

Evaluating the bacterial diversity from the southwest coast of India using fatty acid methyl ester profiles

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Abstract

The fatty acid composition of bacterial isolates remains stable under standardized culture conditions, which makes it a useful taxonomic marker. The present study aims to characterize the diversity and quantity of fatty acid methyl esters (FAME) profiles of cultivable bacterial isolates collected along the southwest coast of India. Based on the similarity indices (range: >0.3 - 0.7) of the FAME profiles, the isolates were aggregated into 10 families, 11 genera and 19 species of cultured isolates. The following classes of bacteria were found: Bacilli, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, and Actinobacteria, which also included a few pathogens such as *Pseudomonas*, *Staphylococcus* and *Bacillus* sp. The hydroxyl FAMEs 2-hydroxydodecanoic acid (C_{12:0} 2OH), 2-hydroxypentadecanoic acid (C_{15:0} 2OH), 3-hydroxy 14-methylpentadecanoic acid (C_{16:0} iso 3OH), 3 hydroxy hexadecenoic acid (C_{16:0} 3OH) and 3-hydroxy 15-methylhexadecanoic acid (C_{17:0} iso 3OH), as well as the unsaturated FAMEs (11Z)-11-hexadecenoic acid (C_{16:1} ω5c), were exclusively associated with the isolates from Mangalore samples. Similarly, FAMEs 2-hydroxydecanoic acid (C_{10:0} 2OH), 9-methyldecanoic acid (C_{11:0} iso), undecanoic acid (C_{11:0}), tridecanoic acid (C_{13:0}), 10-methylhexadecanoic acid (C_{16:0} 10-CH₃) and (7Z)-7-hexadecenoic acid (C_{16:1} ω9c) occurred only in the isolates from Trivandrum samples. However, the isolates from Goa did not possess a signature FAME profile. The reproducibility of the GC-MIDI bacterial identification system was evaluated using 16S rRNA gene sequencing techniques for selected isolates.

Keywords: Bacterial FAME signatures, 16S rRNA, GC-MIDI, bacterial identification

Introduction

The uniqueness of the fatty acids present in a microbial species enables the compounds to be used as chemotaxonomic biomarkers [1]. Microbial acyl chains constitute the hydrophobic part of the cell membrane [2] and are enzymatically synthesized through the type II fatty acid biosynthesis pathway [3]. The plasma membrane of bacterial cells acts as a semipermeable barrier that facilitates the entry of nutrients and metabolites while preventing the undesirable molecules from passing into the cell [4]. Mutation or gain/loss of plasmids does not affect the cellular fatty acid pattern [5], which opens the possibility of creating a huge library of different microbes with which unknown bacteria can be compared and identified [6]. The Sherlock MIDI System employs this method for the identification and characterization of bacteria. The computerized system identifies fatty acids (9:0-20:0) and compares the individual percentages with libraries containing stored databases [1].

The Sherlock System presents the results based on a Similarity Index (SI), which is an expression of how close an unknown fatty acid profile is when compared with the mean fatty acid composition of the strains present in the library. By this comparison, the system enlists the closest match to the unknown strain [6]. The fatty acids are converted to fatty acid methyl esters (FAMES) to overcome the difficulty in analysing highly polar lipids using gas chromatography [7]

Fatty acids with carbon numbers ranging between 6 and 14 are described as medium-chain fatty acids (MCFAs) [8]. In bacterial cells, no significant changes in the cellular fatty acid composition could usually be detected within a short period as it is highly conserved [5]. The fatty acid composition of a strain is stable under highly standardized culture conditions. Over 300 fatty acids have so far been identified in cultivable bacteria. The differences in the position of double bonds, chain length and the binding of functional groups among these fatty acids have allowed them to be utilized as taxonomic markers [9,6]. The use of FAME signatures to identify human [10-13], fish [14], plant [15] and other animal [16] pathogens has been widely reported. However, it should be noted that a single bacterial species may exhibit differences in the fatty acid composition depending on the environment to which it is exposed [17], since it is an adaptive mechanism that helps the bacteria to survive in the changing environment. Thus, it is important to maintain uniform growth conditions while attempting to taxonomically classify bacteria based on their membrane lipid composition. This study aims to decode the diversity and quantity of medium-chain fatty acids in bacteria isolated from different transects along the southwest coast of India. We also strive to

ascertain the presence of any signature FAME profiles that are unique to the oxic and anoxic layers.

Materials and methods

Environmental sampling and parameters

Sampling was carried out on the research vessel Sindhu Sankalp (cruise no. SSK113) in January 2018. Seawater samples were collected from three different locations along the southwest coast of India, from the stations: Goa (Lat. 15°22.99'N, Long. 72°46.81'E), Mangalore (Lat. 12°50.79'N, Long. 73°56.80' E) and Trivandrum (Lat. 08°34.00' N, Long. 76°10.32' E) (Fig. 1). Niskin sampler bottles (12 L each) attached to a CTD sampler (Sea-Bird Scientific, 2016: conductivity-temperature-depth sensor) were used for this purpose. The sampling zone extended from the water surface to a depth of 600 m, which included the chlorophyll maxima layer, in all the three stations. Environmental parameters viz., conductivity, depth, temperature, dissolved oxygen, turbidity, and fluorescence were measured using sensors mounted on the CTD devise (Sea-Bird Scientific, USA).

Isolation and enumeration of bacteria

One-hundred microlitres of each sample were pipetted and directly inoculated on nutrient agar plates (HiMedia, India). The spread plate method was adopted, and the inoculated plates were incubated at 28°C for a maximum of 48 hours. Colonies with unique morphologies were chosen for sub-culturing. Each strain was further reviewed on trypticase soy base agar (TSBA), a medium suggested for the cultivation of aerobes for FAME MIDI analysis, as per the manufacturer's protocol (MIS Operating Manual, September 2012)

Fatty acid extraction and FAME analysis

A loopful of bacterial cells, weighing approximately 40 mg, was harvested from the 3rd quadrant (as these cells are in the rapid growth stage and are hence best suited for FAME extraction and quantification [18]) using a 4mm inoculation loop and transferred to the bottom of a clean glass tube with Teflon screw caps. The FAMES were prepared and analysed from whole-cell fatty acids of bacterial strains according to the methods detailed in the MIS operating manual (Sherlock Microbial Identification System version 6.2, MIDI, Inc., Newark, DE, USA). Briefly, the fatty acids released by the process of saponification using NaOH were esterified with 6N HCl. The FAMES were extracted using 1:1 (v/v) solution of methyl-tert-

butyl ether and hexane, which led to the separation into an upper organic phase and a lower aqueous phase. The latter was pipetted out and discarded. This step was followed by a base wash with NaOH, and the upper phase (two-thirds volume) was transferred into a GC vial (2 ml) and sealed (with Teflon-lined cap). The gas chromatograph (7890A; Hewlett Packard, Rolling Meadows, IL) containing a fused silica column and a flame ionization detector was used to quantify FAMES. Based on the equivalent chain lengths, individual FAMES were recognized using Sherlock's pattern recognition software (Sherlock Microbial Identification System version 6.2 Microbial ID, Inc, Newark, DE, USA). Prior to each batch of analysis and after every 10 samples, calibration standards were analysed. The generalized approach for naming FAMES based on their structural properties can be found elsewhere [18,19,6,1].

DNA extraction, amplification, 16S rRNA sequencing, and phylogeny

Eight bacterial isolates were selected from three transects for cross-validating the FAME bacterial identification system with molecular identification through 16S rRNA gene sequencing. DNA was isolated following the procedure of the DNAeasy Power Lyzer Microbial Kit (Qiagen, India). The extracted DNA was stored at -20°C until further analysis. PCR amplification was performed with a total volume of 25µl (Nexus Mastercycler, Eppendorf, USA). Takara GT PCR Master Mix (Takara Bio, Japan) was used to amplify the DNA. The reaction mixture consisted of 12.5 µl of PCR Master Mix, 1 µl each of forward and reverse primers, 1 µl of template DNA and 9.5 µl of distilled water. The primer pair (27F 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R 5'-GGTTACCTTGTTACGACTT-3') was used for 16S rRNA gene amplification, and the PCR conditions specified by Miller et al. [20] were adopted. The PCR products were then visualized on 1.5% agarose and sequenced using Genetic Analyser (Applied Biosystem, 3130XL, USA). The forward and reverse sequences were manually checked using Bioedit and prepared the contig files for respective bacterial isolates [21]. The prepared contigs were used as query sequences in NCBI BLAST. The five most similar sequences were selected from BLAST hits and aligned using Clustal W and the phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis (MEGA) ver. 7 [22-25].

Statistical analysis

The FAME profiles of the 51 bacterial morphotypes were determined from the relative masses of individual FAMES created for each isolate by adopting the method used by Duran et al. [26].

Results and discussion

Comparison of bacterial diversity from different stations using FAME profiles

From the three transects, only 51 isolates were selected for FAME analysis. An initial screening was performed through observation and only morphologically different colonies were picked. However, not all of them were able to grow on TSBA, which resulted in the final selection of 51 isolates. The relative mass ranges and their prevalence (ratio of the isolates containing the FAME to the total number of isolates within the station) were determined to find whether the FAME signatures were transect-specific (Table 1). Since the relative mass, of the FAME C16:0, was unity after normalization of the area responses, it was not included in the tabulation [26].

Even though the sampled locations were relatively close, unique FAME profiles could be observed among the three stations. Mangalore and Trivandrum isolates had a unique FAME signature, whereas those from Goa did not have any signature FAME profile (Table 1). The hydroxyl FAMES 12:0 2OH, 15:0 2OH, 16:0 iso 3OH, 16:0 3OH, 17:0 iso 3OH, as well as the unsaturated FAMES 16:1 w5c, were found only within the isolates from Mangalore samples, among which, 12:0 2OH was most prevalent (47.4%), while 17:0 iso 3OH and 18:1 w5c were least prevalent (10.5%). Similarly, FAMES 10:0 2OH, 11:0 iso, 11:0, 13:0, 16:0 10-methyl and 16:1 w9c was detected only in the isolates from the Trivandrum samples, among which 11:0 was most prevalent (29.4%), while 10:0 2OH and 11:0 iso were least prevalent (5.9%). Nonetheless, the Goa isolates did not contain unique FAME profiles unlike those from the rest of the stations. For instance, FAMES 16:1 w7c alcohol, 16:1 w11c and 12:1 3OH were equally prevalent in Mangalore and Trivandrum stations but were absent in the Goa isolates. According to Haack et al. [27], 16:1 w7c alcohol and 16:1 w11c are characteristic of *Bacillus sp.* In our study too, *Bacillus sp.* was found to be more prevalent in Mangalore and Trivandrum than in Goa. However, for one Goa isolate identified as *Bacillus alcalophilus*, the SIM index was rather low (0.17) when compared with the *Bacillus sp.* isolated from Mangalore and Trivandrum. This may be attributed to the presence of certain FAMES exclusively in the isolates from the latter two stations and their complete absence in the Goa isolates.

Taxonomy of culturable isolates

Sherlock MIDI assigns species nomenclature by quantitatively assessing the FAME profiles apart from detecting the presence/absence scenarios. For a strong species diagnosis, an SI

value of 0.7 or above was considered valid. Among the SIM indices allotted to the samples, only 13.72% (7 nos.) could be identified to the species level (Table 2), demonstrating the prevalence of uncommon bacterial isolates in the coastal waters. Among the identified isolates, five belonged to *Pseudomonas aeruginosa* GC-subgroup A and the remaining fell under *Pseudomonas putida*-biotype A and *Staphylococcus epidermidis*-GC subgroup. Relaxing the 0.7 SI to 0.3 allowed the aggregation of the FAME profiles into 10 families, 11 genera and 19 species of cultured isolates. Upon comparison with literature, it was found that most of the isolates could be classified as either human pathogens [28-40] or environmental samples [41-45] (Table S2). Owing to the presence of isolates belonging to the class of human pathogens, we validated our findings with 16S rRNA identification and the results were congruent.

All sampled stations had both oxic and anoxic layers of waters whose environmental parameters have been listed in Table 3. Dissolved oxygen (DO) content of the oxic layer varied from 3.54 to 6.05 mg/L over the depth of 5-100 m, and in the anoxic layers, the DO varied from 0 to 0.64 mg/L (100-600m) (Table 3). There was a total of 21 and 30 bacterial isolates from the oxic and the anoxic layers, respectively. The research to ascertain the influence of DO on the profiles did not yield any FAME signatures that were unique to oxic or anoxic layers. The current study also helped in profiling the FAME signatures of *Pseudomonas aeruginosa* GC-subgroup A isolated from the oxic (M6) and anoxic layers (M7, M8, M9 and M10) of Mangalore station (Table S1); however, no unique FAME signatures were found based on the oxygen availability in the water column. This observation highlights the fact that the FAME profiles of bacteria chiefly depend on the nutrient media in which they are grown and DO has no significant role in it.

16S rRNA gene sequencing identified the bacterial isolates G4, T14, and M13 as *Staphylococcus* sp., G9, G10 and T11 as *Pseudomonas* sp. and T17, T8, T3 and T1 as *Bacillus* sp. The phylogram built using the sequences aided in grouping the species from the present study with those of the GenBank reference sequences in their respective clades (Fig. 2). The taxonomy of the GC-MIDI system and GenBank BLAST analysis was found to be congruent. Although the SIM indices were with a comparatively low (0.4 and 0.58 for G9 and T1, respectively), the bacterial identification through GC-MIDI was accurate up to the species level those isolates. And whereas for the other six bacterial isolates, the results of the MIDI and 16SrRNA identification were matched up to the genus level. These results suggest that employing the FAME profiles for bacterial identification is more reliable up to the genus

level when compared with the species-level identification for any preliminary evaluation of coastal bacterial diversity.

Even though the SI value of the strain G1 was only 0.4 and hence could not be regarded as an appropriate match according to the standard MIDI protocol (MIS Operating Manual September 2012), it is worth noting that the 16S rRNA data also yielded identical results up to the species level. This result validates the efficiency of GC-MIDI, a tool that has been widely employed in recent studies [46-55]. Moreover, Fykse et al. [56] had carried out a similar study to establish the congruency of GC-MIDI and 16S rRNA data, and it was observed that 70% of the isolates demonstrated identical results in both methods.

The identification of a huge number of environmental bacterial isolates directly through 16S rRNA sequencing as part of preliminary screening is not a cost-effective method. Therefore, GC-MIDI analysis of fatty acids comes handy for initial screening, and it narrows down the number of samples to be later subjected to 16S rRNA sequencing. The results of this investigation prove that the FAME method of bacterial identification is both simple and economical [57]. Based on the MIDI software SI values in the range of 0.7-0.9 can be considered for a valid genus-level identification and those below 0.7 need to be further confirmed using 16S rRNA analysis for environmental monitoring. The Sherlock System has been described by previous studies to be a sensitive and cost-effective tool for the preliminary screening of some bacterial species as well as for substantiating the molecular identification performed using 16S rRNA approaches. Nonetheless, the method has its own limitations for accurate, high-confidence identification of environmental bacteria, which invariably can be achieved only through high-throughput sequencing.

Conclusions

The FAME profiles of 51 cultivable marine bacterial isolates from three different transects along the southwest coast of India exhibited qualitative and quantitative transect-specific differences under the study conditions. However, the parameters influencing such variations remain largely unknown. It was further noticed that most of the isolates are human pathogens, and the route through which they enter the deep-sea waters is yet to be investigated. More research is needed to determine the mechanisms responsible for the transect specificity and to assess the temporal and spatial FAME profiles.

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TABLES

Table 1: Relative mass ranges and relative occurrences of FAMEs within each transect sampled

Table 2: Species naming attempted by Sherlock-MIDI based on the FAME profiling

Table 3: Physicochemical parameters of the sampling locations

Figure Legends

Fig. 1: Sampling stations along the coastal waters of southwest India

Fig. 2: The evolutionary history was inferred by using the Maximum Likelihood method and the Tamura 3-parameter model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All other sequences (denoted by accession numbers followed by species name) were obtained from GenBank, including the 18S rRNA sequence of *Candida ooitensis*, which was used as the outgroup.

Table 1: Relative mass range and relative percentage of FAMES within each transect sampled

FAME	GOA (15) ^a Range (relative percentage)	MANGALORE (19) Range (relative percentage)	TRIVANDRUM (17) Range (relative percentage)
10:0	0-0.03 (26.7) ^b	0-0.04 (52.6)	0-0.66 (35.3)
10:0 2OH	0	0	0-0.15 (5.9)
10:0 3OH	0-0.11 (60)	0-0.23 (57.9)	0-0.08 (11.8)
11:0 iso	0	0	0-0.45 (5.9)
11:0	0	0	0-0.35 (29.4)
12:0	0-0.3 (80)	0-0.35 (68.4)	0-0.73 (58.8)
12:0 2OH	0	0-0.23 (47.4)	0
12:1 3OH	0	0-0.6 (42.1)	0-0.24 (5.9)
12:0 3OH	0-0.1 (66.7)	0-0.23 (42.1)	0-0.26 (17.6)
13:0 iso	0-0.1 (53.3)	0-0.11 (31.6)	0-0.22 (76.5)
13:0 anteiso	0-0.04 (26.7)	0-0.1 (10.5)	0-0.06 (17.6)
13:0	0	0	0-0.38 (23.5)
14:0 iso	0-0.99 (33.3)	0-0.31 (31.6)	0-2.33 (76.5)
14:0 3OH/16:1 iso I	0-0.1 (13.3)	0-0.18 (5.3)	0-0.08 (11.8)
14:0 2OH	0	0-0.49 (26.3)	0-0.17 (5.9)
14:0	0-0.42 (100)	0.02-0.26 (100)	0-0.82 (94.1)
15:0 iso	0-4.03 (66.7)	0-12.3 (52.6)	0-19.63 (94.1)
15:0 iso 3OH	0	0-9.47 (21.1)	0-0.08 (5.9)
15:0 anteiso	0-7.32 (40)	0-15.17 (52.6)	0-47.54 (76.5)
15:0 2OH	0	0-0.02 (31.6)	0
15:0	0-0.13 (86.7)	0-0.25 (89.5)	0-0.33 (58.8)
16:0 iso	0-1.36 (33.3)	0-2.8 (52.6)	0-4.64 (76.5)
16:0 iso 3OH	0	0-0.03 (15.8)	0
16:0 3OH	0	0-0.12 (10.5)	0
16:0 2OH	0-0.08 (6.7)	0-0.12 (15.8)	0
16:0 10-methyl	0	0	0-0.03 (11.8)
16:1 w7c alcohol	0	0-0.52 (15.8)	0-1.38 (35.3)
16:1 2OH	0-0.08 (13.3)	0-0.1 (10.5)	0-0.18 (5.9)
16:1 w5c	0	0-0.13 (31.6)	0
16:1 w7c/16:1 w6c	0-1.13 (86.7)	0-0.05 (94.7)	0-1.13 (58.8)
16:1 w9c	0	0	0-0.19 (11.8)
16:1 w11c	0	0-0.15 (15.8)	0-0.43 (35.3)
17:0 iso	0-1.21 (80)	0-2.73 (52.6)	0-6.9 (94.1)
17:0 iso 3OH	0	0-0.14 (10.5)	0
17:0 anteiso	0-1.6 (33.3)	0-5.15 (52.6)	0-21.98 (76.5)
17:0 cyclo	0-0.5 (66.7)	0-0.21 (52.6)	0-0.17 (23.5)
17:0	0-0.13 (53.3)	0-0.1 (42.1)	0-0.41 (41.2)
17:1 anteiso w9c	0-0.04 (13.3)	0	0-0.11 (23.5)
17:1 iso w9c	0	0-0.08 (15.8)	0
17:1 iso w10c	0	0-0.42 (21.1)	0-0.59 (11.8)
17:1 w7c	0-0.05 (26.7)	0-1.7 (31.6)	0

17:1 w6c	0	0-0.11 (15.8)	0
17:1 w8c	0.01-0.08 (26.7)	0-0.03 (36.8)	0-0.15 (17.6)
17:1 iso I/anteiso B	0	0- 0.27 (15.8)	0-0.49 (11.8)
17:0 2OH	0	0-0.03 (10.5)	0
18:0 iso	0-0.42 (26.7)	0-0.94 (10.5)	0-1.77 (35.3)
18:3 w6c (6,9,12)	0-0.07 (13.3)	0-0.03 (10.5)	0
18:1 w7c	0.03-1.96 (93.3)	0-4.36 (89.5)	0-1.5 (52.9)
18:1 w7c 11-methyl	0-0.43 (40)	0-0.57 (21.1)	0-0.12 (11.8)
18:1 w5c	0	0-0.13 (21.1)	0
18:1 w9c	0-0.17 (33.3)	0-0.11 (26.3)	0-2.03 (35.3)
18:2 w6 9c/18:0 ante	0-0.02 (13.3)	0-0.06 (10.5)	0
18:0	0.02-3.19 (100)	0.02-3.83 (100)	0.03-6.38 (100)
19:0 iso	0-0.51 (46.7)	0-0.97 (10.5)	0-6.91 (35.3)
19:0 anteiso	0-0.74 (26.7)	0-1.66 (10.5)	0-12.01 (41.2)
19:0 cyclo w8c	0-0.36 (60)	0-0.06 (73.7)	0-0.11 (17.6)
19:0	0-0.27 (26.7)	0-0.27 (5.3)	0-0.65 (35.3)
20:0 iso	0-0.22 (20)	0-0.23 (5.3)	0-0.56 (17.6)
20:0	0-5.22 (33.3)	0-4.98 (15.8)	0-11.01 (41.2)

^aNumber of isolates analysed from the transect

^bPercentage isolates carrying that particular FAME (not indicated)

Values in bold case, are unique fatty acids which are absent in only one station or two stations

Table 2: Species naming attempted by Sherlock-MIDI based on FAME profiling

Stations	Isolates ^a	Peak named (%)	Similarity index ^b	Suggested species name
GOA	G1	95.9		NO MATCH ^c
	G2	94.6	0.38	<i>Streptococcus mitis</i>
	G3	99.3		NO MATCH
	G4	99.4	0.65	<i>Staphylococcus epidermidis</i> -GC subgroup B
	G5	96.9	0.61	<i>Brevundimonas vesicularis</i>
	G6	99.2	0.17	<i>Bacillus alcalophilus</i>
	G7	96.1	0.27	<i>Variovorax paradoxus</i> -GC subgroup B
	G8	86.7		NO MATCH
	G9	99.3	0.40	<i>Pseudomonas stutzeri</i>
	G10	99.4	0.61	<i>Pseudomonas stutzeri</i>
	G11	98.1	0.55	<i>Escherichia coli</i> -GC subgroup D
	G12	97.2	0.51	<i>Escherichia coli</i> -GC subgroup D
	G13	99.3	0.34	<i>Pseudomonas stutzeri</i>
	G14	99.3	0.57	<i>Staphylococcus epidermidis</i> -GC subgroup B
	G15	99.7	0.56	<i>Cellulosimicrobium cellulans</i> -GC subgroup B
MANGALORE	M1	99.5	0.12	<i>Aquaspirillum polymorphum</i>
	M2	99.6	0.46	<i>Microbacterium imperial</i>
	M3	99.2	0.43	<i>Staphylococcus schleiferi</i>
	M4	99.6	0.50	<i>Virgibacillus pantothenicus</i>
	M5	98.8	0.25	<i>Photorhabdus luminescens</i> -luminescens
	M6	99.0	0.89	<i>Pseudomonas aeruginosa</i> -GC subgroup A
	M7	99.2	0.92	<i>Pseudomonas aeruginosa</i> -GC subgroup A
	M8	99.3	0.87	<i>Pseudomonas aeruginosa</i> -GC subgroup A
	M9	99.6	0.92	<i>Pseudomonas aeruginosa</i> -GC subgroup A
	M10	99.0	0.95	<i>Pseudomonas aeruginosa</i> -GC subgroup A
	M11	97.0	0.76	<i>Pseudomonas putida</i> -biotype A
	M12	99.5	0.39	<i>Sphingobium herbicidovorans</i>
	M13	99.8	0.49	<i>Staphylococcus schleiferi</i>
	M14	99.7	0.58	<i>Staphylococcus hominis</i> -hominis
	M15	94.9	0.45	<i>Bacillus clausii</i>
	M16	97.6	0.55	<i>Pseudomonas aeruginosa</i> -GC subgroup A
	M17	99.4	0.39	<i>Pseudomonas stutzeri</i>

	M18	99.8	0.13	<i>Pseudomonas aeruginosa</i> -GC subgroup A
	M19	99.8	0.14	<i>Brevundimonas vesicularis</i>
TRIVANDRUM	T1	85.4	0.58	<i>Bacillus megaterium</i> -GC subgroup A
	T2	99.7	0.40	<i>Staphylococcus schleiferi</i>
	T3	99.5	0.74	<i>Staphylococcus epidermidis</i> -GC subgroup B
	T4	98.4	0.39	<i>Staphylococcus schleiferi</i>
	T5	96.1	0.55	<i>Staphylococcus hominis-hominis</i>
	T6	99.9	0.47	<i>Bacillus clausii</i>
	T7	92.1	0.18	<i>Bacillus alcalophilus</i>
	T8	99.4	0.52	<i>Marinobacterhydrocarbonoclasticus</i>
	T9	99.2	0.48	<i>Marinobacterhydrocarbonoclasticus</i>
	T10	95.3	0.15	<i>Bacillus</i> -GC group 22
	T11	98.0	0.55	<i>Pseudomonas balearica</i>
	T12	98.3		NO MATCH
	T13	99.8	0.34	<i>Bacillus</i> -GC group 22
	T14	99.8	0.48	<i>Staphylococcus hominis-hominis</i>
	T15	100.0	0.63	<i>Staphylococcus hominis-hominis</i>
T16	96.2		NO MATCH	
T17	95.8	0.50	<i>Bacillus alcalophilus</i>	

^aG, M and T represents the cultivable bacterial isolates retrieved from Goa, Mangalore and Trivandrum, respectively and number indicates the isolate number.

^b The Similarity Index is a numerical value, which expresses how closely the fatty acid composition of an unknown compares with the mean fatty acid composition of the strains used to create the Sherlock MIDI library entry listed as its match.

^c NO MATCH indicates low value of similarity index which do not find any matching with the reference library.

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Table 3: Physicochemical parameters of sampling locations

Environmental parameters/ sampling stations	Oxic sampling parameters			Anoxic sampling parameters		
	Goa (Samples G1 to G6)	Mangalore (Samples M1 to M6)	Trivandrum (Samples T1 to T9)	Goa (Samples G7 to G15)	Mangalore (Samples M7 to M19)	Trivandrum (Samples T10 to T17)
Dissolved Oxygen (mg.L ⁻¹)	5.90 ± 0.0	5.41 ± 0.62	5.56 ± 0.44	0.06 ± 0.01	0.08 ± 0.05	0.56 ± 0.08
Depth (m)	5-54	5-100	5-100	200-590	200-600	200-561
Temperature (°C)	27.91 ± 0.03	28.2 ± 0.54	28.43 ± 0.29	12.58 ± 1.21	10.23 ± 1.37	11.84 ± 1.27
Salinity (psu)	35.78 ± 0.04	36.22 ± 0.38	34.74 ± 1.06	35.44 ± 0.04	35.28 ± 0.06	35.16 ± 0.02
Density (mg.L ⁻¹)	1023.13 ± 0.09	1023.43 ± 0.47	1022.28 ± 0.83	1028.59 ± 0.75	1026.44 ± 0.30	1028.45 ± 0.73

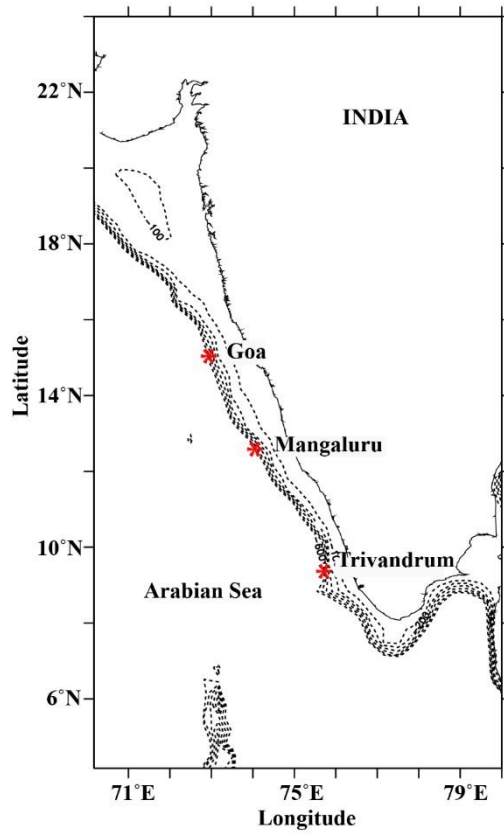


Fig. 1

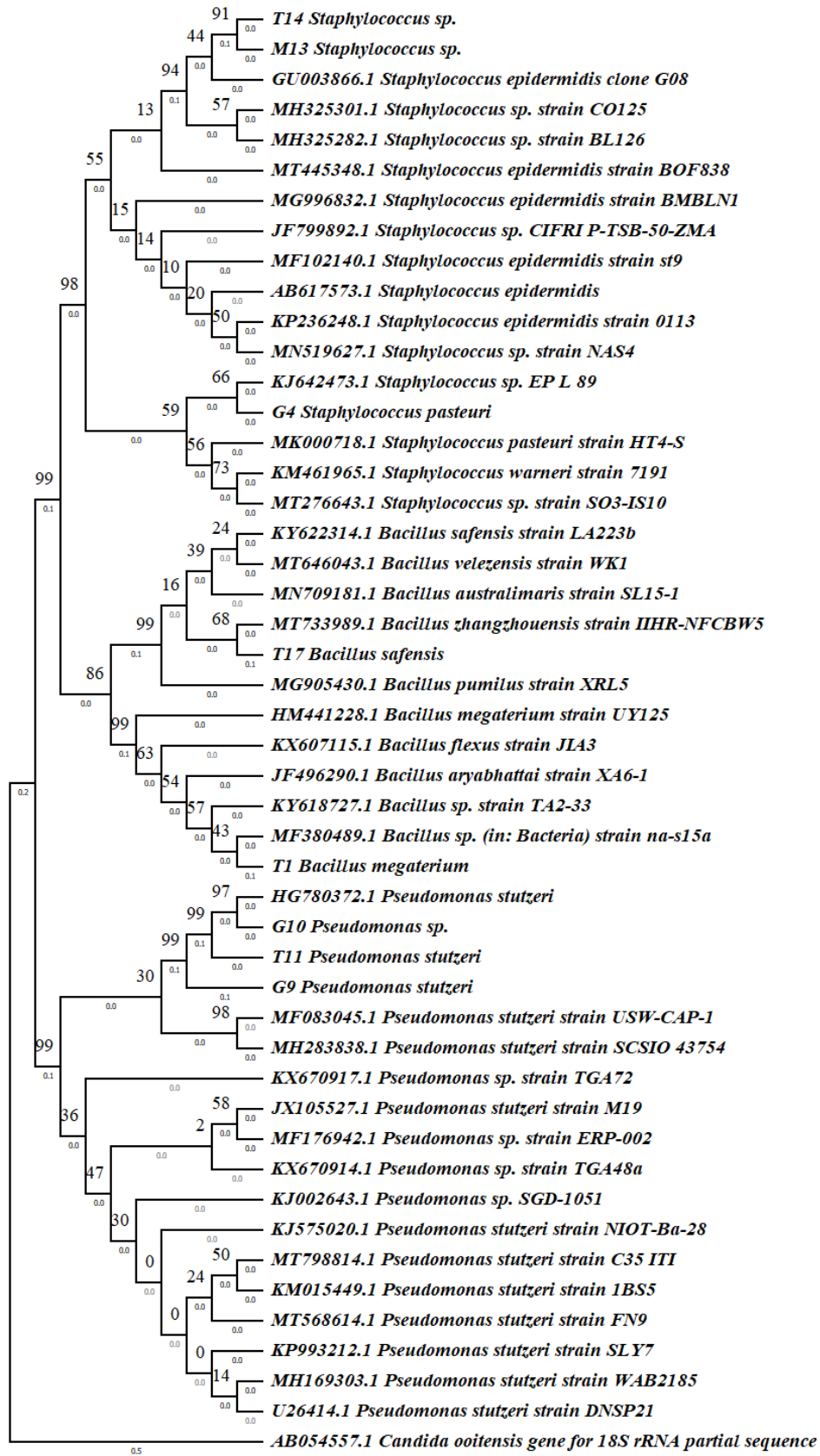


Fig. 2