

SUMMARY

The prokaryotic diversity from the vast marine habitats of the Indian subcontinent have not been systematically analyzed and reported till date. In the present study, different types of marine samples were collected from eight different sampling sites of the Central West coast of the country. The different marine samples were collected from the mangrove, saltpan (from the state of Goa) and the intertidal regions of coastal beaches (from the state of Goa and Maharashtra). The sediment soil was collected from mangrove and saltpan sites, while macroalgae were collected from the intertidal regions. These marine samples collected from these regions were subjected to culture dependent and independent analysis. Some highlights of work are detailed below:

1. The soil samples collected from the mangrove and saltpan habitat were subjected to physicochemical analysis with respect to pH, conductivity, salinity, metals, inorganic forms of elements such as nitrogen, sulphur, phosphorus, total organic carbon and dissolved solids (Table 4.1). The slightly acidic pH (5.3-6.1) of Salim Ali soil indicates more predominance of sulphur oxidizing bacteria (SOB) whose end product of metabolism i.e. sulphate with its subsequent aeration lead to acidic conditions. Moreover the higher percentage of sodium chloride (8%) in saltpan as compared to mangrove soil (1.2-1.4%) indicates more saline conditions (Table 4.2). The agglomerative hierarchical clustering based on Euclidean distance with respect to physicochemical parameters also signifies the difference in the ecology of both the habitats (Fig. 5.1).
2. The maximum number of colony forming units (CFU) values were recorded for macroalgal samples as compared to mangrove sediments (Table 4.2; Fig. 5.2), but the characterization of the isolates revealed limited diversity in algal samples (Table 5.4, 5.11). The range of CFU obtained in our study is comparable to earlier reports that have also mentioned similar diversity patterns (Chan and McManus, 1969; Lewis et al., 1985; Jensen et al., 1996; Largo et al., 1997; Hempel et al., 2008). Thus a higher CFU does not guarantee a diverse nature of cell types within the limitations of media conditions utilized in this study (Table 4.2; Fig. 5.2).
3. A sum total of 719 bacterial and archaeal isolates were purified and preserved from the twelve marine samples. Nearly, half of the marine isolates (belonging to samples SAOS, DI, CDRSL and SAB) were subjected to preliminary characterization based on ribosomal protein profiles (MALDI-TOF MS), fatty acid

profiles (FAME) and partial/complete 16S rRNA gene sequencing. The remaining isolates were identified based on only partial/complete 16S rRNA gene sequencing. The preliminary characterization taxonomically placed the strains till genus level and also facilitated identification of putative novel taxa.

- The reliability of identification on the basis of similarity index in FAME and log(score) in MALDI-TOF MS among the marine isolates of mangrove (SAOS and DI) and macroalgae (SAB and CDRSL) were found to be more and less similar (Table 6.1). The congruence of the three techniques (MALDI-TOF, FAME and sequencing) with respect to identification of the isolates at the level of genus revealed that the correlation coefficient among FAME/MALDI, sequencing/FAME and sequencing/MALDI was in the range of 87-98% for mangroves (Table 5.1; Fig. 5.2, 5.4) and 58-76.5% for macroalgal isolats (Table 5.1; Fig. 5.5, 5.6).

Table 6.1 Percentage distribution of characterization patterns with respect to FAME and MALDI-TOF MS

Sampling site	FAME (similarity index)			MALDI-TOF MS (log score)		
	Good match	Atypical strain	No match	Genus level identification	Species level identification	Non reliable identification
Mangrove	34%	38%	27.8%	24.7%	15%	60.3%
Macroalgal	26.3%	47.3%	26.3%	23.1%	10.5%	66.2%

- A total of 42 genera with 127 different species belonging to four different phyla (*Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroidetes*) and 46 genera with 136 different species belonging to three different phyla (*Proteobacteria*, *Actinobacteria* and *Firmicutes*) were characterized from the mangrove and macroalgal habitats respectively, while 9 genera with 26 different species belonging to three different phyla (*Proteobacteria*, *Firmicutes* and *Euryarchaeota*) were isolated from the saltpan habitat. In case of mangrove habitat, 36% of the characterized species were earlier isolated from similar marine habitats, while the corresponding number was 34.5% for macroalgal isolates. Surprisingly, 8.6% and 11% of the characterized species from the mangrove and macroalgae respectively showed closest sequence similarity to strains isolated from forensic/clinical samples. In case of saltpan sample, 53.5% of the characterized species were earlier

isolated from similar ecosystems. The alpha diversity indices showed that the differences in Simpson index of diversity to be statistically significant among the mangrove/saltpan and macroalgae, while the other parameters based on Shannon, observed species and Chao1 index were not significant. The highest number of species were detected in sediment soils collected from Salim Ali mangrove habitat (SAOS), while for macroalgal samples, *Sargassum sp.* collected from Anjuna beach (SAB) reported highest diversity. The PCA plot based on species composition data indicated that the three habitats (mangrove, saltpan and macroalgae) cluster separately and can be defined by specific endemic prokaryotic communities (Fig. 5.12).

6. The culture dependent analysis of all the three habitats resulted in the identification of 22 novel taxa (13 gram-stain negative and 9 gram-stain positive) both at the genus and species level (i.e. 3% of the total isolates). The percentage distribution of putative novel taxa at the phylum level showed that 50% belonged to *Proteobacteria* (41% belonged to γ -*Proteobacteria* and 9% belonged to α -*Proteobacteria*), followed by 32% *Firmicutes* and 9% each of *Actinobacteria* and *Euryarchaeota*. Out of these, four novel species have been published under the etymology of *Domibacillus mangrovi* SAOS 44^T/*Domibacillus epiphyticus* SAB 38^T (Verma et al., 2017), *Luteimonas padinae* CDR SL 15^T (Verma et al., 2016) and *Marinomonas epiphytica* SAB 3^T (Ojha et al., 2017). This is the first report of description of novel bacterial taxa from the surface of macroalgae in the country. Additionally 9 more isolates based on partial 16S rRNA sequence characterization were found to be putative novel taxa belonging to genus *Bacillus* (DI 90D, SAB-20 R2A; Table 4.4, 4.5), *Vibrio* (LLKUN-4 SWC, CDRSL-1 VNSS; Table 4.6; Table 4.13), *Demequina* (OG-105 MA; Table 4.8), *Citrobacter* (SAB 4D; Table 4.5), *Streptomyces* (CDRSL-26 MA; Table 4.6), *Massilia* (CDRSL-7 R2A; Table 4.6) and *Micrococcus* (CDRSL-16 TSBA; Table 4.6).
7. A total of 296 marine isolates were screened for the polymer hydrolysis potential against six different substrates. In mangrove samples, cellulose, xylan, pectin, gelatin, casein and starch degraders were identified in the range of 37.7-52.1%, 60.8-61.2%, 50.7-51%, 52-53.6%, 41.8-56.5% and 64.2-65.2%, while in macroalgal samples, the hydrolysis potential were in the range of 38.8-50%, 46.3-59.7%, 32.8-51.6%, 48.4-49.2%, 20.9-24.2%, 47.8-72.6% respectively. More diverse group of bacteria belonging to genera *Bacillus*, *Kocuria*, *Planomicrobium*,

Streptomyces and *Vibrio* were found to have hydrolysis potential in macroalgal samples while members of only four genera belonging to *Bacillus*, *Vibrio*, *Microbulbifer* and *Micrococcus* showed the hydrolysis potential in mangrove samples.

8. The culture independent analysis through amplicon sequencing of V3-V4 region of the 16S rRNA gene from all the twelve marine habitats indicated the prokaryotic taxa which were not cultured on axenic media. Among the mangrove, saltpan and macroalgal the total identified phyla corresponds to 59, 46 and 14 while the genera count corresponds to 288, 232 and 80 which is far higher than obtained in culturing. At the species level, the different corresponding numbers among the mangrove/saltpan and macroalgal samples were in the range of 1653-4343 and 225-618 respectively, about 2 to 10 times higher than reported in culturing.
9. The OTU's of the identified families that were abundant and exclusively detected in mangrove/saltpan samples belonged to *Piscirickettsiaceae*, *Helicobacteriaceae*, *Halothiobacillaceae*, *Desulfobacteraceae*, *Balneolaceae* and *Fusobacteriaceae*, while for the macroalgal samples these belonged to members of *Pseudoalteromonadaceae* and *Halomonadaceae*. The list of specific genera specific to mangrove/saltpan at a frequency of >1 % OTU abundance belonged to *Thiomicrospira*, *Gaetbulbacter*, *Halothiobacillus*, *Sulfurimonas*, *Psychrilyobacter* and *Desulfococcus*, while for macroalgae, these were identified as belonging to unclassified genera of family *Halomonadaceae* family, *Pseudoalteromonas*, *Cobetia*, *Vibrio*, *Halomonas*, *Marinobacter*, *Cellulophaga* and *Octadecabacter*.
10. The Sulphur oxidizing bacteria (SOB; *Thiomicrospira sp.*) were detected in high abundance while OTU's belonging to taxa of sulphate reducing bacteria (SRB; *Desulfococcus sp.*, *Desulfofaba sp.*, *Desulfosarcina sp.* etc.) occurring in a smaller scale thereby indicating the presence of both oxygenic and anoxygenic conditions prevailing in the mangrove habitats. The presence of both SOB and SRB completes the circle of sulphur cycle which acts in close association with carbon and nitrogen fluxes. Thus the microbial communities present in these niches play a major role in the nutrient cycling between inorganic and organic forms of elements. Similarly, in case of saltpan sample, the halophilic taxa playing a major role in sulphate reduction were found predominant and few of these are involved in the detoxification of the saline soil through bioremediation of heavy metals. In

case of macroalgal samples, the members known to play important roles in quorum sensing (*Cobetia sp.*), release of low and high molecular weight bioactive compounds and inhibitory extracellular agents (*Pseudolateromonas sp.*) were predominant.

11. The total number of OTU's in culture independent analysis were much higher in mangrove/saltpan samples compared to macroalgae (Table 5.1). Moreover the differences in alpha diversity indices based on Shannon, Simpson and Chao1 among the mangrove/saltpan and macroalgal samples were statistically significant with the maximum number of OTU's detected in mangrove soil collected from Salim Ali mangrove habitat (SAOS), while the corresponding value for macroalgal samples belonged to *Sargassum sp.* collected from Kunkeshwar beach (LLKUN). It is interesting to note that in culturing methodology, maximum number of isolates were preserved from the SAOS sample (Table 4.1). The fewer OTU's being detected for macroalgae indicates the competitive, stressful and specialized environment (host-microbe interaction) present on the macroalgal surface that leads to selection of limited prokaryotic diversity. However since very few algal samples were collected in this study and seasonal and replicate analyses was not considered therefore validation of these results would require a thorough metagenomic analyses of a greater algal diversity obtained from different locations and analyzed keeping in perspective the spatial and temporal gradients.
12. Interestingly, a total of 43 genera characterized through culture dependent analysis were not detected in metagenomic sequences. Among the mangroves, the genera belonging to *Defluviimonas*, *Kocuria*, *Lysinimicrobium*, *Micrococcus* and *Terrabacter* of the phylum *Actinobacteria*, genera *Mangrovimonas* and *Tenacibaculum* of the phylum *Bacteroidetes*, genera *Domibacillus*, *Fictibacillus*, *Lysinibacillus* and *Planomicrobium* of the phylum *Firmicutes* and genera *Acinetobacter*, *Catenococcus*, *Oleigrimonas* and *Yangia* of *Proteobacteria* were not identified in metagenomics. Similarly, in case of macroalgal samples, the genera *Brachybacterium*, *Cellulomonas*, *Cellulosimicrobium*, *Isoptricola*, *Janibacter*, *Kocuria*, *Ornithinimicrobium*, *Sanguibacter* and *Streptomyces* of the phylum *Actinobacteria*, genera *Brevibacillus*, *Domibacillus*, *Fictibacillus*, *Gracilibacillus*, *Oceanibacillus* and *Paenibacillus* of *Firmicutes*, genera *Aeromonas*, *Brevundimonas*, *Catenococcus*, *Cedecea*, *Citrobacter*, *Enhydrobacter*, *Enterobacter*, *Kosakonia*, *Pantoea*, *Roseomonas* and *Salinimonas* of

Proteobacteria were not detected in macroalgal samples. The probable reason for omission of these phylotypes could be either due to PCR biasness, efficiency of the lysis procedure (since cell walls of Gram-stain-positive bacteria are more difficult to break mechanically) or removal of these sequences during the pre-processing step.

13. The total prokaryotic community composition detected through culture independent approach can be used in the future endeavours to study the shifts in the microbial populations in the wake of climate change, anthropogenic disturbances and rising ocean temperatures. Some of the members of genera such as *Haliea*, *Clostridium* and *Fusibacter* represent bioindicators of oil contamination in mangrove ecosystems. It is a well known fact that the members of genus *Haliea* [member of Oligotrophic Marine γ -*Proteobacteria* (OMG) group] are known to show significant decrease while the members of genus *Clostridium* and *Fusobacteria* (phylum *Firmicutes*) showed a significant increase in oil contaminated area. Similarly in our analysis, the Divar island mangrove showed no OTU's corresponding to *Haliea*, while the percentage of *Clostridium* (0.4% of OTUs) and *Fusibacter* (0.6% of OTUs) were found to be higher as compared to other mangrove systems (0-0.1% OTUs of *Clostridium* and 0.07-0.07% OTU's of *Fusibacter* in other mangrove systems). Thus the mangrove habitat of Divar Island showed to be under the influence of oil contamination as compared to rest of the mangrove systems.
14. The cataloguing of microbial consortia of a particular environment is indeed helpful not only from understanding the ecology of an environment but the information can also be utilized for increasing the number of cultured isolates from these ecosystems. Since in our analysis, a total of 29 and 15 different phyla with cultured representatives were characterized from mangrove/saltpan and macroalage samples respectively (Table 5.8 and 5.9), so the existing information from the particular cultured taxa can be utilized to target specific groups of bacteria having a role in degradation potential or bioactive molecules production. Moreover attempts can also be made in culturing the uncultivated lineages which do not represent any cultured representatives through innovative culturing conditions.