



RESEARCH ARTICLE

METAGENOMICS BASED MICROBIAL BIOPROSPECTING STUDIES AT SELECTED BEACHES OF MUMBAI

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Abstract

Background: Bioprospecting studies play a crucial role in identifying species of specific value in different fields of science. Marine ecosystems are rich biodiverse areas with environmentally and economically viable microbes. The microbes play a prominent role in the sandy beach ecosystem. The microbial composition may reflect the richness, pollution levels, food chain intricacies, and distribution of dependent species. Primary biofilm constituting marine bacteria initiates the colonization pattern and survival of organisms in a food pyramid. Culturable microbes and their industrial applications constitute a major chunk of microbial research till recently. Metagenomics, a new innovative, and powerful approach helps to study the biomes of environmental systems, especially the missing and unculturable species.

Materials and methods: Soil samples collected from the upper 5cm stretch of intertidal sand sediments of Versova, Juhu, and Mahim beaches. Sampling was done fortnightly for three months. The pooled samples were analyzed using metagenomics.

Results: The study indicated the richness and distribution of 262 bacterial genera. The common occurrence of 132 genera was observed across three stations. 23 specific genera were noticed at Juhu Beach. A wide variety of microbes were observed across the Versova beach with the predominance of ammonifying bacteria and their metabolic pathways.

Conclusions: Rich biodiversity was seen at Juhu and Mahim beaches as well as the presence of common species. The Versova beach showed lower biodiversity at the phylum level and represented more varieties of bacteria.

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Introduction:-

Bioprospecting studies of the marine environment are needed to address future problems in the areas of food, medicine, health, and economic upliftment of society across world. The marine environment is rich in biological diversity and, comprises nano-sized nanoplankton to huge size whales. Marine microbial bioprospecting has gained interest due to the economic value of the metabolites, and microorganisms of high biotechnological potential (1). Most of the current microbial studies are of generic representation based on Culturable species identification.

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Metabarcoding techniques are often used for marine studies considering the environmental DNA or RNA samples extracted from the soil/sand /sediment and analyzed with known Universal primers to assess the biodiversity, ecosystem quality, and climate change (2) (3). In contrast, Metagenomics uses brute force sequencing, and all DNA sequences present in a sample are read by sequence-it-all technique, focusing on select universally conserved sequences such as 16S and 18S r RNA genes. The advantage is to obtain lower complexity data and a higher number of sequences attributed to a given organism or a set of related organisms.

Marine environmental studies such as quantitative marine risk assessment studies (4), prokaryotic diversity of coastal dunes (5), enzymes and bioactive compounds (6), Oxygen minimum zones of Arabian Sea and Bay of Bengal (7), continental shelf regions of the Arabian Sea (8) and tarball associated bacteria in the marine beaches of Goa and other deep sea and shore sand of marine environment (9)(10)(11)(12) are to name a few which used this technique. Varieties of studies were conducted to study the microbial biodiversity of coastal marine waters about polluted sewage waters (13), oil spills (14) and, beach aquifers (15). The successional patterns of bacteria exhibited in beach sands (14), the comparative account of microbial communities across different geographical regions (16), and the association with abiotic factors are some of the studies worth mentioning during this decade. In the Indian context, the need to study biodiversity is more. The study aimed to compare the biodiversity of bacterial species at selected stations. Similarly to compare the biodiversity of bacteria at Versova beach identified based on isolated, cultured biochemically recognized, and metagenomically identified bacterial genera of Mumbai coast.

Materials and Methods:-

Sample collection:

The three stations selected across the Mumbai coast are well-known, frequently visited, and located almost equidistant and separated in between by creeks. The Juhu beach was located between Versova and Mahim beaches. The Samples were collected at 3 selected stations for 3 months during low tide (Fig.1). Sterile glass assay tubes were submerged such that the upper 5 cm surface of the sand sediment along with water could be collected into the sterile tube and sealed immediately. Samples were kept in the dark till further analysis. The samples thus collected each time were pooled representing each station. 10 g of the sample was transferred to an amber-colored bottle under aseptic conditions. The samples were transported immediately to Credora Life Sciences, Bangalore for Metagenomics analyses based on molecular biology techniques and bioinformatics (17)

Soil sample analysis:

Total count of bacterial species: The samples collected from Versova Beach were simultaneously cultured in laboratory conditions. 1 gm of soil sample was added to 10ml of sterile saline and serially diluted up to 10^{-7} . The three dilutions of 10^{-4} , 10^{-5} , and 10^{-6} were used for the spread plate technique on nutrient agar. The colony-forming units were isolated, counted, and reported as CFU/ml. The isolated bacterial colonies were streaked on selective agar media for further analysis followed by biochemical analyses.

Metagenomics studies:

1gm of soil was inoculated in Nutrient broth and kept for enrichment followed by identification by biochemical analysis as well as CFU of total bacteria. The sample kept in the dark was considered for the metagenomics study. The DNA was extracted, quantified V3-V4 sites amplified, and the library was prepared. The Amplicons were sequenced (Illumina MiSeq), and quantified. The generated data was analyzed after QC (FASTQC and MULTIQC) DNA trimming (TRIMGALORE), Merging, chimeric check, and OTU calling using QIIME/MOTHUR/KRAKEN/BRACKEN workflows. The results were analyzed, and the top 10 genera were identified. Species with specific metabolic pathways were identified based on bioinformatics analytical techniques. The SILVA/GREENGENES/NCBI databases were used during the study.

Biodiversity indices were estimated to compare the biodiversity, evenness, richness, and commonness between the selected stations. Shannon- Weiner, Simpson diversity indices were used to understand the diversity, and evenness index to assess the commonness between the stations. Jaccard index and Sorenson's coefficient were considered to assess the overlap of the species. The formulae of

$$\text{Shannon index } H = -\sum [(p_i) * \log(p_i)]$$

$$\text{Simpson Index : } D = \sum n_i(n_i - 1) / N(N - 1)$$

Where p is the proportion (n/N) of individuals

Evenness index (E) = $H' / H_{\max}$.

(Where E = Evenness index, H' = Diversity index, $H_{\max} = \ln S$, S = Number of species found)

Sorenson's Coefficient (CC) = $2C / S_1 + S_2$

Where, C is the number of common species of two stations; S_1 and S_2 are the total number of species present in each of the two stations

Jaccard's index (J) = $(x \cap y) / (x \cup y) * 100$ where, $x \cap y$ = number of species shared between samples $x \cup y = N$ = total number of species found (18)



Fig.1:- Station locations.

Results:-

The water quality analysis-based bacterial counts represented 5×10^7 CFU/ml. The water quality parameter represented salinity approx. 28‰, phosphate 15 µg/ml, and Nitrite -3.02 µg/ml. The metagenomic analysis of the sand sample is based on 0.4 Million reads representing 54% GC content of V3-V4 Amplicon region of 16S r RNA with 200bp.

The bacterial colonies were isolated using culture-based studies from Versova Beach. 9 Bacterial genera such as *Vibrio*, *Pseudomonas*, *Escherichia*, *Enterobacter*, *Salmonella*, *Klebsiella*, *Citrobacter*, *Proteus*, and *Shigella* were cultured in laboratory conditions and identified based on biochemical techniques. These genera at Versova Beach belonged to Enterobacteriaceae, Vibrionaceae, and Pseudomonadaceae respectively.

The Metagenomic study at 3 selected stations revealed a representation of 30 phyla, 52 classes, 113 orders, 223 families, and 262 genera of bacterial population. The diversity of the bacteria varied with station location and composition. The maximum threshold of OTU was supported by *Pseudoalteromonas*, followed by *Vibrio*, *Paracoccus*, *Methylophaga*, and *Halomonas* spp. During the current study *Acidobacteria*, *Balneolata*, *Chloroflexi*, *Firmicutes*, and *Proteobacteria* were predominant among the 30 bacterial phyla observed. *Proteobacteria* was noticed in all three stations. Mahim samples indicated more diversity than the others and a gradual decrease was observed in Juhu and followed by Versova. The other bacterial phyla are represented by *Saccharibacteria*, *Zixibacteria*, and *Chlamydiae*. The diversity of bacteria at the class level was more at Mahim represented by 30 classes followed by Juhu and Versova beaches. *Gammaproteobacteria* was predominant at all three stations and a decreasing trend of classes was observed from Versova (97%), and Juhu (50%) to Mahim (40%). Overall, 3 selected stations together were represented by 113 orders. The study indicated a peculiarity of the predominance of 3

different orders, each of which showed dominance in each, different station, and the diversity of the orders showed a decline from Mahim, Juhu to Versova. 223 families represented the microbiome of the selected stations.

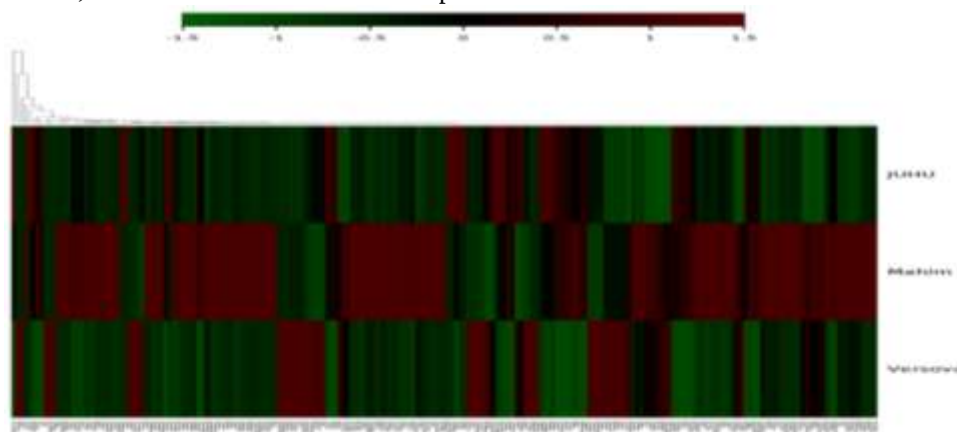


Fig. 2:- Heat map representation of bacterial genera distribution across the 3 stations

The Heat map indicated a richness of genera at Juhu followed by Mahim and, Versova. Juhu has shown maximum diversity than the other two stations with genera common with either of the neighboring stations (Fig.2)

A Comparative analysis of bacterial distribution at the generic level was performed. The total distribution of 390 bacterial genera among the 3 stations was analyzed. Venn diagram, indicated that 33.8% (132) genera were common among all the three stations. Juhu and Mahim represented 200 common genera and, 35 were common at Juhu and Versova

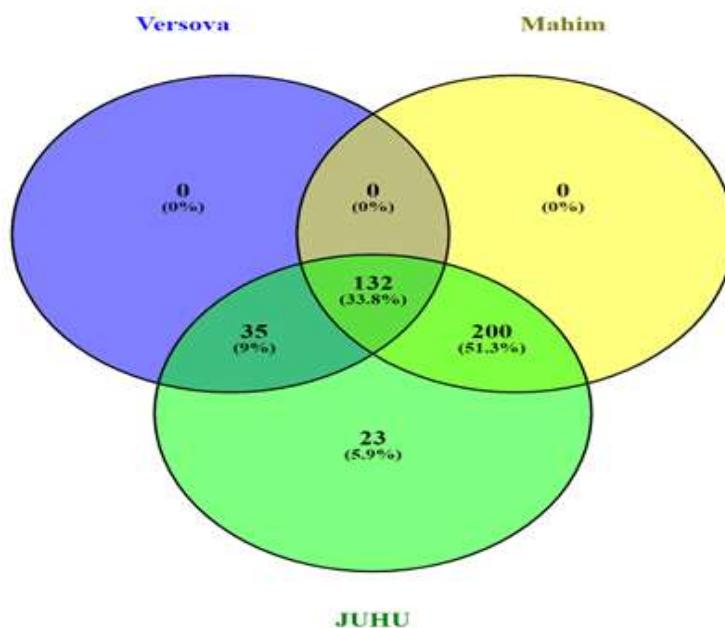


Fig. 3:- Ven-diagram indicating the common and unique species of 3 selected stations.

The 35 genera were represented by *Achromobacter*, *Acidimicrobium*, *Advenella*, *Alistipes*, *Arachidicoccus*, *Burkholderia*, *Castellaniella*, *Chelatococcus*, *Cupriavidus*, *Delftia*, *Dolosigranulum*, *Enterobacter*, *Eubacterium*, *Finigoldia*, *Gemella*, *Kosakonia*, *Kribbella*, *Leptospira*, *Luteibacter*, *Ornithinimicrobium*, *Panacibacter*, *Phascolarctobacterium*, *Rahnella*, *Ralstonia*, *Rhodobacter*, *Rothia*, *Saccharospirillum*, *Saccharothrix*, *Salmonella*, *Shigella*, *Sphaerobacter*, *Streptosporangium*, *Syntrophomonas*, *Tannerella* and *Xanthomonas*; while there no common genera were noticed between Versova and Mahim. Juhu was the only station with 23 exclusive genera

viz., *Alcaligenes*, *Arcticibacterium*, *Azospira*, *Blastochloris*, *Candidatus*, *Hodgkinia*, *Candidatus*, *Pelagibacter*, *Cutibacterium*, *Euzebya*, *Halocynthiibacter*, *Herbaspirillum*, *Kytococcus*, *Methylothermus*, *Pediococcus*, *Photobacterium*, *Pontibacter*, *Pontimonas*, *Proteus*, *Pseudovibrio*, *Rufibacter*, *Sutterella*, *Synechococcus*, *Thalassococcus* and *Veillonellasp.*, in contrast to Mahim and Versova.

Culture-based identification techniques indicated the presence of 9 Bacterial genera represented by *Vibrio*, *Pseudomonas*, *Escherichia*, *Enterobacter*, *Salmonella*, *Klebsiella*, *Citrobacter*, *Proteus*, and *Shigella* represented by Enterobacteriaceae, Vibrionaceae, and Pseudomonadaceae were identified by biochemical analysis

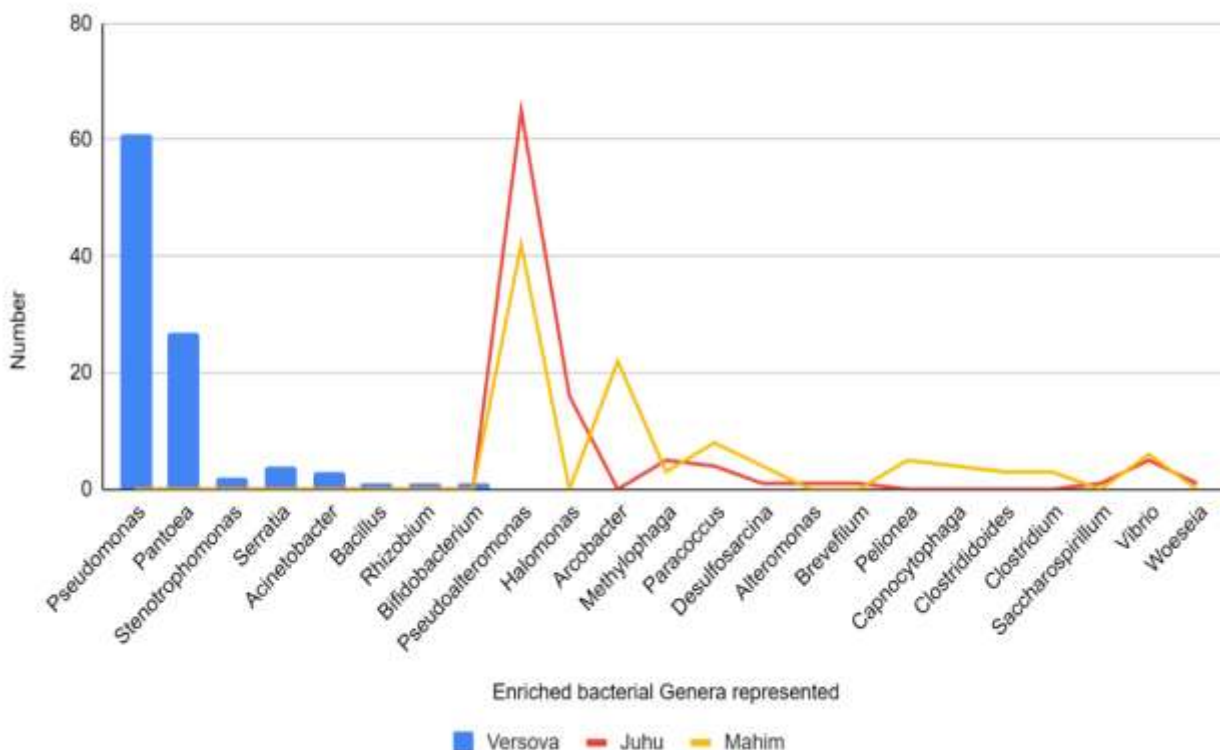


Fig.4:- Enriched bacterial genera represented at selected stations.

23 enriched bacterial genera represented all three selected stations (Fig-4). The Enriched bacterial species analysis included the first ten topmost species that have contributed maximum to the biodiversity at each selected station. Overall, there were no common enriched genera noticed at all 3 stations. *Pseudoalteromonas* spp. predominance was observed (42 and 65% respectively) at the Mahim and Juhu stations. followed by *Vibrio*, *Methylophaga*, *Paracoccus*, and *Desulfosarcina* (1 to 8%). In contrast, *Pseudomonas* spp. (61%) was the predominant species only at Versova and absent at Juhu and Mahim. Only 2 species viz., *Pseudomonas* and *Pantoea* spp. contributed 88% of the population in comparison to 3 species at Juhu and 5 species together represented the biodiversity at Mahim. (Fig.3) ,

The metabolic pathways of bacterial species indicated wide application sources from these areas. The analysis indicated different types of microorganisms with the metabolic activity of Ammonia oxidizers, Alkaline degraders, Atrazine metabolizers, Carbon fixers, cellulose degraders, chitin degraders, chlorophenol degraders, aromatic hydrocarbon degraders, dehalogenators, gramicidin producers, lignin degraders, iron oxidizers, naphthalene degraders, nitrite reducers, nitrogen fixers, propionate metabolizers (V), Selenite reducers, Streptomycin producers, sulfate reducers, sulfide reducers, sulfur oxidizers, sulfur reducers, syntrophic, and xylan degraders. Of these, bacteria alkaline degraders at Mahim, Propionate metabolizers at Versova, and Iron oxidizers at Juhu and Mahim were specially located while the others were noticed at all three selected stations. The distribution of these useful bacteria showed remarkable variation across the Versova, Juhu, and Mahim shores. A decreasing trend of the composition of Ammonia oxidizer, Atrazine metabolism, Nitrite reducer, and Dehalogenators was observed. On the

contrary, the bacterial contribution showed a remarkable pattern in the case of Sulfate reducer, Sulphide oxidizer, sulfur oxidizer, Xylan degrader, chitin degraders, and unknown (Fig.3).

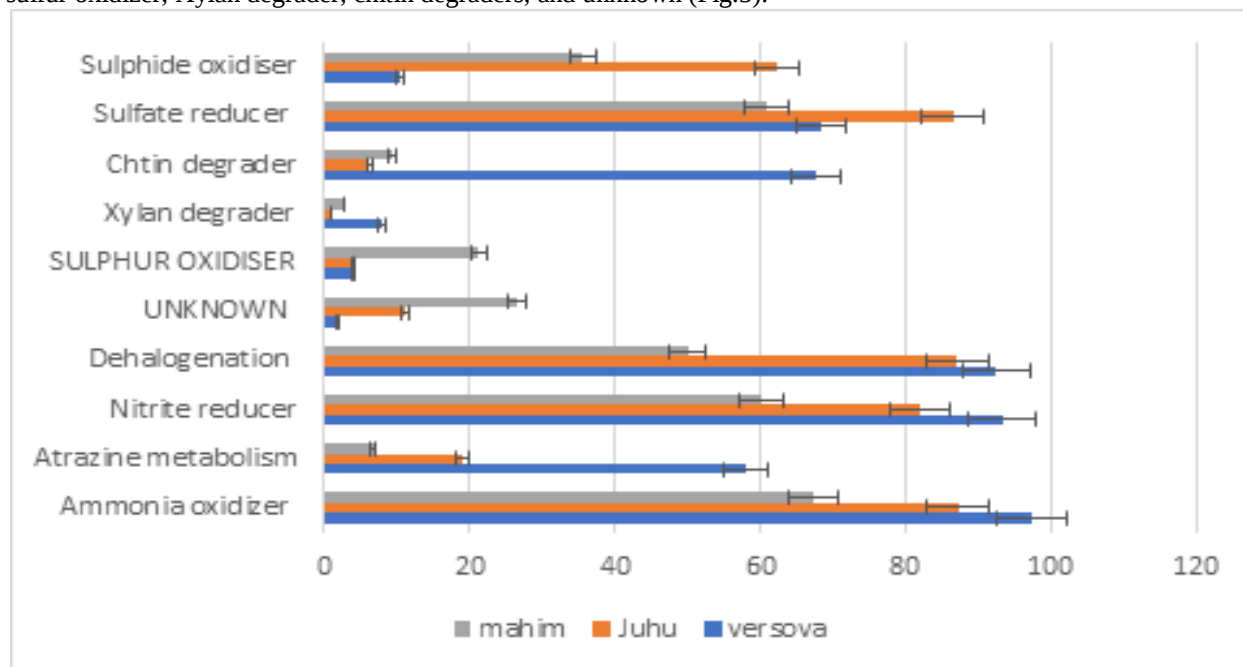


Fig. 5:- Metabolic pathways of bacteria at the 3 selected stations.

The overall analysis of the data based on factorial analysis and clusters concerning the species distribution and prevalence indicated, a close relationship between Juhu and Mahim stations and the distinctness of Versova Beach from the rest of the two.

Biodiversity index	Stations		
	Mahim	Juhu	Versova
Simpson's index (D)	0.243	0.455	0.448
Shannon Weiner index (H)	1.791	1.232	1.105
Evenness (E)	0.778	0.535	0.532
Simpsonian Index (CC)	0.44	0.44	-
Jaccard index (J)	20	20	-

Table 1:- Biodiversity indices at the 3 selected stations.

Biodiversity indices based on enriched genera indicated moderate diversity ($1 < H' \leq 3$) at all the stations. Overall, the dominance of the bacterial genera was close to moderate levels numerically. The evenness index indicated a stable community at Mahim ($0.75 < E$) and an unstable community at Juhu and Versova ($0.5 < E > 0.75$), indirectly indicating the predominance of one species. The Simpsonian coefficient (0.44) and Jaccard index (20%) indicated a commonness of the species at moderate levels between Mahim and Juhu. (Table-1) stations.

Discussion:-

Previous microbial studies from the intertidal regions considered culture-based studies and, biochemical or molecular biology-based identification techniques. A similarity of the bacterial genera that contributed majorly to the composition was noticed in the case of Juhu and Mahim. However, the representation at Versova Beach was distinct from that of these two.

Metagenomics-based sandy beach research revealed the sandy shores as reservoirs of pathogenic bacteria(19). The metagenomic analysis confirmed the culture-based studies with the presence of *Pseudomonas*, *Pantoea*, *Stenotrophomonas*, *Serratia*, *Acinetobacter*, *Bacillus*, *Rhizobium*, *Bifidobacterium* genera of bacteria at Versova beach. The microbial communities varied spatially with three domains of life especially due to microhabitat and intertidal location(20). Juhu Beach represented sulfide oxidizers, and sulfate reducers while, Versova represented

chitin digesters, xylan degraders, dehalogenation, nitrite reducers, atrazine metabolism, and ammonia oxidizers. The previous studies indicated that the taxonomic distribution of the bacterial species and the observed pathways may change with depth, location, and especially the silicate concentration(21). The rich bacterial biodiversity and evenness of their distribution at Mahim Creek were observed. Simpsonian and Jaccard index indicated the presence of the same species metagenomics analysis also confirmed the relation between Mahim and Juhu could be due to the estuarine conditions. comparison to the sandy beaches needs further studies to translate the bacteria of technological value into industrial targets. These studies indicate the biodiversity of Versova, Juhu, and Mahim sandy beaches and the possible sources of bio-degraders that can be used for bioremediation studies.

The metagenomic studies of sandy beaches also help to characterize antibiotic-resistant pathogens and guide time-to-time analyses. These enable to establishment of epidemiological information and improve public health surveillance. The microbial communities were assessed which enabled to mapping of the biotechnologically important bacterial communities. The different microbial metabolic pathways of oxidizers (Ammonia, iron), degraders (cellulose, Chitin, Chlorophenol, aromatic hydrocarbons, lignin, naphthalene,) fixers (carbon, nitrogen) can be efficiently used in bioremediation and purifying the polluted conditions. Similarly, gramicidin producers can be used in the pharma industry.

Conclusion:-

A clear variation in the composition of bacterial populations is evident among the selected stations. The abiotic and biotic factors impact result in the prominence of specific types of biodegraders. Simultaneously the origin of antibiotic-resistant varieties also results due to molecular evolution processes. The current study indicated the importance of metagenomic studies to assess culturable and unculturable species prevalence in huge concentrations along with the metabolic pathways followed at the selected locations. Continuous monitoring of beaches using metagenomics can be considered to improve the status of the pure environment, reduce environmental pollution, monitor the impact of global warming conditions

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Conflict Of Interest:

Nil.

Limitations:

Water quality samples were not sent for the metagenomic analysis to compare with sediment samples.

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