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Iron Bacterial Phylogeny and their Execution Towards Iron Availability in Equatorial Indian Ocean and Coastal Arabian Sea

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Abstract

Based on distinct colony morphology, color, size, shape and certain other traits, 92 bacterial isolates were investigated to understand their managerial ability on iron from the Arabian Sea and Equatorial Indian Ocean samples. The ARDRA (amplified rDNA restriction analysis) applied to eliminate the duplication of the bacterial strains, resulted 39 different banding patterns. The 16S rRNA gene sequencing data indicate the dominancy of three phylogenetic groups, α-Proteobacteria (10.25%), γ-Proteobacteria (35.89%) and Bacilli (53.84%) in these waters. *Marinobacter* and *Bacillus* were the only common genera from both of the regions. *Pseudoalteromonas*, *Halomonas*, *Rheinheimera, Staphylococcus* and *Idiomarina* were some of the other genera obtained from the Arabian Sea. *Erythrobacter*, *Roseovarius*, *Sagittula* and *Nitratireductor* were found mostly in Equatorial Indian Ocean. In addition, 16S rRNA gene sequence data of some of our iron bacterial strains belong to novel species and one isolate ASS2A could form a new genus. Close to 23% of the isolates were able to produce high affinity sets of ligands like siderophores to mediate iron transport into the cell. The current study indicated that the Equatorial Indian Ocean species were well adapted to oxidize iron as an electron acceptor and the Arabian Sea species preferably go through siderophore production.

Key words: Iron bacteria, 16S rRNA gene, Equatorial Indian Ocean, Arabian Sea, Siderophores

Introduction

Iron (Fe) is the fourth most abundant element in the Earth's crust, and the second most abundant element that is redox-active in near-surface aqueous habitats, providing it the most vital metal in the environment (Cornell and Schwertmann, 1996; Edwards *et al*., 2004). The largest source of iron in the oceans is probably the atmosphere and this comes mainly from the wind erosion of soils to form dust. Iron is involved in many key biological processes like photosynthesis, N_2 fixation, methanogenesis, H_2 production and respiration, oxygen transport, gene regulation and DNA biosynthesis (Andrews *et al*., 2003). Microorganisms are involved in the precipitation and solubilization of iron by growing them at the expense of energy gained from the oxidation of reduced iron or by reduction of ferric ions (Cameron *et al*., 1984). Iron-oxidizing bacteria (FeOB) have been noticed in a wide range of environments and exposed to increase the rate of Fe oxidation by up to four orders of magnitude compared with the rate of strictly abiotic oxidation (Sogaard *et al*., 2001). FeOB are important catalysts of Fe cycling; on the other hand little is known about their diversity and distribution in various environments (Wang *et al*., 2009). Secretion of siderophores, the iron solubilizing material produced by the marine bacteria to facilitate the iron uptake into microbial cells has been reported by Trick (1989) and Okujo *et al.* (1994). Marine bacteria hold more iron per biomass than phytoplankton, the major primary producers (Tortell *et al*., 1996). For instance the bacterium *Thiobacillus ferrooxidans* gains energy from ferrous ion oxidation, but a wide variety of bacteria may deposit ferric iron without necessarily obtaining energy from the process.

Most of the iron in the seawater is present in the oxidation state Fe(III) which in alkaline conditions of sea should readily form insoluble iron oxides (Turner and Hunter, 2001). Although heterotrophic bacteria require up to one micro molar iron for growth, the total amount of iron in surface ocean water is sub nano molar. Dissolved iron level in open ocean water is about 20 pmol – 1 nmol/l (Wu and Luther, 1994), which is much lower than the concentration required for most of the microorganisms for their growth. This limiting amount of iron has implications in the biogeochemical cycling of

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carbon and in limiting phytoplankton growth. Marine bacteria can successfully compete for this limited nutrient using a specialized iron transport system, including the production and release of siderophores. Chemically siderophores are low molecular weight metabolites with masses of < 2000 Da that have high affinity for ferric ion (Schalk *et al.*, 2011). The role of these compounds is to scavenge iron from the environment and to make the mineral which is almost always essential, available to the microbial cell (Reid and Butler 1991; D'Onofrio *et al.*, 2010). In this study we planned to investigate the culturable fraction of iron bacterial diversity from the Arabian Sea (AS) and Equatorial Indian Ocean (EIO) which are of having moderate and low iron concentrations to compare and to understand how these two different environments fulfill the iron requisite of the bacteria present over.

Experimental

Materials and Methods

Sampling details. EIO Sampling was carried out during the cruise track of Boris Petrov ABP#37 from June 09 to July 10, 2009; stations were occupied at one degree intervals across the equator along 83°E from 1°N to 5°S. Fifty five sediment samples and 98 water samples were collected and analyzed. AS water and sediment samples were collected at different locations of Off Goa during the cruise by coastal research vessel Sagar Sukti (SASU#185, August 2009) along 15°N between 72 and 73°E.

Isolation of iron bacteria. Iron Bacteria were isolated using the media M622- HiMedia, Mumbai (g/l: Glucose 0.15, Ammonium sulphate 0.5, Calcium nitrate 0.01, Dipotassium phosphate 0.05, Magnesium sulphate 0.05, Potassium chloride 0.05, Calcium carbonate 0.1, Vitamin B12 0.00001, Thiamine 0.0004, Agar 10.0). Appropriately diluted samples were spread plated on Iron bacterial isolation medium prepared in half strength seawater and incubated for 3–5 days at 30°C. Bacterial colonies appeared on the media plates were isolated and purified for further analyses.

Genomic DNA extraction and PCR amplification of 16S rRNA gene. The genomic DNA was extracted from all the isolates using DNeasy kit (69506, Qiagen) according to the manufacturer's protocol and 16S rRNA genes of the bacterial isolates were amplified using 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') universal forward and reverse 16S rDNA primers (Lane, 1991). The PCR reaction mixture contained 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTP's, 1 U of Taq DNA polymerase (Genei, Bangalore), 10 pmol of each forward and reverse oligonucleotide primers and approximately 25–50 ng of genomic DNA. The amplification profile consisted of an initial denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 1 min. This was followed by a final extension step of 72°C for 5 min. The samples were held at 4°C until further analyses were done.

Amplified rDNA restriction analysis (ARDRA). The ARDRA technique was carried out to identify the variations in the banding patterns among the bacterial groups. 16S rRNA gene was digested using the four base-cutting restriction enzyme *Alu* I with appropriate buffers (Fermentas) according to the manufacturer's protocol. The 20 µl of the above reaction mixture was incubated at 37°C for 3 h. Restriction fragments were separated using 2.5% TAE-agarose gel at 60 V for 2 h and viewed on Alphaimager-2200 gel documentation system (Alpha Innotech, USA).

16S rRNA gene sequencing and phylogenetic analysis. DNA sequencing for these samples were performed in 3130 xl genetic analyzer (Applied Biosystems, USA) at National Institute of Oceanography. Nucleotide sequences thus obtained were assembled using DNA-Baser V.3 software. The PINTAIL program (Ashelford *et al.*, 2005) was used to check chimera formations in our sequences. The nucleotide sequences of the isolates obtained were compared with the sequences available in GenBank database using BLAST software (www.ncbi.nlm.nih.gov) and 16S rDNA sequence similarity of ≥ 97% were considered as a phylotype. Further sequence alignment and comparison was performed using multiple sequence alignment program Clustal X 1.81 (Thompson *et al.*, 1997). Neighbor-joining method was employed to construct the Phylogenetic tree using MEGA4 software (Tamura *et al.*, 2007) and the maximum likelihood method was adopted for calculating the evolutionary distance (Tamura *et al.*, 2004). The topology of the phylogenetic tree was evaluated by bootstrap analysis with 1,000 replications. The sequences were submitted to NCBI-GenBank and accession numbers were assigned from JQ905060 to JQ905098.

Screening of siderophore producing bacteria. To screen the low molecular-weight Fe(III) specific ligands (siderophores), Chrome azurol sulphonate (CAS) plate method was used (Schwyn and Neilands, 1987) and the media contains (g/L) CAS – 0.726; Hexadecyltrimethylammonium bromide (HDTMA) – 0.1456; Iron solution (1 mM $FeCl_3$, 10 mM HCl) – 2 mL; Peptone – 5.0; Yeast extract – 1.0 and Agar – 20.0 prepared in 50% seawater. The bacterial cultures were spot inoculated in the medium and incubated for 24–72 h. Appearance of orange/yellow zone around the colony indicated siderophore production.

Characterization of siderophores. Cell free culture supernatants were examined for various types of siderophores *i.e.*, hydroxamate nature by $FeCl_3$ and tetrazo-

lium tests (Neilands, 1981; Snow, 1954), catecholates by Arnow's test (Arnow, 1937) and carboxylate siderophores by spectrophotometric test (Shenker *et al.*, 1992).

Quantification of siderophores. Quantitative estimation of siderophores was done by CAS-shuttle assay (Payne, 1994). In which 0.5 mL of culture supernatant was mixed with 0.5 ml of CAS reagent, and absorbance was measured at 630 nm against a reference consisting of 0.5 ml of uninoculated broth and 0.5 ml of CAS reagent. Siderophore content in the aliquot were calculated by using the formula: % siderophore units = (Ar-As)/Ar (Where, Ar = absorbance of reference at 630 nm (CAS reagent) and As = absorbance of sample at 630 nm)

UV-Visible spectrophotometer scanning. Bacterial cultures were grown in TYES medium (g/l: Tryptone 4.0, Yeast Extract 0.04, $CaCl_2 . 2H_2O$ 0.2, $MgSO_4 . 7H_2O$ 0.5, Glucose 0.05) for 6 days at 30°C. The cells were removed by centrifugation at 6000 × g for 15 min. The pH of the supernatant were adjusted to 2.0 with 12N HCl and extracted with 0.4 volume of ethyl acetate using separating funnel. The extracts were then concentrated by using rotary vacuum evaporator (Roteva, Equitron). Dried sample was re-suspended with 1 ml of methanol and subjected to scan from 200–800 nm under UV-Visible spectrophotometer (UV-2450, Shimadzu). Base line was corrected with the solvent methanol.

Results and Discussions

A total of 92 bacterial colonies were isolated from the iron media based on morphological characteristics like colour, size, shape, texture, Gram's and spore staining. Most of the colonies were circular, white or dull white and only few were accounted for yellow. Around 95% of the isolates were rod shaped and very few were in coccid nature (Fig. 1). Seventy percent of the EIO isolates were gram positive, spore producers and in contrast 92% of the AS isolates were gram negative (data not shown).

Approximately 1500 nucleotides long PCR amplified 16S rDNA of the bacterial isolates were differentiated for its phylotypes using ARDRA analysis with *Alu*-I endonuclease (AG↓CT). ARDRA of the culturable iron bacterial isolates sorted them into various phylotypes (39 restriction patterns) which were then sequenced. Identifications based on 16S rRNA gene sequence comparison to BLAST analysis of the iron bacteria are shown in Table I. Chimera check indicated that there were no anomalies detected from the sequences. The 16S rRNA gene sequences expressed three different bacterial classes, each one represented by different families: Bacilli (Bacillaceae and Staphylococcaceae), α-Proteobacteria (Rhodobacteraceae, Erythrobacteraceae and Phyllobacteriaceae) and γ-Proteobacteria (Pseudoalteromonadaceae, Alteromonadaceae, Alcanivoracaceae, Pseudomonadaceae, Idiomarinaceae, Chromatiaceae and Halomonadaceae). 16S rRNA gene sequencing analysis exhibited 13 different genera belong to *Halomonas, Rheinheimera, Staphylococcus, Marinobacter, Idiomarina, Alcanivorax, Erythrobacter, Roseovarius, Sagittula, Nitratireductor, Pseudoalteromonas, Pseudomonas* and *Bacillus* (Table I).

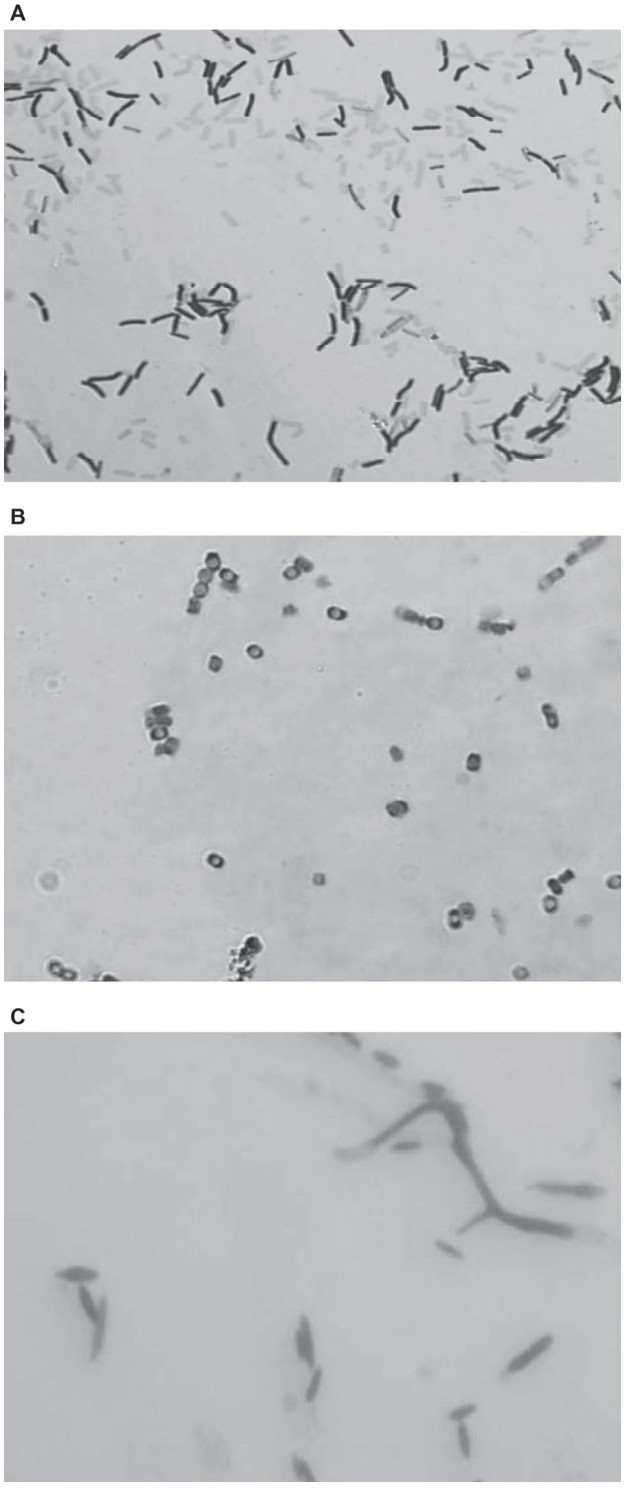
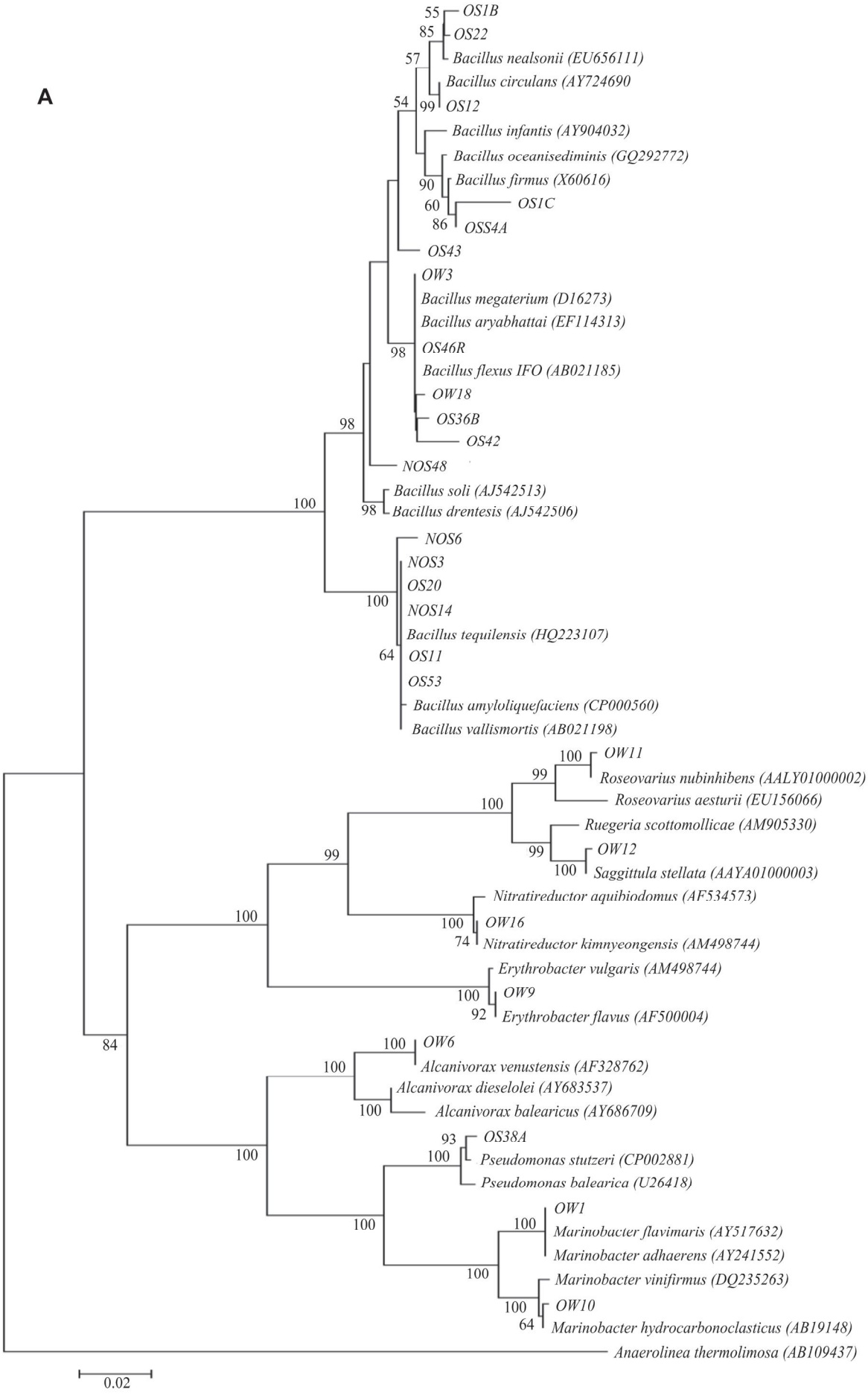


Fig. 1. Morphology of few representative bacteria (A: OS1B, B: ASW2C, C: NOS6).

Iron bacterial 16S rRNA gene similarity levels were mostly ≥97% when compared with the published


A
OS1B
OS22
Bacillus nealsonii (EU656111)
Bacillus circulans (AY724690
OS12
Bacillus infantis (AY904032)
Bacillus oceanisediminis (GQ292772)
Bacillus firmus (X60616)
OS1C
OSS4A
OS43
OW3
Bacillus megaterium (D16273)
Bacillus aryabhattai (EF114313)
OS46R
Bacillus flexus IFO (AB021185)
OW18
OS36B
OS42
NOS48
Bacillus soli (AJ542513)
Bacillus drentesis (AJ542506)
NOS6
NOS3
OS20
NOS14
Bacillus tequilensis (HQ223107)
OS11
OS53
Bacillus amyloliquefaciens (CP000560)
Bacillus vallismortis (AB021198)
OW11
Roseovarius nubinhibens (AALY01000002)
Roseovarius aesturii (EU156066)
Ruegeria scottomollicae (AM905330)
OW12
Saggittula stellata (AAYA01000003)
Nitratireductor aquibiodomus (AF534573)
OW16
Nitratireductor kimnyeongensis (AM498744)
Erythrobacter vulgaris (AM498744)
OW9
Erythrobacter flavus (AF500004)
OW6
Alcanivorax venustensis (AF328762)
Alcanivorax dieselolei (AY683537)
Alcanivorax balearicus (AY686709)
OS38A
Pseudomonas stutzeri (CP002881)
Pseudomonas balearica (U26418)
OW1
Marinobacter flavimaris (AY517632)
Marinobacter adhaerens (AY241552)
Marinobacter vinifirmus (DQ235263)
OW10
Marinobacter hydrocarbonoclasticus (AB19148)
Anaerolinea thermolimosa (AB109437)
0.02

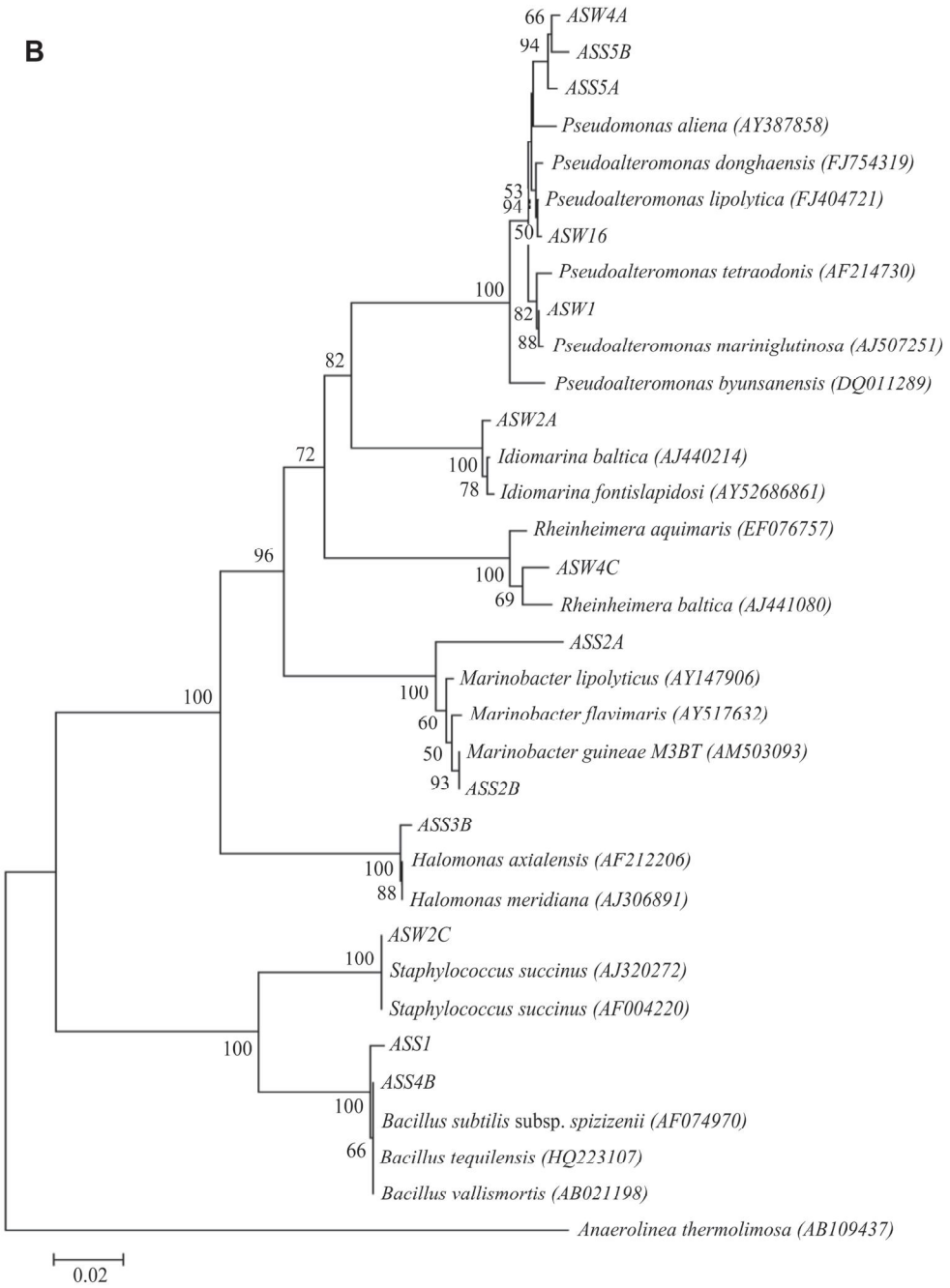


Fig. 2. The Neighbor-Joining tree constructed based on evolutionary distances and computed using the Maximum Composite Likelihood method representing relationship between the 16S rRNA sequence of iron bacteria (a. Equatorial Indian Ocean; b. Arabian Sea). Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at the tree branch points. *Anaerolinea thermolimosa* is used as an out group. Bacteria with accession numbers provided in the phylogenetic tree were retrieved from GenBank database to know the relationship of our bacteria.

GenBank database. But some of the Arabian Sea isolates like ASW4a and ASS5a were matched only 96.8% with the existing database. Since the similarity indexes are showing lower than 97%, this could expect to form a new species under the genera *Pseudoalteromonas*. Further one of the isolate ASS2A from the Arabian Sea observed the similarity level of 94.7% may form a potential new genus in the family Pseudoalteromonadaceae. Additional experiments like Fatty Acid Methyl Ester analysis and DNA-DNA relatedness have to be done to confirm this. Even though more than 97% similarity level were observed for some of the Indian Ocean isolates like OW6, OW10, OW11, OW12, OW18, OS1B, OS1C and OS42, these species were reported very rarely in the literatures. Above results clearly indicate many novel and rare bacterial species on Iron bacteria to be included in the diversity data bases.

Much less information is available about iron oxidizing bacteria from the marine environment because their habitats appear limited primarily to deep sea

Table I
16S rRNA gene identity of iron bacteria from EIO and AS.

Sr. No	Strain no.	Bacteria identified by 16S rRNA gene	Phyla/Class	Accession no.	Similarity %
1	OW1	*Marinobacter flavimaris*	γ-Proteobacteria	JQ905060	99.6
2	OW3	*Bacillus flexus*	Bacilli	JQ905061	99.2
3	OW6	*Alcanivorax venustensis*	γ-Proteobacteria	JQ905062	100
4	OW9	*Erythrobacter flavus*	α-proteobacteria	JQ905063	99.9
5	OW10*	*Marinobacter hydrocarbonoclasticus*	γ-Proteobacteria	JQ905064	97.7
6	OW11	*Roseovarius nubinhibens*	α-Proteobacteria	JQ905065	99.8
7	OW12	*Sagittula stellata*	α-Proteobacteria	JQ905066	98.2
8	OW16*	*Nitratireductor kimnyeongensis*	α-Proteobacteria	JQ905067	100
9	OW18	*Bacillus aryabhattai*	Bacilli	JQ905068	98.9
10	OS1B	*Bacillus nealsonii*	Bacilli	JQ905069	98.3
11	OS1C	*Bacillus oceanisediminis*	Bacilli	JQ905070	98.6
12	OS11	*Bacillus tequilensis*	Bacilli	JQ905071	97.8
13	OS12	*Bacillus circulans*	Bacilli	JQ905072	99.7
14	OS20	*Bacillus tequilensis*	Bacilli	JQ905073	100
15	OS22	*Bacillus nealsonii*	Bacilli	JQ905074	98.7
16	OS36B	*Bacillus aryabhattai*	Bacilli	JQ905075	99.3
17	OS38A*	*Pseudomonas stutzeri*	γ-Proteobacteria	JQ905076	98.2
18	OS42	*Bacillus megaterium*	Bacilli	JQ905077	97.7
19	OS43	*Bacillus nealsonii*	Bacilli	JQ905078	97.6
20	OS46R	*Bacillus flexus*	Bacilli	JQ905079	99.0
21	OS53	*Bacillus vallismortis*	Bacilli	JQ905080	100
22	OSS4A	*Bacillus oceanisediminis*	Bacilli	JQ905081	99.6
23	NOS3	*Bacillus vallismortis*	Bacilli	JQ905082	100
24	NOS6	*Bacillus tequilensis*	Bacilli	JQ905083	99.3
25	NOS14	*Bacillus vallismortis*	Bacilli	JQ905084	100
26	NOS48	*Bacillus soli*	Bacilli	JQ905085	97.9
27	ASW1*	*Pseudoalteromonas mariniglutinosa*	γ-Proteobacteria	JQ905086	99.8
28	ASW2A	*Idiomarina baltica*	γ-Proteobacteria	JQ905087	99.5
29	ASW2C	*Staphylococcus succinus*	Bacilli	JQ905088	99.9
30	ASW4A	*Pseudoalteromonas* sp.	γ-Proteobacteria	JQ905089	**96.8**
31	ASW4C*	*Rheinheimera aquimaris*	γ-Proteobacteria	JQ905090	98.7
32	ASW16	*Pseudoalteromonas lipolytica*	γ-Proteobacteria	JQ905091	98.3
33	ASS1	*Bacillus tequilensis*	Bacilli	JQ905092	98.8
34	ASS2A	Uncultured *Marinobacter*	γ-Proteobacteria	JQ905093	**94.7**
35	ASS2B*	*Marinobacter guineae*	γ-Proteobacteria	JQ905094	97.9
36	ASS3B*	*Halomonas axialensis*	γ-Proteobacteria	JQ905095	98.7
37	ASS4B	*Bacillus subtilis*	Bacilli	JQ905096	99.8
38	ASS5A*	*Pseudoalteromonas* sp.	γ-Proteobacteria	JQ905097	**96.8**
39	ASS5B*	*Pseudoalteromonas lipolytica*	γ-Proteobacteria	JQ905098	98.1

* Siderophore producing bacteria in this study. Serial numbers 1–26 are Indian Ocean bacteria; 27–39 are Arabian Sea bacteria.

associated with hydrothermal activity or rendered ocean crust that are difficult to study. *Gallionella ferruginea*, *Leptothrix* spp, *Sideroxydans* spp., *Mariprofundus ferrooxydans* and *Thiobacillus ferrooxidans* were well documented iron oxidizing bacteria isolated from various aquatic environments (Emerson *et al.*, 2010). On the other hand *Pseudoalteromonas*, *Pseudomonas*, *Vibrio*, *Halomonas*, *Marinobacter*, *Shewanella* and *Idiomarina* are some of the other organisms which could do better iron oxidation (Sudek *et al.*, 2009). Present investigation conducted from the AS and EIO were also shown that the heterotrophic organisms (Table I) belongs to γ- and α-Proteobacteria were able to perform better iron oxidation than the traditional ones

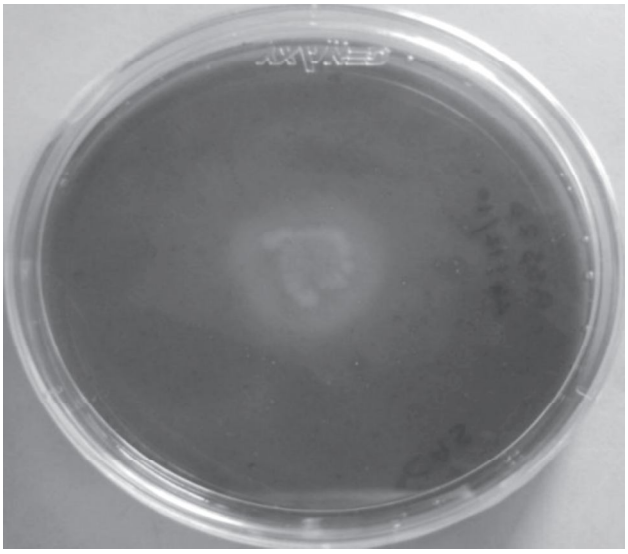


Fig. 3. CAS agar plate showing siderophore production by the strain ASS5B.

(iron bacteria). Similar results were also obtained through γ-Proteobacteria *Marinobacter* and *Halomonas* by Edwards *et al* (2003).

Among the three main classes from across the EIO and AS, samples *Bacillus* spp. being one of the key organisms in marine heterotrophic bacterial community. This is because of their diverse nature and widespread adaptability to the environmental conditions (Parvathi *et al*., 2009). Most of the *Bacillus* spp. obtained here were from the sediment samples at deeper depth (approx 4700 m) and that could be known for its adaptation and survival through spore forming in geologic environments (Vreeland *et al*., 2000). We have noticed an interesting thing that the bacteria belong to α-Proteobacteria obtained only from the EIO water samples (Table I and Fig. 2a) and *Pseudoalteromonas* sp. could retrieve only from AS samples. *Halomonas*, *Rheinheimera, Staphylococcus* were some of the other genera obtained from AS samples (Fig. 2b). *Marinobacter* spp were common in both the study area.

Table II
Siderophore production estimation in TYES broth and in CAS plates.

Test isolates	Isolation source	Siderophore production zone in CAS plates (cm)	% of siderophore production	
			6th day	12th day
ASW1	AS	3	85.3	81.3
ASW4C	AS	1	44.4	26.02
ASS2B	AS	2	87.9	84.07
ASS3B	AS	1	87.5	77.9
ASS5A	AS	3	81.3	71.1
ASS5B	AS	3	78.3	54.16
OW10	EIO	1	78.6	71.5
OW16	EIO	2	59.1	35.8
OS38A	EIO	2	73.3	52.7

Among the 39 strains tested for siderophore production, 9 of them produced yellow/orange colour around the colony (Fig. 3). Maximum diameter of the zone was 3 cm produced by *Pseudoalteromonas* sp. (ASW1, ASS5A and ASS5B) (Table II). Interestingly all the three *Pseudoalteromonas* sp. were affiliated to different 16S rRNA gene sequences which is evidenced in phylogenetic tree. Among the 9 strains produced siderophores 8 of them belong to γ-Proteobacteria and the remaining one to α-Proteobacteria. Bacteria belong to γ-Proteobacteria like *Marinobacter* and *Halomonas* are known for its production of self-assembling amphiphilic siderophores (Martinez *et al*., 2000). Our studies were also in supportive of the above by saying *Marinobacter*, *Halomonas, Pseudoalteromonas, Rheinheimera* and *Pseudomonas* could produce siderophores in a better way. Though the previous reports say the transport of iron through siderophores by *Bacillus* spp. (Dertz *et al*., 2003; Zawadzka *et al*., 2009) we did not see siderophore production from *Bacillus* spp. The reasons are not known.

Siderophore production tested among the 9 strains using various techniques expressed its maximum production from the ASS2b strain with 87.9%. The lowest production was noticed in ASW4c at the rate of 44.4% (Table II). While comparing the siderophore production rates, the AS strain has given much higher when compared to EIO. Quantitative estimation of siderophores have shown that 6th day old culture produces maximum amount of siderophores as compared to 12th day old culture. Further the samples which produced siderophores were scanned to get a rough idea of the compounds present over there. All the 9 strains scanned for siderophore production indicate 2 peaks, one at 306 and the other at 246 nm (Fig. 4) (UV-scan data are not presented beyond 400nm in Figure 4 since there is no peak after). These peaks indicate the compound Azurechelin (Sokol *et al*., 1992). They say that 88% of siderophore production by *Pseudomonas cepacia* had absorbance maxima at 240 and 310 nm. Our reports also indicate the peak in a similar fashion, it could be interpreted that the EIO and AS samples may produced the same compound.

Most of the microbial siderophores are either hydroxamate or catechol or carboxylate types (Miethke and Marahiel, 2007). All the bacteria from the present study were showing the absorption maxima between 190 and 280 (Spectrophotometric Scan) which indicate the presence of carboxylate type. Interestingly, bacterial siderophore production studies conducted by Sullivan *et al*. (2012) also revealed that in general 90% of the bacterial isolates were able to produce carboxylates type siderophores.

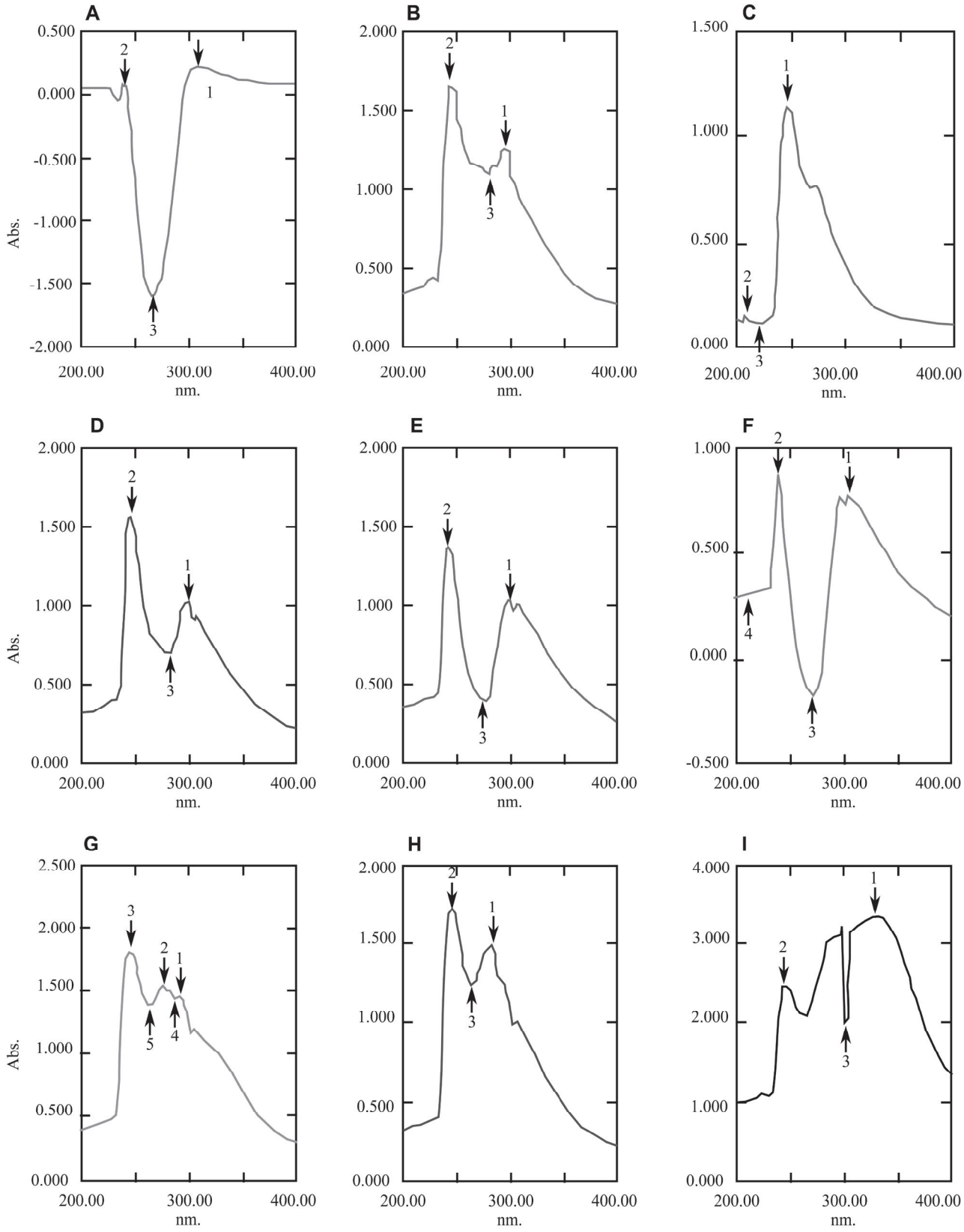


Fig. 4. Scan report for various siderophore producing bacterial strains tested under UV-Visible spectrophotometer (A: ASW1, B: ASW4C, C: ASS2B, D: ASS3B, E: ASS5A, F: ASS5B, G: OW10, H: OW16, I: OS38A).

Importance of heterotrophic bacteria in the cycling of carbon and nutrients, including iron is not fully understood (Tortell *et al.*, 1996). This is especially so from oligotrophic waters (Fukuda *et al.*, 1998). In general, the dissolved Fe concentration in the surface mixed layer was lower due to biological removal and

excess concentration of Fe-binding organic ligands (Nakabayashi *et al.*, 2001 and 2002). Studies by Price *et al.* (1994) and Cochlan (2001) in the eastern Equatorial Pacific Ocean, and Pakulski *et al.* (1996) in Gerlache Strait explained that, even small quantities of iron could increase the heterotrophic bacterial abundance.

Current study on the diversity of culturable iron bacteria from the EIO and AS samples showed that the bacteria retrieved from the selective medium did not indicate any typical iron bacteria like *Thiobacillus.* On the other hand the bacterial groups like *Pseudoalteromonas*, *Halomonas*, *Idiomarina*. *Erythrobacter*, and *Nitratireductor* were retrieved during this study. These kind of heterotrophic organisms were well reported earlier for iron oxidation. Distinct variation on the diversity of iron bacteria were noticed in the analyzed sites indicating *Erythrobacter*, *Roseovarius*, *Sagittula* and *Nitratireductor* from the oligotrophic waters of EIO and *Pseudoalteromonas*, *Halomonas*, *Rheinheimera, Staphylococcus* and *Idiomarina* in nutrient rich waters of AS. Very few isolates like *Marinobacter* and *Bacillus* were common in both the seas.

Iron can be used in two ways by the microorganisms, one as an electron acceptor which oxidize iron directly and the others which produce siderophores to solubilize the iron for its nutritional requirements. Since the isolates *Marinobacter hydrocarbonoclasticus*, *Nitratireductor kimnyeongensis* and *Pseudomonas stutzeri* (EIO) and *Pseudoalteromonas* spp, *Marinobacter guinea*, *Rheinheimera aquimaris* and *Halomonas axialensis* (AS) exhibit to produce siderophores, we assume that these organisms meet their iron requirement through siderophore production. Though *Bacillus* spp reported for siderophore production earlier, our investigations did not come across *Bacillus* spp. for siderophore production in the study area and the reasons are yet to be studied. In general from the current study it may be interpreted that the bacterial species from the oligotrophic waters like EIO try to obtain their iron requirement by oxidation and the bacteria of AS through siderophore production. Further most of the iron solubilising marine bacteria expected to synthesize similar type of compounds in their siderophore. This work reported to bring few new species and genus in bacterial diversity on iron oxidation.

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