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Phylogenetic Analysis of Marine Microbial Communities: Insights from Kanyakumari Coastal Sediments

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ABSTRACT

Marine ecosystems and the microbial diversity therein are still very underexplored because their related microbes are not very culturable, and access to deep-sea environments is limited. This study aims to determine the bacterial community by analysing sediment samples collected from coastal regions of Kanyakumari – Muttom, Sanguthurai, Sothavilai, Thengapattinam and Kanyakumari. From the screens, 160 organisms (S1—S60) were isolated, and species identification was achieved through their morphological and phenotypic characterization by Gram staining. Five high biomass strains (S1, S18, S25, S38 and S59) undertook the advanced 16S rRNA sequencing. The species found were Vibrio alginolyticus, Bacillus stratosphericus, Bacillus subtilis, Enterobacter cloacae, and Micrococcus luteus. The accession numbers for the NCBI database of these strains are KY952688, KY952690, KY952691, KY952694, and KY952695. The results reveal Kanyakumari to be a major reservoir of rich and diverse microbial species with high biotechnological potential.

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1.0 INTRODUCTION

About 71% of our Earth's surface is covered by oceans and is pregnant with the most amazing life forms, in greater numbers than on land. These marine ecosystems can, in turn, be fueled by essential biogeochemical processes within marine ecosystems, such as carbon sequestration, nitrogen cycling and primary production (Sivaganesh & Ruban, 2019). However microorganisms make up about 98 per cent of the biomass in the ocean, but the great diversity and functional roles of

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these organisms have been little explored. Microbial populations occupy all marine niches from surface waters through to the abyssal plains, adapted to the extreme environments of high salinity, pressure and temperature gradients (Manivasagan *et al.,* 2014). However, they are so common that studying marine microorganisms has been hindered by several factors. Ninety-five per cent of marine bacteria are incapable of growth in the laboratory (Hugenholtz & Pace, 1996), so their diversity and utility to human economies are poorly understood. Metagenomics and 16S rRNA sequencing have been developed as molecular tools to identify uncultivable species (Achtman & Wagner, 2008) to overcome these limitations.

Recently, marine microorganisms have proved to be an important source of bioactive compounds that may have pharmaceutical uses. Other compounds, such as bryostatins from Bugula neritina, the sponge, and Conus magus, the cone snail, have also proved to be effective in the treatment of cancer and pain respectively (Chandrasekaran & Kumar, 1997; Newman & Cragg, 2016). Among all those discoveries, microbial symbionts of marine invertebrates such as sponges and corals produce antibiotics, antivirals and anticancer agents. The Bacillus subtilis and Streptomyces species isolated from the Marine Environment have shown the emergence of potent antimicrobial activity against drug-resistant pathogens (Ruban & Gunaseelan, 2011).

Microorganisms in the sea are necessary for the well-being of the sea ecosystems, and they serve as decomposers, nutrient recyclers, and primary producers. Among these roles, they are essential to the mitigation of pollution through the biodegradation of hydrocarbons, heavy metals, and plastics. For instance, Pseudomonas and Alcanivorox hydrocarbon-degrading bacteria have been successful in cleaning up oil spills (Herlihy *et al.,* 1987). For example, halophilic microbes that can live in high-salinity environments are studied for wastewater treatment in coastal areas (Curtis *et al.,* 2002). The present study promises to produce novel marine microbial species that might be useful in medicine or environmental cleanup. They will become immensely useful in understanding the evolutionary history and how these microorganisms are related to each other. This study will help us understand the biodiversity of the Kanyakumari coastline and may also help us in finding unique bacterial strains for their biotechnological significance.

2.0 MATERIALS AND METHODS

2.1 Study Area and Sample Collection

An entire coastal sediment sampling approach has been undertaken in five coastal sites of Kanyakumari coastline (Muttom, Sanguthurai, Sothavilai, Thengapattinam and Kanyakumari beaches) to give a robust dataset of sediment characteristics and possible environmental impacts. Sediment composition, contaminants, and ecological indicators in various depth horizons are analysed from samples, which were carefully collected in depths from 1 to 8 meters and preserved to ensure the integrity of the samples.

2.2 Physicochemical Analysis

This sediment sampling approach and analysis is a robust physicochemical analysis of the Kanyakumari coastline's environmental health and possible effects. Data from multiple coastal sites and different depths will provide insight regarding sediment characteristics and contaminant levels as well as ecological indicators [(pH, electrical conductivity and macronutrient content (nitrogen, phosphorus and potassium)] in this important coastal region. This information can be critical for coastal management, environmental protection programs, and planning for development and conservation in this area in the future (Vaijayanthi & Vijayakumar, 2014).

2.3 Isolation and Cultivation

Serial dilution techniques were used to isolate bacterial colonies. Selective media were used in the process, such as Nutrient Agar and Thiosulfate Citrate Bile Salt Sucrose Agar. Iterative subculture procedures were then used to purify the isolated colonies. The detailed sediment analysis will be crucial in characterising coastal ecosystem health and may help inform policy decisions and planning for conservation. The multiple chemical and ecological parameters investigated in the study should help us fill in the gaps on anthropogenic impacts and coastal dynamics. In addition, the cultivation of bacterial colonies from these sediments may help to isolate and grow important microbial communities that participate in important nutrient cycling and ecosystem functioning.

2.4 Identification of Isolates

The microbial identification process encompassed the following: the shape and size, margin, elevation, and pigmentation exhibited by a colony, which are the colonial characteristics. Biochemical Tests: Microscopy: Gram staining and oxidase, catalase activity, indole, Voges Proskauer, methyl red, and citrate utilisation tests – Table 1, Table 2 *and* Table 3. As exciting and promising as this kind of research is for gaining more knowledge about coastal environments and filling the need for information to steward our shorelines today and into the future, there are many ways to enhance data gathering in this approach. Using a multidisciplinary approach and considering microbial communities, the study may shed light on important processes in nutrient cycling and ecosystem functioning in coastal areas. These results could be helpful in developing more efficient management strategies to preserve and protect vulnerable coastal environments as anthropogenic stresses continue to increase.

2.5 Molecular Characterization

Furthermore, molecular characterisation was performed by sequencing the 16S rRNA gene for 5 selected strains (S1, S18, S25, S38, S59) with high biomass. Analysis of the resulting sequencing data compared their similarities to bacterial strains already known. This research's findings could be implemented to develop innovative strategies for coastal ecosystem management that could radically change the way we conserve and, indeed, make policy. This study may contribute much-needed context to the intricate relationships between microbial communities and nutrient cycling and, by doing so, offer valuable methods for combating the consequences of human influences on coastal environments. Such insights could help develop more resilient and sustainable coastal ecosystems to benefit not only local communities but also global biodiversity.

3.0 RESULTS AND DISCUSSION

3.1 Microbial Diversity in Kanyakumari Coastal Sediments

The microbial richness of the region was extracted by isolating 60 bacterial strains from sediment samples obtained from five different coastal areas of Kanyakumari. Based on the colony morphology and size, morphological variation and metabolic patterns were obtained as confirmed by biochemical tests. In concordance with prior marine literature, although they prove to be the common bacterial type, recent exterior membrane arrangements do offer them a viable salty environment edge (Hobbie, 1988; Curtis *et al.,* 2002). *Bacillus and Micrococcus,* gram-positive strains, produced secondary metabolites of industrial and medicinal relevance, so they also had a high potential (Manivasagan *et al.,* 2014; Newman & Cragg, 2016).

Figure 1

Pure Culture of Strain 1, 18, 25, 38 and 59

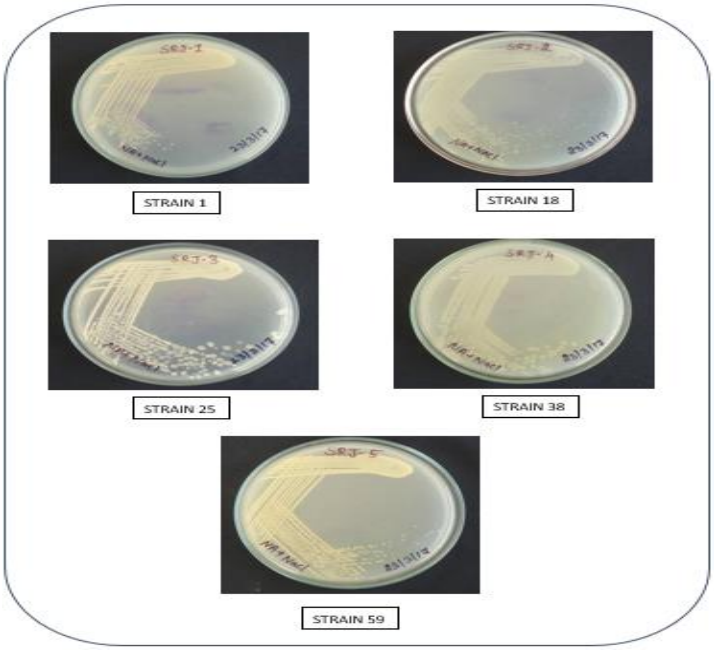


Table 1

Various Physicochemical Parameters in Marine Soil Samples

Sl. No.	Name of the Parameters	Sampling Site at Kanyakumari District				
		Muttom	*Sanguthurai*	*Sothavilai*	*Thengapattinam*	*Kanyakumari*
1	pH	8.75±0.2	9.9±0.4	7.14±0.18	8.1±0.6	7.4±0.41
2	EC (dsm^{-1})	2.90±0.12	61.0±0.13	4.57±0.21	3.12±0.32	19.3±0.28
3	Nitrogen (kg/ha)	85.1±0.29	50.2±0.61	72.2±0.31	47.3±0.41	43.2±0.50
4	Phosphorus (kg/ha)	9.12±0.17	7.45±0.82	6.81 ±0.53	7.01±0.23	5.23±0.22
5	Potassium (kg/ha)	75.0±0.14	68.92±0.21	92±0.26	977±0.13	387±0.25
6	Iron (ppm)	4.3±0.21	11.0±0.2	7.01±0.4	11.2±0.11	7.47±0.17
7	Manganese(ppm)	9.71±0.81	05.2±0.18	8.34±0.21	9.57±0.17	10.52±0.22
8	Zinc (ppm)	3.30±0.22	2.21±0.47	5.14±0.22	8.17±0.25	6.12±0.31
9	Copper (ppm)	5.2±0.2	6.21±0.2	3.05±0.23	4.24±0.22	8.52±0.8
10	Magnesium (mg/kg)	7.71±0.1	3.12±0.4	7.15±0.25	7.22±0.14	3.86±0.28
11	Sodium (mg/kg)	2.96±0.4	5.42±0.14	6.21±0.31	4.52±0.16	7.17±0.53

Table 2

Different Sites of Collected Samples in Varying Dilution Levels 10^{-5} to 10^{-7}, Bacterial Count

Sample	Dilution Factor	No. of Colonies (CFU/ml)
Muttom	10^{-7}	250×10^{-7} CFU/ml
Sanguthurai	10^{-5}	380×10^{-5} CFU/ml
Sothavilai	10^{-7}	190×10^{-7} CFU/ml
Thengapattinam	10^{-6}	185×10^{-6} CFU/ml
Kanyakumari	10^{-7}	190×10^{-7} CFU/ml

Table 3

Biochemical Characterisation

Strain	Gram nature	Cell shape	Methyl red test	Indole	Voges Proskauer	Citrate test	Glucose	Nitrate	Sucrose	Catalase	Oxidase	Urease
S1	-	Curved rod shape	+	+	+	+	+	+	+	+	+	+
S2	+	Curved rod shape	+	-	-	-	-	-	+	+	+	-
S3	+	Straight short rods	-	+	-	-	-	-	-	+	+	-
S4	-	Cocci shape	-	-	+	-	-	+	-	-	-	-
S5	+	Cocci shape	+	+	-	-	-	-	-	+	+	+
S6	-	Straight short rods	_	-	-	+	-	+	-	-	+	-
S7	-	Rod	+	+	-	+	+	+	+	+	-	+
S8	-	Cocci shape	+	-	-	-	-	-	-	-	+	-
S9	-	Straight short rods	+	+	-	-	-	+	-	+	+	-
S10	-	Straight rod shape	-	+	-	+	+	-	+	-	-	+
S11	-	Rod	+	+	-	-	-	+	-	+	+	-
S12	-	Straight short rods	+	+	-	+	-	+	-	+	+	-
S13	-	Straight rod shape	+	-	-	+	-	-	+	-	-	-
S14	-	Cocci shape	+	+	-	+	+	+	+	-	-	+
S15	-	Curved rod shape	+	+	-	-	-	-	-	+	+	-
S16	-	Cocci shape	+	-	+	-	-	+	-	-	+	-
S17	-	Rod	+	+	-	-	-	+	-	+	+	-
S18	-	Rod	-	-	-	-	-	-	-	+	+	-
S19	-	Straight short rods	+	+	-	-	-	+	-	+	+	-
S20	-	Straight short rods	_	-	-	+	-	-	+	-	+	-
S21	-	Cocci shape	+	+	-	-	-	+	-	+	+	-
S22	-	Cocci shape	+	+	-	-	-	+	-	+	+	-
S23	+	Straight short rods	+	+	-	-	+	+	-	+	+	+
S24	-	Straight short rods	+	-	-	+	-	-	+	-	+	-
S25	-	Rod shape	-	-	+	+	-	+	-	+	-	-
S26	+	Straight short rods	_	-	-	+	-	+	-	-	+	-
S27	-	Cocci shape	+	-	-	+	-	-	+	-	+	+
S28	-	Cocci shape	_	+	-	-	-	+	-	+	+	-
S29	-	Rod	+	+	-	-	-	+	-	+	+	-
S30	-	Curved rod shape	+	-	-	+	-	+	-	-	+	-
S31	-	Cocci shape	+	+	-	+	+	-	+	-	-	+
S32	-	Curved rod shape	+	+	-	-	-	+	-	+	+	-
S33	-	Curved rod shape	+	-	-	+	-	+	-	-	+	-
S34	-	Straight short rods	+	+	-	-	-	+	-	+	+	+
S35	-	Cocci shape	+	-	-	-	-	+	-	+	+	-
S36	-	Straight short rods	+	+	-	-	-	+	-	+	+	-

S37	-	Curved rod shape	+	-	-	+	-	-	+	-	+	-
S38	-	Straight rod shape	-	-	+	+	-	+	-	+	+	-
S39	-	Rod	+	+	-	-	-	+	-	+	+	-
S40	-	Curved rod shape	+	+	-	-	-	+	-	+	+	-
S41	-	Straight short rods	+	-	-	+	-	-	+	-	+	-
S42	-	Cocci shape	+	+	-	-	-	+	-	+	+	-
S43	-	Cocci shape	+	-	-	+	-	+	-	-	+	-
S44	-	Curved rod shape	+	-	-	+	-	-	+	-	+	-
S45	-	Curved rod shape	+	+	-	-	-	+	-	+	+	-
S46	-	Cocci shape	+	+	-	-	-	+	-	+	+	-
S47	-	Cocci shape	+	-	-	+	-	+	-	-	+	-
S48	-	Rod	+	+	-	-	-	+	-	+	+	-
S49	+	Straight rod shape	+	-	-	+	-	-	+	-	+	-
S50	+	Rod	+	+	-	-	-	+	-	+	+	-
S51	-	Curved rod shape	+	+	-	-	-	+	-	+	+	-
S52	+	Cocci shape	+	+	-	-	-	+	-	+	+	+
S53	-	Rod	+	-	-	+	-	+	-	-	+	-
S54	-	Cocci shape	+	+	-	+	+	+	+	-	-	+
S55	-	Rod	+	-	-	+	-	-	+	-	+	-
S56	-	Curved rod shape	+	+	-	-	-	+	-	+	+	-
S57	-	Straight short rods	+	+	-	+	+	+	+	-	-	+
S58	-	Cocci shape	+	+	-	-	-	+	-	+	+	-
S59	+	Cocci shape	-	-	+	-	-	+	-	+	+	-
S60	-	Rod	+	-	-	+	-	-	+	-	+	-

3.2 Phylogenetic Insights

Primer3 software was used to design 16S rDNA oligonucleotide primers for the determination of the soil bacterium. The primers were validated in silico and tested in a wet lab. They would produce ~1500 bp, from which the primers could yield an amplicon of the expected size.

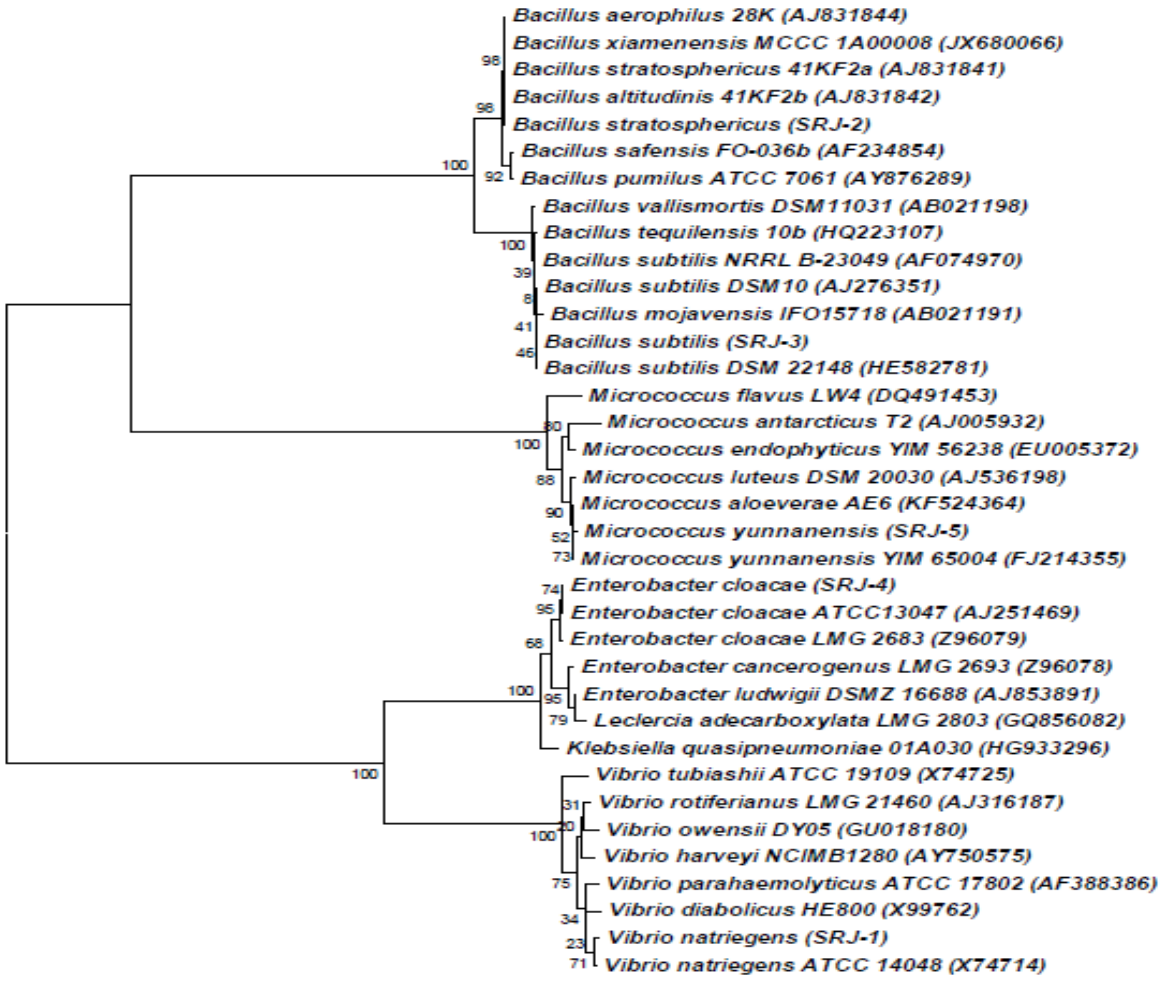
16S rRNA sequencing provided a precise taxonomic classification of the five selected strains:

S1: The results (99% similarity with closely related strains) agree with global databases: S18: *Vibrio alginolyticus, S25: Bacillus stratosphericus, S38: Bacillus subtilis, S59: Micrococcus luteus, Enterobacter cloacae*. At the more coarse level of taxonomic hierarchies, the clustering patterns observed for *Bacillus* and *Vibrio* demonstrated the vital role of bioremediation and bioactive compound production in microbiology (Achtman & Wagner, 2008; Hugenholtz & Pace, 1996), with Bacillus consistently occurring in clades throughout the tree. The observation of the new inclusion, Bacillus stratosphericus, reinforces the ability of microbial strains to adapt to extreme marine conditions. (Chandrasekaran & Kumar, 1997).

The phylogenetic tree was constructed based on comparing the 16s rDNA sequence (S1, S18, S25, S38 and S59). Other sequences with accession numbers were obtained from the gene bank database. The tree topology shown is a rooted tree obtained using a neighbour-joining algorithm. Algorithm with bootstrap values expressed as a percentage of 100% replication Figure *2*.

Figure 2

Phylogenetic Tree of the Isolated Strains



3.3 Applications in Biomedicine

3.3.1 Antimicrobial Activity

However, compounds from Bacillus subtilis and Vibrio alginolyticus had their broad spectra antimicrobial activity. These bacteria have been reported to produce lipopeptides and quorum-sensing inhibitors that inhibit pathogens even when the pathogen resists the drugs (Ruban & Gunaseelan, 2011; Herlihy *et al.,* 1987).

3.3.2 Anti-Cancer Properties

Preliminary assays detected cytotoxic metabolites from Micrococcus luteus. The bioactive compounds isolated from the marine microbe inhibit the growth of the cancer cells, in keeping with the findings of Newman and Cragg (2016).

3.3.3 Drug Discovery

Thus, the identified secondary metabolites indicate that marine bacteria are reservoirs for new drugs. The marine microbial symbionts are well known to have isolated 70% of marine-derived compounds in preclinical trials (Hugenholtz & Pace, 1996; Manivasagan *et al.,* 2014).

3.4 Environmental Remediation Potential

3.4.1 Hydrocarbon Degradation

Bacillus stratospheric and Enterobacter cloacae were extremely effective hydrocarbon degraders and, thus, potential tools for oil spill countermeasures. Herlihy et al. (1987) exhaustively catalogued these enzymatic mechanisms in marine bacteria, and their results agree with their computations.

3.4.2 Salt Tolerance and Wastewater Treatment

The adaptation of isolates to high salinity and salinity spread conditions indicates their suitability for saline wastewater treatment. Earlier, it has been studied halophilic bacteria for the treatment of industrial effluents (Curtis *et al.,* 2002; Boetius *et al.,* 2000).

3.4.3 Plastic Biodegradation

Marine bacteria have recently been found to break down complex polymers, including plastics. Chandrasekaran and Kumar (1997) support the enzymatic potential of Bacillus species for these applications.

3.4.4 Nutrient Cycling

Healthy ecosystems depend on nitrogen fixation, phosphate solubilisation and carbon cycling, and marine bacteria play essential roles in all. Through the results of this study, it was found that the isolates in this study were also involved in nutrient transformation, like Jover *et al.* (2014) and Bech *et al.* (2024).

4.0 CHALLENGES AND FUTURE DIRECTIONS

However, the findings do shine a spotlight on the vastness of marine bacteria and some of the bottlenecks in dealing with such things as non-culturability, scalability, and access to this uncultured marine deep. Therefore, it is these gaps that must be bridged, and advanced molecular techniques like metagenomics and transcriptomics are necessary for the setting of this link (Hugenholtz & Pace, 1996; Achtman & Wagner, 2008). Furthermore, biotechnological inventions to harness these microorganisms at scale for industrial applications require biotechnological inventions (Manivasagan *et al.,* 2014).

5.0 CONCLUSION

The results stress that the Kanyakumari coastline is a unique hotspot for the biodiversity of microbes supportive of biotechnological applications. Unique capabilities for producing bioactive compounds and degrading environmental pollutants were produced by strains identified. Additionally, further research involving these resources, using advanced molecular techniques and scalable cultivation methods, is required to explore these resources for sustainable applications further.

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