RESEARCH ARTICLE

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Preliminary Isolation and Phenotypic Characterization of Bacillus-Like Bacteria from Marine Coastal Soil

G. Alwina [a], Bodika Shynisha [a], B. Sneha [a], Sanjeeb Kumar Mandal [a, *], B. Sumithra [a], Bishwambhar Mishra [a]

[a] Department of Biotechnology, Chaitanya Bharathi Institute of Technology, Hyderabad 500075, Telangana, India

* Corresponding Author: sanjeeb.vit@gmail.com

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Abstract: Marine environments constitute an underexplored source of microorganisms with potential applications in food, aquaculture, and biotechnology. This study aimed to isolate cultivable bacteria from coastal soil collected at Kanyakumari Beach, Tamil Nadu, India, and to perform preliminary phenotypic characterization. Three sample types—dry surface soil, wet soil, and soil collected between rocks—were serially diluted and cultured on solid bacterial medium. Representative colonies from each sample were purified and evaluated for colony morphology, Gram reaction, cellular morphology, arrangement, and motility. More than 80 colonies were observed from each sample type. Three representative isolates, designated D, W, and R, formed medium-sized whitish colonies with predominantly circular morphology. All isolates were Gram-positive, rod-shaped, and motile; cells occurred singly or in short chains. These characteristics support their provisional classification as Bacillus-like bacteria. However, phenotypic observations alone are insufficient for species-level identification or for establishing probiotic functionality. Molecular identification, safety testing, and functional assays—including acid and bile tolerance, antimicrobial activity, adhesion-related properties, antibiotic susceptibility, and haemolysis—are required before any probiotic claim can be made. The present work therefore provides a preliminary culture collection and a foundation for subsequent strain-level evaluation.

Keywords: Bacillus-Like Bacteria, Coastal Soil, Marine Microorganisms, Phenotypic Characterization, Probiotic Screening.

1. Introduction

The human gastrointestinal tract supports a complex microbial community comprising bacteria, archaea, viruses, and unicellular eukaryotes. This microbiota contributes to nutrient metabolism, epithelial homeostasis, colonization resistance, and immune-system development

[1, 2]. Disturbance of the microbial ecosystem has been associated with several gastrointestinal and systemic disorders, which has increased interest in microbiome-directed interventions, including probiotics [3].

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. The concept has developed from early observations on beneficial microorganisms to a field that now requires strain-specific evidence of identity, safety, functional activity, and efficacy [4, 5]. Reported probiotic mechanisms include competitive exclusion of pathogens, production of antimicrobial metabolites, reinforcement of epithelial-barrier function, and modulation of innate and adaptive immune responses [6, 7]. Nevertheless, beneficial effects cannot be inferred solely from genus-level identification because probiotic properties are strain dependent [8]. Commercial probiotic products commonly contain strains of Lactobacillaceae and Bifidobacterium, as well as selected yeasts and other bacterial groups [9]. Candidate strains must also be assessed for undesirable traits, including haemolytic activity, transferable antimicrobial-resistance determinants, toxin production, and other safety concerns [8, 10]. Accordingly, morphological similarity to a recognized probiotic taxon does not itself demonstrate probiotic suitability.

Marine and coastal environments are exposed to salinity, nutrient limitation, temperature variation, and other selective pressures that can support microorganisms with distinctive physiological and metabolic properties. Marine-derived bacteria have therefore attracted attention as sources of bioactive compounds and as potential candidates for aquaculture, food, and therapeutic applications [11, 12]. Bacillus species are of particular interest because many members form resistant endospores and can tolerate environmental stress; however, the genus also contains strains that require careful taxonomic and safety assessment.

The original study isolated bacteria from three coastal-soil microenvironments and characterized representative colonies by macroscopic and microscopic methods. Because no acid tolerance, bile tolerance, antimicrobial activity, adhesion, enzyme, antibiotic-susceptibility, haemolysis, or molecular-identification tests were performed, the investigation is appropriately interpreted as preliminary isolation and phenotypic characterization rather than demonstration of probiotic activity. The objective was therefore to recover cultivable coastal bacteria, document their colony and cellular characteristics, and identify isolates suitable for subsequent strain-level probiotic screening.

2. Materials and Methods

2.1 Sample Collection

Three coastal-soil sample types were collected from Kanyakumari Beach, Tamil Nadu, India: dry surface soil (D), wet soil (W), and soil located between rocks (R). Duplicate samples were collected at an approximate depth of 5–10 cm, placed separately in clean sealed zip-lock

bags, and transported to the Department of Biotechnology laboratory at Chaitanya Bharathi Institute of Technology, Hyderabad, for microbiological processing. The sampling approach was adapted from previously reported soil-microorganism isolation procedures [13].

2.2 Isolation and Purification of Bacteria

Each soil sample was serially diluted in distilled water. The suspensions were vortex-mixed to detach microorganisms from the soil matrix, and aliquots from selected dilutions (10^{-1}, 10^{-4}, and 10^{-5}) were spread onto solid bacterial medium. Fluconazole was added as an antifungal agent at the concentration reported in the source protocol. Plates were allowed to absorb the inoculum for approximately 5 min and were incubated at 37 °C for 48 h. After visible growth, morphologically distinct colonies were selected and repeatedly subcultured to obtain pure cultures. One representative isolate from each sample type was retained and designated D, W, or R. Because the composition and pH of the culture medium were not reported in the source manuscript, these details should be supplied by the authors before final journal submission.

2.3 Macroscopic Characterization

Purified isolates were examined on agar plates for colony size, shape, colour, margin, elevation, and surface texture. Observations were recorded after the stated incubation period under the same culture conditions.

2.4 Microscopic Characterization and Motility

A single colony from each purified isolate was subjected to Gram staining. Cellular morphology and arrangement were observed by light microscopy. Motility was assessed using the hanging-drop method. The combined phenotypic observations were used only for provisional characterization as Bacillus-like bacteria and not for definitive taxonomic identification.

3. Results and Discussion

3.1 Recovery of Bacterial Isolates

More than 80 colonies were observed from each of the three coastal-soil sample types under the stated cultivation conditions. One representative colony from each sample was purified and labelled D, W, or R. Colony counts were reported qualitatively; no colony-forming-unit calculation, replicate variability, or statistical comparison was available. Consequently, the data demonstrate successful cultivation but do not support quantitative comparison of bacterial abundance among the sampling sites.

3.2 Colony Morphology

The colony characteristics of isolates D, W, and R are summarized in Table 1. All three isolates produced medium-sized, whitish colonies, although differences were observed in margin, elevation, and surface texture. Isolate D formed creamy-white, circular, smooth colonies with an entire margin and raised elevation. Isolate W produced off-white, circular, moist colonies with an undulate margin, whereas isolate R produced dull-white, circular, mucoid colonies with an entire margin and convex elevation. These observations indicate partial phenotypic similarity but are not sufficient to establish that the isolates are taxonomically identical or closely related.

Table 1. Colony morphology of the coastal bacterial isolates

Isolate	Size	Shape	Colour	Margin	Elevation	Surface texture
D	Medium	Circular	Creamy white	Entire	Raised	Smooth
W	Medium	Circular	Off-white	Undulate	Raised	Moist
R	Medium	Circular	Dull white	Entire	Convex	Mucoid

3.3 Microscopic Characteristics and Motility

As presented in Table 2, all isolates were Gram-positive, rod-shaped, and motile. Cells of isolates D and W occurred mainly in short chains, whereas isolate R was observed predominantly as single rods. The combination of Gram-positive rod morphology and motility is compatible with Bacillus-like organisms, but it is not diagnostic of the genus or species. Endospore staining, catalase testing, biochemical profiling, and 16S rRNA gene sequencing were not conducted and are required for reliable identification.

Table 2. Microscopic characteristics and motility of the coastal bacterial isolates

Isolate	Gram reaction	Cell shape	Cell arrangement	Motility
D	Positive (purple)	Rod	Short chains	Motile
W	Positive (purple)	Rod	Short chains	Motile
R	Positive (purple)	Rod	Single cells	Motile

3.4 Interpretation and Study Limitations

The three isolates constitute preliminary candidates for further investigation, not validated probiotics. The study did not determine species identity, spore formation, acid or bile tolerance, survival under gastrointestinal conditions, antimicrobial activity, autoaggregation, cell-surface hydrophobicity, adhesion-related properties, enzyme production, antibiotic susceptibility, haemolytic activity, toxin genes, or transferable resistance determinants. No

replicate-level data or statistical analyses were reported. In addition, the use of distilled water as the diluent, the unspecified culture-medium formulation, and incubation at 37 °C may have selectively recovered only a subset of the cultivable coastal microbiota. These limitations should be addressed before the isolates are considered for food, human-health, or aquaculture applications.

4. Conclusion

Three cultivable bacterial isolates were recovered from dry, wet, and inter-rock coastal soil collected at Kanyakumari Beach. The isolates produced medium-sized whitish colonies and were Gram-positive, rod-shaped, and motile, with cells occurring singly or in short chains. These characteristics justify their provisional description as Bacillus-like bacteria. They do not, however, establish species identity, safety, or probiotic functionality. The principal outcome of this study is therefore the establishment of a preliminary isolate set for subsequent characterization. Species-level molecular identification and a structured battery of functional and safety assays are essential before the strains can be described as probiotics or proposed for food, pharmaceutical, or aquaculture use.

5. Future Research

Future work should first confirm strain identity by 16S rRNA gene sequencing and, where necessary, whole-genome analysis. Candidate isolates should then be evaluated for survival under simulated gastric and intestinal conditions, tolerance to acid and bile salts, antimicrobial activity against relevant pathogens, aggregation and adhesion-related properties, and production of beneficial metabolites or enzymes. Safety assessment should include haemolysis, cytotoxicity, antibiotic-susceptibility profiling, screening for virulence determinants, and evaluation of potentially transferable antimicrobial-resistance genes. Only strains that satisfy identity, functionality, safety, stability, and application-specific performance requirements should proceed to formulation studies and controlled in vivo or clinical evaluation.

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