, Ianora A, Tuffin M, et al. Bacteria, fungi and microalgae for the bioremediation of marine sediments contaminated by petroleum hydrocarbons in the omics era. *Microorganisms*. 2021;9(8):1695.
36. Dell'Anno F, Rastelli E, Tangherlini M, Corinaldesi C, Sansone C, Brunet C, et al. Highly contaminated marine sediments can host rare bacterial taxa potentially useful for bioremediation. *Front Microbiol*. 2021;12:584850.
37. Bonugli-Santos RC, dos Santos Vasconcelos MR, Passarini MRZ, Vieira GAL, Lopes VCP, Mainardi PH, et al. Marine-derived fungi: diversity of enzymes and biotechnological applications. *Front Microbiol*. 2015;6:269.
38. Rao TE, Imchen M, Kumavath R. Marine enzymes: production and applications for human health. *Adv Food Nutr Res*. 2017;80:149-163.
39. Zhang XH, Ahmad W, Zhu XY, Chen J, Austin B. Viable but nonculturable bacteria and their resuscitation: implications for cultivating uncultured marine microorganisms. *J Oceanol Limnol*. 2020;38(3):603-617.
40. Bodor A, Bounedjoum N, Vincze GE, et al. Challenges of unculturable bacteria: environmental perspectives. *Rev Environ Sci Biotechnol*. 2020;19:1-22.
41. Pope E, Cartmell C, Haltli B, Ahmadi A, Kerr RG. Microencapsulation and in situ incubation methodology for the cultivation of marine bacteria. *Front Microbiol*. 2022;13:958660.
42. Gutierrez T, Coulon F, Ziervogel K. Editorial: methods in aquatic microbiology. *Front Microbiol*. 2023;14:1338297.
43. Mazur-Marzec H, Andersson AF, Błaszczuk A, Dąbek P, Górecka E, Grabski M, et al. Biodiversity of microorganisms in the Baltic Sea: the power of novel methods in the identification of marine microbes. *FEMS Microbiol Rev*. 2024;48(5):fuae024.
44. Zi GR, Zhang D, He DL, Shu F, Ou Y, Ke C. Current status and prospects of nanopore sequencing technology in the detection of pathogenic microorganisms. *Front Microbiol*. 2026;17:1843102.
45. Chen Z, Grim CJ, Ramachandran P, Meng J. Advancing metagenome-assembled genome-based pathogen identification: unraveling the power of long-read assembly algorithms in Oxford Nanopore sequencing. *Microbiol Spectr*. 2024;12(6):e00117-24.
46. Liu Y, Xu Y, Xu X, Chen X, Chen H, Zhang J, et al. Metagenomic identification of pathogens and antimicrobial-resistant genes in bacterial positive blood cultures by nanopore sequencing. *Front Cell Infect Microbiol*. 2023;13:1283094.
47. Rao T, et al. Methods in aquatic microbiology. *Front Microbiol*. 2023;14:1338297.



HOW TO CITE: Kapil Raj PK, Kenneth N, Manokaran R, Azeena TS, Edwin john JS, Priya S, Sree Devika SJ Isolation, Identification and Genetic Quantification of Bacteria from Sea-Bed Sand – A Comprehensive Review, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 9, 1990-2004, <https://doi.org/10.5281/zenodo.22793431>

