

1 **Isolation, identification and functional characterization of cultivable bacteria**
2 **from Arabian Sea and Bay of Bengal water samples reveals high diversity**

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23 **Abstract**

24 The oxygen minimum zone of the Arabian Sea (AS) and Bay of Bengal (BOB) is rich in organic matter and is an
25 unusual niche. Bacteria present in the oceanic water play an important role in ecology since they are responsible for
26 decomposing, mineralizing of organic matter and in elemental cycling like nitrogen, sulfur, phosphate. This study
27 focuses on culturing bacteria from oxygen minimum zones (OMZ) and non-OMZ regions and their phylogenetic as
28 well as the functional characterization. Genotypic characterization of the isolates using amplified rDNA based
29 16SrRNA sequencing grouped them into various phylogenetic groups such as alpha-proteobacteria, gamma-
30 proteobacteria and unaffiliated bacteria. The cultivable bacterial assemblages encountered belonged to the genus
31 *Halomonas*, *Marinobacter*, *Idiomarina*, *Pshyctobacter* and *Pseudoalteromonas*. Among the enzymatic activities,
32 carbohydrate utilization activity was most predominant (100%) and microorganisms possessed amylase, cellulase,
33 xylanase and chitinase. A large proportion of these bacteria (60%) were observed to be hydrocarbon consuming and
34 many were resistant to ampicillin, chloramphenicol, kanamycin and streptomycin. The high diversity and high
35 percentage of extracellular hydrolytic enzyme activities along with hydrocarbon degradation activity of the culturable
36 bacteria reflects their important ecological role in oceanic biogeochemical cycling. Further assessment confirmed the
37 presence of nitrogen reduction capability in these cultivable bacteria which highlights their importance in oceanic
38 geochemical cycling.

39 **Keywords:**

40 Oxygen minimum zone; 16S rRNA sequencing, enzyme activity; Arabia Sea; Bay of Bengal.

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49 **1. Introduction**

50 Marine microorganisms are exposed to diverse environmental parameters including temperature, pressure, salinity
51 and oxygen concentration [1]. Bioactive compounds discovered from marine microbes are important for
52 biotechnological applications and have medicinal importance [2–5]. More than 15,000 chemical substances including
53 4196 bioactive marine natural products have been isolated from marine organisms [6] highlighting the potential and
54 importance of cultivable marine microorganisms.

55 Oxygen plays an important role in shaping the aquatic ecosystem which controls the spatio-temporal distribution of
56 all living organisms from microbes to higher organisms. Low oxygen (<20 μM) [7] regions can be observed in various
57 aquatic ecosystems including stagnant freshwater, saline lakes [8], and marine basins with restricted circulation [8].
58 In marine regions of high productivity with slow ventilation rates [8], dissolved oxygen concentrations at lower depths
59 drop to very low levels, these regions are referred to as oxygen minimum zones (OMZ). The differential solubility of
60 oxygen and consumption via microbial-mediated oxidative processes are also known to affect the oxygen
61 concentration in marine water.

62 In oceanic OMZ areas, anaerobic respiration by microbes includes use of alternative electron acceptors such as nitrate
63 and sulfate [7]. Thus, presence of OMZ areas influences the chemical composition of the water and induces stress on
64 organisms including alterations in the food web structure [8, 9]. For instance, total reduction of nitrogen and release
65 of nitrogen gas by microbes removes the fixed nitrogen from marine ecosystem [8]. Similarly, sulfur (S) reduction
66 produces hydrogen sulfide and increasing the toxicity of surrounding environment [10, 11]. The Indian Ocean, the
67 Arabian Sea and Bay of Bengal covers the 59% of the total global OMZ area [12]. Some previous studies on OMZ
68 have concentrated primarily on the diversity of microorganisms in waters/sediments from Arabian sea [13–15] and
69 Bay of Bengal [16–21]. In addition, the identified cultivable bacteria during algal blooms in AS region were shown
70 to have denitrifying capability, indicating relationship between water column characteristic and microbial diversity
71 [22]. Additionally presence of active nitrogen cycle [23–25], sulfate reduction [26] and methane metabolism [27] from
72 Arabian sea have been reported. In a recent study [28] where abundance of microbial genes associated with N_2
73 production have been quantified which supported the presence of denitrifiers and anammox microbial population in
74 Bay of Bengal OMZ area. BOB –OMZ is weaker due to less detritus along the western boundary [29] than AS-OMZ.
75 Unlike Arabian sea, Bay of Bengal receives immense fresh water runoff as well as average sediment load (1.1×10^9
76 tonnes) [30, 31] from major rivers. Thus, overall AS-OMZ and BOB-OMZ differ in various aspects including salinity,
77 DO concentrations and sediment load.

78 Denitrification is a dissimilatory process which involves the oxidized nitrogen compounds as alternative electron
79 acceptors for energy production during very low oxygen concentration. Nitrogen oxides are reduced to gaseous
80 products (NO , N_2O , and N_2) which are released, leading to a loss of fixed nitrogen to the environment. In marine
81 coastal sediments, denitrification removes 40 to 50% of external inputs of dissolved inorganic matter [32] resulting in
82 an unbalanced nitrogen budget in the ocean [33, 34]. Accumulation of NO and N_2O contributes to global warming
83 and the destruction of the ozone layer. Also the mangrove studies reveals that the anthropogenic activities lead to the

84 bioaccumulation of different metals which in turn affects its associated biota [35]. Thus, there is a need for
85 understanding the community of denitrifying bacteria as they influence the environmental conditions.

86 In addition to 16S rDNA, several functional genes were shown to be useful in the investigation of microbial
87 communities [36–38]. Functional genes provide a resolution below species level and this approach may indicate
88 functional diversity in the environment. An approach involving 16S rDNA alone does not appear to be suitable to
89 investigate communities of denitrifying bacteria, as denitrification is widespread among phylogenetically unrelated
90 groups [39]. Present study investigates the cultivable bacterial diversity from OMZ and non-OMZ areas of AS and
91 BOB. Microorganisms have been cultured and characterized based on 16SrRNA sequencing. Further functional
92 characterization includes the substrate utilizing properties. Hence this study deals with comparative taxonomic as well
93 as different substrate utilization properties of cultivable bacteria from Indian OMZ and Non-OMZ areas. .

94 **2. Materials and Methods**

95 **2.1. Sample collection and isolation of microorganisms**

96 Water samples from OMZ and Non-OMZ regions of Arabian Sea and Bay of Bengal were collected in Apr/May 2015
97 (Cruise No. 340,) and in December 2015 (Cruise No. 346) Sagar Sampada, CMLRE, MOES respectively. CTD
98 profiler was used for collection of Water samples and measuring the DO concentration.

99 Water samples (100µl) were spread on Zobell's media containing 5% sea salt and incubated at 12°C in dark for 7
100 days. Morphologically different colonies were isolated and purified. These pure single isolates were then used for
101 subsequent analysis including Gram staining and substrate utilization assays. Isolates were also examined for their
102 growth at 12°C and 37°C temperature so as to find out the optimum temperature required for their growth.

103 **2.2. Identification of microorganisms**

104 In total, 40 single colonies from Arabian Sea and 31 distinct single colonies from Bay of Bengal were isolated. From
105 all the 71 colonies genomic DNA was isolated using QIAprep® kit according to the manufactures protocol. This was
106 followed by 16S rRNA PCR using 27F in combination with1492R/1390R/519R set of primers (Online resource 1).
107 Total 25 µl of total PCR reaction with 1 µl DNA (50–100 ng), 1 µl each of primers (10 pmol µl⁻¹), 2.5 µl 10× Taq
108 polymerase buffer (Promega), 0.5 U Taq DNA polymerase (Promega) and 200 µM of each dNTPs (Promega) was set
109 up. The PCR conditions were, 5min at 95°C for initial denaturation, 35 cycles of 30 s at 95°C, 45 s at 48°C or at 55°C,
110 120 s at 72°C and a final extension for 10 min at 72°C. PCR product was checked using 1% agarose gel electrophoresis
111 and purified using Promega (A9282) kit according to manufacturer's instructions. Sanger dideoxy method was used
112 for sequencing all the PCR products of 16S rDNAand the taxonomic identification of each bacteria was carried out
113 using NCBI BLAST tool.

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115 **2.3. Screening for ability to use different Substrates**

116 **2.3.1. Enzyme Assays**

117 All the 71 isolates were screened for extracellular production of amylase, xylanase, agarase, cellulase and chitinase
118 enzymes. For agarase and amylase, 1% agar and 1% starch plates were used, respectively and clear zones produced
119 by the isolates were identified using Gram's iodine with potassium iodide staining. For cellulose, 1% CMC and for
120 xylanase, 1% Birchwood xylan plates were used and clear zones were observed after 2/3 days using Congored. For
121 Chitinase assay, 1% colloidal chitin plates were used and clear zones were directly observed after 3 days of incubation.
122 Bacterial isolates which showed clear zones in plate assays were used for colorimetric quantitative assays for amylase,
123 xylanase and cellulase. Initially, the cultures were grown in minimal medium using 1% soluble starch, 1% birch-wood
124 xylan and 1% CMC as a sole carbon source for amylase, xylanase and cellulase, respectively. After 72 hrs of
125 incubation, cell free supernatant was used for colorimetric assay. For cellulase, xylanase and amylase assay 20µl of
126 1% CMC, 1% xylan and 1% starch was added in 96 well plate with 20µl cell supernatant from each isolate. Plates
127 were incubated for 1hr. Further 160µl of DNS solution was added in each well and incubated at 100°C for 20 min
128 followed by reading at 570nm in multimode plate reader (Perkin Elmer). All the quantitative assays were done in
129 triplicates and the activity was estimated using the standard graph of glucose.

130 **2.3.2. Hydrocarbon degradation**

131 For Hydrocarbon degradation assay, isolates were grown in Bushnell-Hass medium (Himedia- M350) with kerosene
132 as a sole carbon source. Microbes which were able to grow in this medium were considered as positive for kerosene
133 degradation.

134 **2.3.3. Biochemical Test for Carbohydrate utilization**

135 Carbohydrate utilization kit (HiCarboTMKB009) containing 35 different carbohydrates was used to study the
136 utilization of different carbohydrates. The kit contains 35 wells of different carbohydrates with a negative control.
137 50µl of freshly grown culture was inoculated in each well and color change was observed according to the
138 manufactures instructions.

139 **2.3.4. Sulfur Reduction Test**

140 Barr's medium was used to test the sulfur reducing capacity of different isolates. The medium contains different sulfate
141 salts which turn black when utilized by any organisms. Isolates were inoculated in 10 ml of medium and kept in dark.
142 Color change from white to black indicated the positive reaction and confirmed the sulfur reduction. *Desulfovibrio*
143 *desulfuricans* was used as a positive control.

144 **2.3.5. Nitrate reduction test**

145 Nitrate reducing ability was assessed by Nitrate/Nitrite colorimetric assay kit (780001) from “Cayman Chemicals”
146 with slight modification in manufactures protocol. Bacterial culture was suspended in 500µl of 1X PBS buffer,
147 lysozyme (400µg/ml) was added in each tube followed by sonication. Samples were incubated at 37°C for 1hr and
148 centrifuged at 14,000 rpm for 20 min at 4°C. Supernatant was removed and protein concentration was quantified. Total
149 cell protein (50 µg) for each sample was used along with 80µl of assay buffer (from the kit). Then 20µl of Nitrate
150 standard (From Kit, concentration) was added in each well. Assay plate was incubated at RT in dark for 1hr, 50µl of
151 Griess reagent I followed by 50µl of Griess reagent II was added in each well and colour development was observed.
152 If pink color appeared, it indicated the presence of nitrite and the test was considered positive. In case no colour
153 developed, pinch of zinc dust was added to the well. Development of colour after addition of zinc showed that nitrate
154 was not reduced and the test is negative but if no colour appeared, it indicated the total reduction of nitrate and
155 confirmed that nitrogen is released from the sample indicating positive test.

156 **2.4. Antibiotic resistance**

157 All seventy-one isolates were screened for Ampicillin, Chloramphenicol, Streptomycin and Kanamycin resistance by
158 disc diffusion method. Bacterial culture (100µL) was spread on Zobell’s agar plate and discs were placed on the plate.
159 On discs, 10 µL of each antibiotic (100µg/mL concentration) was added. Presence of clear zone around disc after
160 overnight incubation indicated sensitivity to antibiotic.

161 **3. Results and Discussion**

162 **3.1. Cultivable microbes from AS and BOB OMZ-Non-OMZ area**

163 Initial screening of samples led to the identification of 71 distinct types of microbes (Online resource 2). Detailed
164 sampling information of the water samples collected from AS and BOB has been mentioned in Table I. Isolates were
165 examined for their growth at two different temperature i.e. 12°C and 37°C and it was observed that all the isolates
166 were able to grow on both the temperature. Interestingly it was observed that at 12°C the growth was much slower
167 than at 37°C. In future studies however, it will be interesting to understand the metabolic difference in bacteria grown
168 at these temperatures.

169 The bacteria were characterized and subjected to 16S rRNA sequencing followed by BLASTn homology search. All
170 these 16S rRNA sequences were deposited in NCBI GenBank under accession numbers MH217583, MH256039 to
171 MH256108 (Table II). Each 16S rRNA was subjected to MEGA6 analysis with 10 iterations and 1000 bootstraps to
172 identify phylogenetic relationship. 16S rRNA based analysis has identified 71 bacteria belonging to genus
173 *Halomonas*, *Marinobacter*, *Idiomarina*, *Pshyctobacter*, *Pseudoalteromonas* along with two more which are
174 represented only by the single sequences those were *Thallassospira* (in Bay of Bengal) and *Paenibacillus* (in Arabian
175 sea). In case of both Arabian Sea and Bay of Bengal the distribution was not specific with respect to either depth,
176 collection site and oxygen concentrations. For example, bacteria from genus *Halomonas* or *Pseudoalteromonas* could
177 be isolated from almost all samples in BOB and AS waters. However, some bacteria like *Thallassospira* was observed

178 only at BOB waters and bacteria from genus *Paenibacillus* was isolated only from AS. Thus, the initial 16SrRNA
179 analysis revealed that from AS in total 6 genera while from BOB bacteria belonging to 2 genera were successfully
180 cultured, indicating greater diversity in AS than BOB.

181 **3.2. Characteristics of bacteria**

182 *Halomonas spp.* has been isolated from hydrocarbon contaminated industrial brine [40], sea water [41], hypersaline
183 lake, hydrothermal vent, saltern fermented food. Members of this genus are involved in bioremediation process [41],
184 some *spp.* can produce biosurfactants. We could identify different *Halomonas* species such as *Halomonas*
185 *aquamarina*, *Halomonas axialensis*, *Halomonas meridiana* and *Halomonas denitrificans* from different location and
186 water depths. Few of these bacteria were reported to have specific characteristics that can be useful in various
187 applications. Such as *Halomonas axialensis* has been shown to reduce nitrate in both aerobic and anaerobic condition
188 and is also oxidase positive [42]. It is known to harbor 20 aldehyde dehydrogenase genes involved in 14 metabolic
189 pathways of aldehyde degradation[43]. *Halomonas meridiana* has genes such as choline dehydrogenase and betaine
190 aldehyde dehydrogenase which are responsible for production of osmo-protectants. Genes responsible for osmolyte,
191 ecotine production (ectABC) and for utilization of ecotine (eutED) have also reported in its genome [44].

192 Many bacteria from *Marinobacter* genus were also isolated from BOB and AS waters. Interestingly, many species
193 from *Marinobacter* genus are known to degrade petroleum and a few can tolerate metalloids. One of the isolate was
194 identified as *Marinobacter hydrocarbonoclasticus* which is known to grow on aromatic hydrocarbons and can produce
195 extracellular surface-active compound by forming biofilm on n-alkanes, fatty alcohols, apolar lipids, wax esters and
196 triglycerides. Another isolate *Thalassospira tepidiphila* was initially found in petroleum-contaminated seawater and
197 was reported to degrade polycyclic aromatic hydrocarbons. It also shows degradation of naphthalene, phenanthrene,
198 dibenzothiophene and fluorene (mixed into crude oil) [45]. We have also isolated *Pseudoalteromonas shioyasakiensis*
199 which was reported to show halo-alkali and thermo tolerant properties in addition to chitinase production [46]. Same
200 *spp.* isolated from Indonesian marine areas, shows polyaromatic hydrocarbon degradation activity. In current report,
201 some of the isolates of *Pseudoalteromonas* were seen to possess hydrocarbon degradation property. Few microbes
202 from *Idiomarina* genus were also identified and bacteria from this genus are known for their chitin degradation activity
203 and production of biodiesel from *Jatropha* oil [47]. Thus, microbes isolated from Arabian Sea and Bay of Bengal
204 belong to various genus and species which were reported to have many industrial and biological applications.
205 Therefore these isolates were further assessed for various chemical and enzymatic properties.

206 **3.3. Carbohydrate utilizing ability of bacteria**

207 Carbohydrates are important part of a global carbon cycle. Marine surface water contains up to 21% carbohydrates in
208 the form of dissolved organic carbon. Polysaccharides and monosaccharide are reported to be present at for 3-4 μM
209 concentration in Pacific, Atlantic and Antarctic Ocean [48]. Carbohydrate utilization is a central pathway for energy
210 production in bacteria (Aluwihare and Repeta, 1999; Arnosti, 2000, Zoppini et al, 2010). Marine heterotrophic bacteria
211 contribute carbon cycle either by the remineralisation of organic carbon or by the production of new bacterial biomass.

212 Previous reports have demonstrated the presence of carbohydrate degrader's, belonging to *Bacillus* , *Vibrio*,
213 *Marinobacter*, etc from estuarine sources near Arabian sea [45]. The characteristic ability of microorganisms to utilise
214 different carbon sources is important in the understanding the marine microbial ecology [49]. Among the bacterial
215 isolates, most of the bacteria were able to utilize multiple carbohydrates while only a few bacteria (C1 from AS and
216 A13, A16 and A23 from BOB) were unable utilize any carbohydrate (Online resource 3). Further, amongst Bay of
217 Bengal isolates, citrate was preferentially used followed by malonate (Fig 1). While Arabian Sea isolates were
218 observed to utilize more complex carbohydrates such as esculin and malonate (Online resource 3). This underlines the
219 basic difference between energy production pathways and complexity of the bacterial metabolic abilities between
220 various isolates from two different Indian oceans. Further a few microbes were observed to utilize only one carbon
221 source, namely, A1 (*Halomonas sp. MCCB 340*), A3 (*Halomonas meridiana* strain NIOSSD020#411), A4
222 (*Marinobacter hydrocarbonoclasticus* strain ss36) from BOB utilized citrate as a carbon source (Online resource 3)
223 while G3 (*Pseudoalteromonas shioyasakiensis* strain QY19), K10 (*Pseudoalteromonas sp. Bac186*), M1
224 (*Pseudoalteromonas shioyasakiensis* strain SE3), M2 (*Halomonas sp.*), M4 (*Idiomarina sp. PR53*), M6
225 (*Pseudoalteromonas shioyasakiensis* strain SE3) and T2 (*Halomonas sp. whb34*) could use single carbohydrate for
226 energy production (Online resource 3). Thus, it could be observed that similar type of bacteria isolated from two
227 different location or conditions have entirely different preferences for carbon sources and energy production pathways.

228 **3.4. Enzyme assays**

229 Ocean contains several carbon sources as nutrients. Most of the primary production in marine environments goes into
230 the detrital food which is consumed by heterotrophic microorganisms. The complex macromolecular detritus must be
231 degraded initially into simpler substrates by enzymes which can be utilized by other living organisms. These
232 extracellular enzymes are released into the environment either by secretion or to some extent by cell lysis [50, 51]. In
233 addition to microbial diversity, the types of extracellular enzymes, their magnitude and nature can reflect the probable
234 consumption and diversity of available organic matter in a particular ecosystem. Both aerobic and anaerobic bacteria
235 are known to produce such extracellular enzymes degrade organic matter [52]. Aerobic heterotrophic bacteria often
236 possess degradative pathways for structurally complex organic matter [52–54] and thus can play a significant role in
237 maintenance of nutrient cycling. From this study, we report that in both Bay of Bengal and Arabian Sea amylase,
238 agarase producers and hydrocarbon utilizing micro-organisms are predominant in number. Among the 71 isolates, 42
239 isolates showed amylase activity (Fig 2 Online resource 4) and 17 isolates showed agarase activity (Fig 2, Online
240 resource 4).

241 Further, 6 isolates from BOB (Fig 2, Online resource 4) and 28 from AS (Fig 2, Online resource 4) also showed
242 cellulose utilizing capability (Fig 2). In comparison, microbes with chitinase and xylanase activity were less in number
243 (Fig 2, Online resource 4). Groups of the micro-organisms known as the hydrocarbonoclastic bacteria (HCB) play an
244 important role in the biodegradation and removal of petrochemical pollutants in the global ocean. Oil spill is one of
245 the serious issues among the contaminants in the ocean where the presence and activities of these bacteria has been
246 detected. Oil (hydrocarbons) is spilled in the ocean from the ships. Thus, in addition to bacterial diversity, evaluating

247 the ability of bacteria to utilize various organic substrates is an important aspect to understand the functional marine
248 ecology. Over 175 genera, distributed across several major bacterial classes such as Alpha, Beta, and
249 Gammaproteobacteria, Actinomycetes, Flavobacteria include representatives of HCB species [55]. Out of 71 isolates,
250 32 bacteria showed promising hydrocarbon utilization activity (Fig 2, Online resource 4).

251 In OMZ regions, organisms are known to use nitrate and sulfate as a terminal electron acceptor and as one of the
252 energy sources. 3 isolates from AS demonstrated sulfur reduction ability (Fig 2, Online resource 4) whereas 2 from
253 BOB and 3 from AS showed nitrate reduction activity (Fig 2, Online resource 4). Among all the isolates, very few
254 isolates show multiple enzymatic activities. A1 (*Halomonas* sp. MCCB 340) from Bay of Bengal was hydrocarbon
255 degrading as well as had nitrate and sulfur reducing activity (Fig 2, Online resource 4). Enzymatic activities of each
256 isolates from BOB & AS are summarized (Fig 2, Online resource 4). A recent study from the Arabian sea, also
257 suggested that some bacteria are capable for complete nitrate reduction [56]. Bacteria of from genera such as
258 *Halomonas* and *Pseudoalteromonas* are known to be involved in nitrogen cycle [57, 58]. *Pseudoalteromonas*,
259 *Halomonas* and *Paenibacillus* species are well known for nitrate reduction, with the presences of NirS gene [59–61].
260 In addition, presences of denitrification process, supporting the presences of denitrifier microbial populations in BOB
261 and Arabian sea regions, is recently reported [28, 56]. Also, a metagenomics analysis of OMZ and non-OMZ regions
262 of Bay Bengal suggests the difference in bacterial abundance. Functional analysis revealed the presence of
263 assimilatory Sulphur reducing genes in the non-OMZ areas whereas the dissimilatory sulphate reducing genes were
264 abundant in OMZ regions. Also, our study has revealed the distinct bacterial diversity of Bay of Bengal when
265 compared to OMZ regions of Peru and Chile. This work also reports the presence of bacteria involved in nitrogen
266 metabolism [62]. Therefore, current analysis with support from the previous studies confirms the presences of nitrate
267 reducing cultivable bacteria in BOB and Arabian Sea areas.

268 **3.5. Antibiotic assay**

269 Bacteria acquire antibiotic resistance naturally or through horizontal gene transfer from other organisms and by contact
270 with antimicrobial agents. Many of the isolates were found to resist higher concentration (100µg/mL) of ampicillin,
271 chloramphenicol, kanamycin and streptomycin antibiotics. Ampicillin susceptibility to 100µg/mL concentration was
272 only shown by 9 AS bacteria and 20 BOB bacteria (Fig 3, Online resource 5) and a few were weakly resist 100µg/mL
273 concentration of ampicillin and chloramphenicol respectively (Fig 3, Online resource 5). Susceptibility to 100µg/mL
274 concentration of chloramphenicol was shown by 11 bacterial isolates from AS and 1 bacterial isolate (BOB).
275 Resistance to 100µg/mL kanamycin and streptomycin was detected in all 71 isolates (Fig 3). Therefore, our results
276 revealed that AS and BOB is reservoir of chloramphenicol, kanamycin and streptomycin resistant bacteria and this
277 genetic trait is predominant in these isolates.

278 The OMZ and Non-OMZ areas of the Indian Ocean thus harbor diverse bacteria with different catabolic efficiencies.
279 In conclusion, current study has identified 71 distinct bacteria from AS and BOB water samples which differ in
280 dissolved oxygen concentration. The identified bacteria belong to different genera and species which have varied
281 physical and biochemical properties including carbohydrate utilization, hydrocarbon utilization. The supply of organic

282 material to the OMZ and Non-OMZ water column is an important factor as macro- as well as micro- life forms survive
283 on this organic matter. Therefore, future studies including detailed investigation of currently identified cultivable
284 bacteria and their potential applications and exploration of more bacterial diversity from Indian Ocean is warranted
285 and is likely to provide rich and diverse microbial populations. Also, this study identifies the denitrifying microbial
286 population which will be useful in studying the nitrogen cycle in the Arabian Sea and Bay of Bengal. Overall, the
287 current study provides basic identification of bacterial diversity and their characterization in Indian oceans.

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292 **Author contributions**

293 DDD, SNR and MP designed the experiments; YMP, RB, AMK and PGM carried out the wet lab work; SNR, YMP,
294 RB, carried out bioinformatics and statistical analysis, DDD and SNR interpreted the results and wrote the manuscript.

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301

302 **Conflict of Interest:** The authors declare that they have no conflict of interest.

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481 **Figure Legends**

482 **Figure 1** Several carbohydrate utilizing capacities of bacterial isolates from Arabian sea
483 and Bay of Bengal.

484 **Figure 2** Bacterial isolates capable to produce various substrate degrading enzymes.

485 **Figure 3** Number of bacterial isolates showing resistances to different antibiotics.

486

487 **Table I**

488 A)

Location	Latitude	Longitude	Depth (m)	Oxygen concentration (ml/L)
Calicut	11.377	74.712	1000	0.5559
Calicut	11.621	74.903	189	0.28106
Calicut	11.37	75.128	96	0.51704
Goa	15.755	72.819	1001	0.3527
Goa	15.755	72.819	503	0.04355
Goa	15.707	73.009	205	0.07799
Goa	15.616	73.228	93	1.64047
Kochi	10.176	75.907	94	0.35533
Kochi	10.078	75.634	250	0.35533
Kochi	10.044	75.529	500	0.35533
Mangalore	12.826	74.339	102	0.6854
Mangalore	13.058	74.071	176	0.14161
Mangalore	13.005	74.012	983	0.47906
Mangalore	13.005	74.012	200	0.13913
Trivandrum	8.649	76.662	110	0.38569
Trivandrum	8.724	76.428	196	0.34403
Trivandrum	8.626	76.229	500	0.46625
Trivandrum	8.626	76.229	750	0.46665

489 B)

Location	Latitude	Longitude	Depth (m)	Oxygen concentration (ml/L)
Station1	1956.762N	8652.630E	70	0.55

Station2	1956.762N	8652.630E	70	0.55
Station3	1956.762N	8652.630E	70	0.55
Station4	1956.762N	8652.630E	70	0.55
Station5	1921.658N	8546.100E	510	0.07
Station6	1707.369N	8328.174E	40	0.53
Station7	1707.369N	8328.174E	40	0.53
Station8	1956.762N	8652.630E	320	0.03
Station9	1508.014N	8033.868E	912	0.52
Station10	1508.014N	8033.868E	912	0.52
Station11	1811.692N	8427.742E	468	0.04
Station12	1707.369N	8328.174E	302	0.05
Station13	1707.369N	8328.174E	302	0.05
Station14	1557.937N	8142.164E	359	0.11
Station15	1800.468N	8421.505E	408	0.07
Station16	1800.468N	8421.505E	408	0.07
Station17	1931.112N	8553.380E	550	0.05
Station18	1931.112N	8553.380E	550	0.05
Station19	1508.014N	8033.868E	633	0.09
Station20	1508.014N	8033.868E	633	0.09
Station21	1557.937N	8142.164E	910	0.41
Station22	1947.570N	8649.754E	509	0.05
Station23	1947.570N	8649.754E	509	0.05
Station24	1707.369N	8328.174E	302	0.05
Station25	1707.369N	8328.174E	302	0.05
Station26	1947.570N	8649.754E	20	0.623
Station27	1947.570N	8649.754E	20	0.623
Station28	1800.468N	8421.505E	817	0.28
Station29	1800.468N	8421.505E	817	0.28
Station30	1921.658N	8546.100E	340	0.074
Station31	1921.658N	8546.100E	340	0.074

490

491 **Table I** Details of sampling site and Physico-chemical parameters of samples collected from A)
492 Arabian Sea and B) Bay of Bengal waters.

493

494 **Table II**

495 A)

Bacteria	Identified as	NCBI Accession Number
C1	<i>Marinobacter sp.</i>	MH256039
C2	<i>Halomonas sp.</i>	MH256040
C3	<i>Pseudoalteromonas sp.</i>	MH256041
C4	<i>Halomonas meridiana strain sz31</i>	MH256042
G1	<i>Marinobacter salsuginis K-W5</i>	MH256043
G2	<i>Pseudoalteromonas sp. Bac178</i>	MH217583
G3	<i>Pseudoalteromonas shioyasakiensis strain QY19</i>	MH256044
G4	<i>Halomonas axialensis strain- ACH-L-8</i>	MH256045
G5	<i>Halomonas meridiana</i>	MH256046
G6	<i>Psychrobacter celer strain B_IV_3825</i>	MH256047
G7	<i>Paenibacillus sp.</i>	MH256048
K1	<i>Halomonas denitrificans strain SVD I</i>	MH256049
K3	<i>Pseudoalteromonas sp. Bac186</i>	MH256050
K4	<i>Idiomarina seosinensis</i>	MH256051
K5	<i>Halomonas sp. GX18B9-2</i>	MH256052
K6	<i>Pseudoalteromonas sp. ECSMB51</i>	MH256053
K7	<i>Pseudoalteromonas sp. ECSMB70</i>	MH256054
K8	<i>Pseudoalteromonas sp. ECSMB70</i>	MH256055
K9	<i>Pseudoalteromonas shioyasakiensis strain NIOSSD020#192</i>	MH256056
K10	<i>Pseudoalteromonas sp. Bac186</i>	MH256057
K11	<i>Pseudoalteromonas lipolytica strain chem16</i>	MH256058
M1	<i>Pseudoalteromonas shioyasakiensis strain SE3</i>	MH256059
M2	<i>Halomonas sp.</i>	MH256060
M3	<i>Psychrobacter celer strain K-W15</i>	MH256061
M4	<i>Idiomarina sp. PR53</i>	MH256062
M5	<i>Idiomarina sp. PR53</i>	MH256063
M6	<i>Pseudoalteromonas shioyasakiensis strain SE3</i>	MH256064
P	<i>Psychrobacter</i>	MH256065
T1	<i>Marinobacter sp. H054</i>	MH256066
T2	<i>Halomonas sp. whb34</i>	MH256067
T3	<i>Pseudoalteromonas lipolytica strain chem16</i>	MH256068
T4	<i>Halomonas sp. R-28817</i>	MH256069
T5	<i>Pseudoalteromonas sp.</i>	MH256070
T6	<i>Psychrobacter celer strain 7K1</i>	MH256071
T7	<i>Pseudoalteromonas sp. NA133</i>	MH256072
T8	<i>Psychrobacter celer strain B_IV_3L25</i>	MH256073
T9	<i>Psychrobacter sp. LZB026</i>	MH256074
T10	<i>Halomonas sp. H2-42</i>	MH256075

T11	<i>Pseudoalteromonas sp. NPYQ10A</i>	MH256076
T12	<i>Psychrobacter celer strain 7K1</i>	MH256077

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497 B)

Bacteria	Identified as	NCBI Accession Number
A1	<i>Halomonas sp. MCCB 340</i>	MH256078
A2	<i>Halomonas meridiana strain NIOSSD020#411</i>	MH256079
A3	<i>Halomonas meridiana strain NIOSSD020#411</i>	MH256080
A4	<i>Marinobacter hydrocarbonoclasticus strain ss36</i>	MH256081
A5	<i>Halomonas meridiana strain NIOSSD020#411</i>	MH256082
A7	<i>Halomonas meridiana strain TVG01-C008</i>	MH256083
A8	<i>Marinobacter salsuginis strain Xmb040</i>	MH256084
A9	<i>Halomonas meridiana strain Na6MA-1</i>	MH256085
A12	<i>Marinobacter salsuginis strain Xmb040</i>	MH256086
A13	<i>Marinobacter salsuginis strain Xmb040</i>	MH256087
A14	<i>Halomonas meridiana partial 16S rRNA gene, strain Chem17</i>	MH256088
A16	<i>Marinobacter salsuginis strain Xmb040</i>	MH256089
A17	<i>Marinobacter salsuginis strain Xmb040</i>	MH256090
A18	<i>Marinobacter salsuginis strain Xmb040</i>	MH256091
A20	<i>Halomonas CDJ15-A06</i>	MH256092
A21	<i>Halomonas aquamarina strain NIOSSD020#448</i>	MH256093
A22	<i>Halomonas meridiana strain NIOSSD020#411</i>	MH256094
A23	<i>Marinobacter salsuginis strain K-W5</i>	MH256095
A24	<i>Halomonas sp. MCCB 340</i>	MH256096
A25	<i>Thalassospira tepidiphila strain SMT41 (only R)</i>	MH256097
A27	<i>Marinobacter salsuginis strain NIOSSD020#173</i>	MH256098
A28	<i>Halomonas sp. MCCB 340</i>	MH256099
A29	<i>Halomonas sp. MCCB 340</i>	MH256100
A30	<i>Halomonas sp. B-4211</i>	MH256101
A31	<i>Halomonas meridiana strain NIOSSD020#411</i>	MH256102
A32	<i>Halomonas meridiana strain NIOSSD020#411</i>	MH256103
A33	<i>Halomonas sp. MCCB 340</i>	MH256104
A34	<i>Halomonas sp. B-4211</i>	MH256105
A35	<i>Halomonas meridiana strain NIOSSD020#411</i>	MH256106
A36	<i>Halomonas axialensis strain NIOSSK079#24</i>	MH256107
A37	<i>Halomonas meridiana strain NIOSSD020#411</i>	MH256108

498

499 **Table II** Identified bacteria and corresponding NCBI gene sequence ID for A) Arabian sea B) Bay
500 of Bengal

501 **Supplementary Material Legends**

502 **Supplementary material 1** List of primers used in this study

503 **Supplementary material 2** Colony characterization of AS and BoB isolates

504 **Supplementary material 3** Carbohydrates utilized by AS and BoB isolates

505 **Supplementary material 4** Enzyme activity shown by AS and BoB isolates

506 **Supplementary material 5** Antibiotic resistance shown by AS and BoB isolates

507