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Retreived bacteria from *Noctiluca miliaris* (green) bloom of the northeastern Arabian Sea

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Abstract In recent years, seasonal blooms of the dinoflagellate *Noctiluca miliaris* have appeared in the open-waters of the northern Arabian Sea (NAS). This study provides the first characterization of bacteria from a seasonal bloom of green *Noctiluca* of NAS (20°N–17°N and 64°E–70°E), during the spring-inter-monsoon cruise of *Sagar Sampada* 253, in March 2007. Bacterial growth as assessed by most-probable number (MPN) and plate counts, revealed 'variable-physiotypes' over a wide range of salinities (0%–25% w/v NaCl), pH levels (5–8.5), and organic nutrient strengths, in comparison to non-bloom waters. MPN indices of bacteria in surface waters of bloom stations *DWK and *PRB, corresponded to (3.08–4.41)×10^3 cells/mL at 3.5% NaCl (w/v), and (2.82–9.49)×10^2 cells/mL at 25% (w/v) NaCl in tryptone-yeast extract broth (TYE). Plate counts were (1.12–4)×10^6 CFU/mL at 0% (w/v) NaCl, (1.28–3.9)×10^6 CFU/mL at 3.5% (w/v) NaCl, and (0.4–7)×10^4 CFU/mL at 25% NaCl (w/v) on TYE. One-tenth-strength Zobell's gave (0.6–3.74)×10^5 CFU/mL at pH 5 to (3.58–7.5)×10^5 CFU/mL at pH 8.5. These bacteria were identified to the genera *Bacillus*, *Cellulomonas*, *Staphylococcus*, *Planococcus*, *Dietzia*, *Virgibacillus*, *Micrococcus*, *Sporosarcinae*, *Leucobacter*, and *Halomonas*. The identity of three strains (GUFBSS253N2, GUFBSS253N30, and GUFBSS253N84) was confirmed through 16S rDNA sequence homology as *Bacillus cohnii*, *Bacillus flexus*, and *Bacillus cereus*. The ~2–3-fold higher plate counts of culturable bacteria from the open-waters of the NAS indicate that these bacteria could critically determine the biogeochemical dynamics of the bloom and its milieu. The role of these bacteria in sustaining/terminating the bloom is under evaluation.

Keyword: northeastern Arabian Sea (NAS); green *Noctiluca* bloom; retrievable bacteria

1 INTRODUCTION

Algal blooms of the dinoflagellate *Noctiluca miliaris* (synonymous to *Noctiluca scintillans*) have been frequently detected in the seas around India (Harrison et al., 2011). However, green *Noctiluca* blooms in the far offshore waters of north-eastern Arabian Sea (NAS) have only occurred recently, with the first reports during the winter monsoon of 2003–2004 (Matondkar et al., 2004). A green *Noctiluca* bloom was also observed during the March 2007 cruise of the Fishery and Oceanographic Research Vessel (FORV) *Sagar Sampada* 253 (Gomes et al., 2008). *Noctiluca miliaris* is a high biomass harmful algae (Furuya et al., 2006; Ferreira et al., 2012), thus, the emergence and expansion of such seasonal blooms of green *Noctiluca* could create high-organic (eutrophic) conditions in the open-waters of the NAS. As the importance of water-column bacteria in both organic matter utilization and sustaining a strong microbial-loop in the Arabian Sea has been underscored during the last decade (Azam et al., 1994; Madhupratap et al., 1996; Ducklow et al., 2001), the ability of these bacteria to grow and utilize organic matter during blooms of green *Noctiluca* will also be critical for the biogeochemistry of the basin.

Blooms of phytoplankton are known to favor specific bacterial communities (Sapp et al., 2007). Previous studies of red *Noctiluca* have shown intracellular bacteria within the highly acidic

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cytoplasm (pH ~3.5), and such bacteria-containing *Noctiluca* red cells are referred to as "turbid" cells (Nawata and Sibaoka, 1976; Seibold et al., 2001). This prompted the culturing of bacteria from red *Noctiluca* on low nutrient 0.1× Zobell's medium, adjusted to several low pH conditions from 3.1–7.9 (Kirchner et al., 2001; Seibold et al., 2001). Unlike the heterotrophic red forms, green *N. miliaris* contains numerous photosynthetic algal symbionts, *Pedinomonas noctilucae* (Elbrachter and Qi, 1998), which are also acidophilic and require an optimum pH of 4.5–5 in culture (Furuya et al., 2006). Thus, similar bacterial associates that can adapt or grow over a range of low pH conditions in the green *Noctiluca* bloom milieu may exist.

As the average salinity of most marine environments varies between 10 and 35 (1%–3.5% w/v NaCl), true marine heterotrophic bacteria are known as "slight" or "marine" halophiles, growing best in media containing 1%–3% NaCl (Kushner et al., 1988). Other groups include the "non-halophiles" (requiring <1% NaCl), the "moderate" halophiles (optimally requiring 5%–20% NaCl), and the "extreme" halophiles (including archaea), which are mostly restricted to hypersaline environments, and optimally require 20%–30% NaCl (Kushner et al., 1988). Several marine heterotrophic bacteria can also grow in a wide range of salt concentrations (between 0%–25% NaCl), and are referred to as "halotolerant" or "euryhaline" halophiles (Ventosa et al., 1998). In recent times, evidence for a cosmopolitan distribution and high abundance of such moderately halophilic/halotolerant heterotrophic bacteria from several non-hypersaline marine environments has come to light. Examples of these bacteria have been found from the pelagic ocean (Mimura and Nagata, 2000) to the extreme deep-sea and hydrothermal vent ecosystems (Kaye et al., 2003; Kaye et al., 2011), including marine sediments (Diaz et al., 2000; Raghavan and Furtado, 2004), and from high-organic oil-spill environments (Diaz et al., 2000; Al-Awadhi et al., 2007). The reasons for such widespread occurrence of halophilicity or halotolerance among common heterotrophic bacteria from non-hypersaline marine environments are not well known (Ventosa et al., 1998; Kaye et al., 2004), although it may be linked to the physiological ability of these bacteria to tolerate osmotic-stress in different econiches (Trousselier et al., 1998; Wood et al., 1999). Unlike the haloarchaea, which prefer a "salt-in" strategy to adjust intracellular osmotic balance in hypersaline environments, the marine halophilic/halotolerant heterotrophic bacteria synthesize or accumulate organic compatible solutes to prevent water loss from their cytoplasm (Ventosa et al., 1998). This commonly occurs in conditions of high extracellular osmolality because of high or fluctuating salinity and/or high organic matter/solute concentrations (Csonka, 1989; Ghoul et al., 1990; Kempf and Bremer, 1998; Empadinhas and Da-Costa, 2008). As the emergence of green *Noctiluca* blooms in the NAS represents such an extreme high-organic biomass event (Ferreira et al., 2012), the response of native bacteria to increasing bloom organic matter, changing redox conditions, and fluctuating osmolarities in the high mucus micro-environment of the bloom will also be critical for the NAS biogeochemistry.

The present study is designed to assess the culturable bacterial load from the green *Noctiluca* bloom of the NAS. Using a combination of most probable number (MPN) estimation and plate count methods, the bacterial load from bloom and non-bloom areas was estimated selectively using an organic nutrient medium containing 3.5% NaCl (for marine halophiles), 0% NaCl (for non-halophiles), and 25% NaCl (for extreme halophiles/halotolerant forms). Plate counts of bacteria cultured on low nutrient medium with a pH gradient of 5–8 were also examined. A selection of the most commonly isolated forms was identified using metabolic parameters and 16S rDNA sequences. These findings are discussed in light of the relative bacterial load of the culturable physiotypes and their importance.

2 MATERIAL AND METHOD

2.1 Study site

To locate visible bloom waters, FORV *Sagar Sampada* 253 was navigated between 20°N–17°N latitude and 64°E–70°E longitude in the NAS from March 1st–15th of 2007. Sampling stations for this study were designated as shown in Fig.1. Co-ordinates were as follows: Central Arabian Sea (CAS: 21°50.31′N; 64°07.31′E); Dwarka (*DWK: 21°50.32′N; 66°09.38′E); Kathiawar (KTW: 21°50.28′N; 65°03.64′E); Porbander (*PRB: 21°50.63′N; 67°03.55′E); Chorwad (CHD: 21°00.40′N; 65°03.93′E); Somnath (SMN: 20°59.62′N; 66°00.90′E), and Dapoli (DPL: 17°55.25′N; 70°19.20′E).

2.2 Physicochemical study

In situ wind-speed was recorded from the shipboard meteorological facility while temperature and salinity

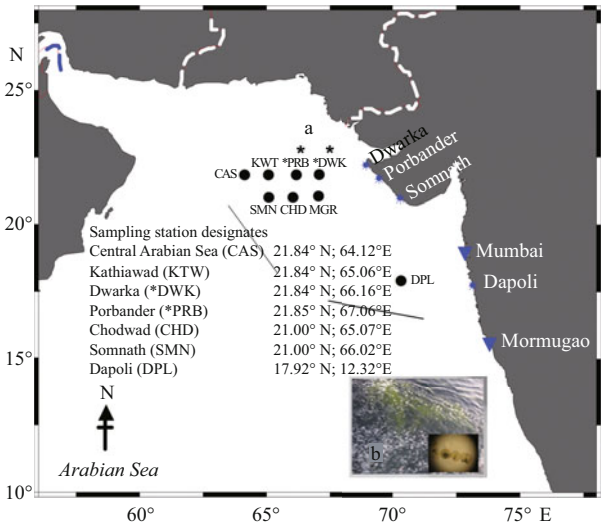

Fig.1 Map showing sampling stations during the cruise of the *Sagar Sampada* 253 in the northeastern Arabian Sea (NAS)

a. Stations marked with asterisk indicate the presence of *N. miliaris* bloom; b. Green *Noctiluca* bloom patch with inset of *Noctiluca* cells observed under 20× magnification.

data were obtained using Sea Bird Electronics, (Washington, USA) CTD sensors. Water samples were analyzed onboard for pH using an automated pH meter, Labindia instruments Pvt Ltd, Gurgaon, India). Dissolved oxygen (DO) was estimated by the modified Winkler's method using a Dosimat titrator (Metrohm) and expressed in μmol/L (Strickland and Parsons, 1972; Knap et al., 1994).

2.3 Primary productivity

Primary productivity was estimated onboard using a simulated in situ ^{14}C method. Carbon fixed was determined using a liquid scintillation counter Wallac-1404 (Perkin Elmer, Massachusetts, USA) after quench corrections and expressed in terms of mg C/(m^3·d) (Knap et al., 1994). Contour lines of physicochemical and biological data in the study area were plotted using the Ocean Data View software version 4.4 (Schlitzer, 2012) and Surfer 8 (Golden Software, Inc., Colorado, USA).

2.4 Collection of surface water samples for bacteriological analysis

Surface water samples were collected from each sampling station (Fig.1) using Go-Flo 1.7 L Niskin sampling bottles (General Oceanics, Florida, USA) and drained into sterile containers. *Noctiluca miliaris* from individual water samples was concentrated on sterile 0.22 μm Nucleopore filters (Millipore, Massachusetts, USA) and used for microbiological analyses. Using a low pump pressure of ~5 mm Hg, the volume of filtered water varied from 15–20 mL at the *DWK and *PRB bloom stations, to 200 mL at the DPL non-bloom reference station, located below 19° N latitude in the NAS. An additional sample of *Noctiluca* bloom was collected from station *PRB, designated PRB-1, and analyzed directly as an unfiltered sample for presence of bacteria.

2.5 Estimation of culturable bacterial load

The bacteria retained on the individual filter discs were dislodged under aseptic conditions in 3.5% NaCl (w/v), as it represented ambient salinity (~35). This suspension was used as an inoculum for enumerating bacteria present at each station as detailed below:

2.5.1 Most probable number

An MPN method, previously used for selectively estimating extremely halophilic bacteria from Arabian Sea sediments using a tryptone yeast extract medium (TYE) (Raghavan and Furtado, 2004), was modified for our study. In brief, the basal TYE medium was modified to provide: (a) ambient salinity conditions of the bloom area with 3.5% NaCl (~35) (referred to as 3.5% TYE) and (b) hypersalinity conditions with 25% NaCl (referred to as NTYE). A three-tube MPN series was set-up (Kaye and Baross, 2000) for each of these conditions. Aliquots of 0.5, 0.05, and 0.005 mL of the sample suspension were inoculated separately into two sets of nine tubes, with each of these two sets consisting of a first, second, and third subset of three tubes each. The first set contained 3.5% NaCl (w/v), while the second set was prepared with 25% NaCl (w/v) in a nutrient rich TYE medium. The inoculated tubes were incubated at 25°C and growth in MPN tubes was visually monitored for turbidity against an uninoculated control. MPN counts were deduced by calculating from the McCrady's Chart (Cruickshank, 1973; Raghavan and Furtado, 2004).

2.5.2 Bacterial plate counts

Plate counts were obtained after serial dilution of the sample and plating on: (a) TYE with 3.5% (w/v) NaCl (3.5%TYE) as in situ salinity, TYE with 25% (w/v) NaCl (NTYE), and TYE medium with 0% (w/v) NaCl (TYE), and (b) 0.1× marine Zobell's 2216E (ZB0.1) medium, adjusted to pH 5, 6, 7, and 8, with

all combinations containing 3.5% NaCl as the in situ salinity.

Isolates retrieved from TYE, 3.5% TYE, and NTYE were purified by sub-culturing and further checked for osmotolerance by their ability to grow on basal TYE medium with 0% NaCl, 3.5% NaCl, and 25% NaCl (Giebel et al., 2011), respectively.

2.6 Phenotypic characterization

Morphological, biochemical, and enzymatic potentials of the isolates were examined as described previously (Smibert and Krieg, 1994; Reva et al., 2001; Basu et al., 2011).

2.7 Genomic DNA extraction, polymerase chain reaction (PCR), and16S rDNA sequencing

Genomic DNA was extracted from three isolates (GUFBSS253-2; GUFBSS253-30, and GUFBSS253-84) using the cetyl-trimethylammonium bromide (CTAB)-NaCl method (Ausubel et al., 1995). The 16S rDNA gene was amplified from genomic DNA using the universal bacterial primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-ACGGCTACCTTGTTACGACTT-3′) (Green et al., 2004). 50 μL PCR reaction mixture was prepared with 25 μL of 2× red-dye PCR master-mix containing DNA polymerase, dNTP's and PCR-buffer (Genei, Bangalore, India) was diluted with 20 μL PCR-grade water, 10 pmol/L each of primer (8f and 1492R) and 10–30 ng of DNA template following manufacturer's instructions (Genei, Bangalore, India). The thermo-cycler program (Takara Bio. Inc., Otsu, Japan) was as follows: denaturation at 95°C for 3 min, 35 cycles of 95°C for 1 min, 48°C for 1 min, and 72°C for 90 s, followed by a final elongation step of 72°C for 5 min. The amplified DNA was purified using a PCR-purification kit (Genei, Bangalore, India). The purified 16S rDNA amplicons of ~1.5 kb were then bidirectionally sequenced using the Bigdye terminator sequencer method on an ABI-3730 DNA analyzer (Applied Biosystems, California, USA). Electropherograms were imported in ChromasPro, ver 1.5 (www.technelysium.com.au). The naive Bayesian classifier algorithm of RDP-II (Wang et al., 2007) was used to identify the aligned sequences. The sequences from the GUFBSS253-2, GUFBSS253-30, and GUFBSS253-84 cultures were submitted to GenBank (www.ncbi.nlm.nih.gov/genbank/) and assigned the accession numbers JN315891–JN315893.

3 RESULT

3.1 Bloom detection

Green *Noctiluca* blooms, conforming to the microscopic description by Sweeny (1976), were visible at stations *DWK and *PRB in the NAS. Green *Noctiluca* cells were also detected in the surface water samples from stations CAS, KTW, CHD, and SMN, although the bloom was not visible. Station DPL, located furthest away from the bloom area, was considered to be the reference site.

3.2 Physico-chemical characteristics

The wind speed of the region varied between 1–7.4 m/s (Fig.2a), the sea surface temperature was between 25.6–27.8°C (Fig.2b), the pH was between 8.29–8.40 (Fig.2c), and the salinity ranged from 36.0–36.8 (Fig.2d). The highest surface DO of 233 μmol/L was measured at KTW, while the DO decreased to 204 μmol/L at DPL (Fig.2e). The DO at bloom stations varied between 211 μmol/L at *DWK to 227 μmol/L at *PRB.

3.3 Primary-productivity

Organic matter production by the bloom, measured in terms of surface primary productivity, varied from as high as 858.2 mgC/(m^3·d) at *DWK and 337 mgC/(m^3·d) at *PRB, to 45.2 mgC/(m^3·d) at CAS (Fig.2f).

3.4 Culturable bacterial-load

Bacteria capable of growth in nutrient-rich TYE medium, 3.5% TYE, and NTYE were detected at each of the aforementioned stations. As seen in Table 1, the viable counts varied from station to station. The highest MPN index of those growing in NTYE was 9.49×10^2 cells/mL at bloom station *DWK, with the lowest index, 6.92×10 cells/mL, found at station CAS. Similarly, the total MPN index in 3.5% TYE was the highest at bloom stations *DWK and *PRB ($>3.08\times10^3$ cells/mL and 4.41×10^3 cells/mL, respectively), while it ranged from $>4.5\times10^3$ –3.74×10^2 cells/mL at stations without bloom. Although turbidity as a result of bacterial growth in some MPN tubes was observed to be uniform, growth in several MPN tubes could be visibly distinguished into: (i) pellicle (surface) (ii) column (homogenous), and (iii) submerged (bottom). Thus, although total MPN index for *DWK was 9.49×10^2 cells/mL, only 1.48×10^1 cells/mL and 1.44×10^1 cells/mL were found at the surface and throughout the nutrient column,

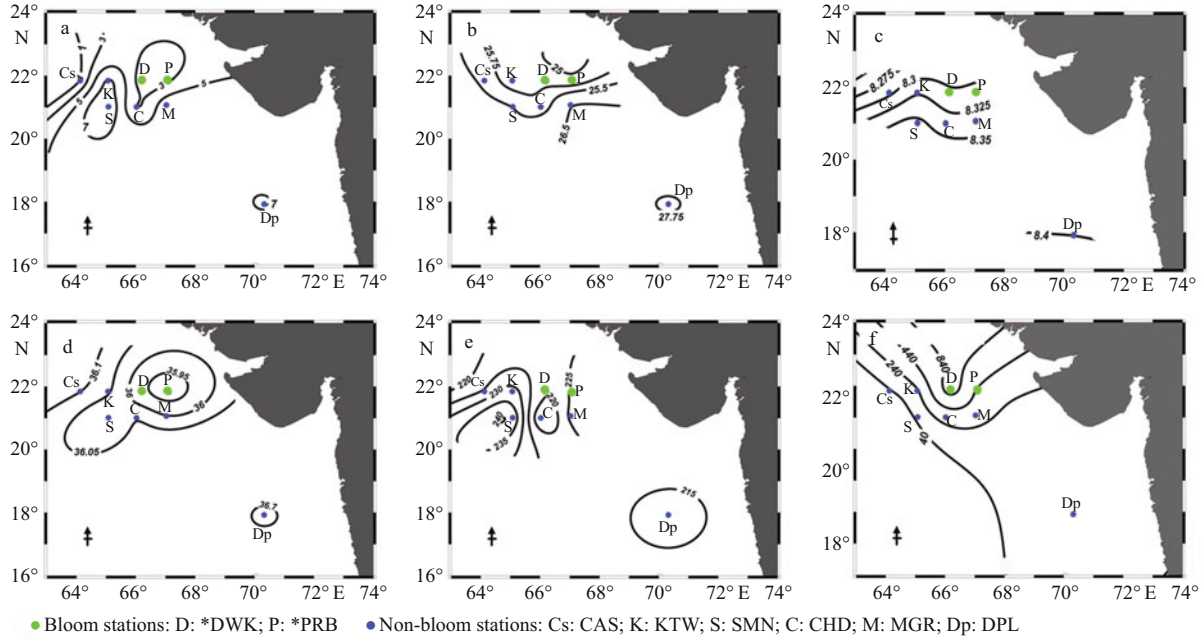

Fig.2 Hydrographic characteristics of northeastern Arabian Sea surface waters during March 2007

a. wind speed (m/s); b. temperature (°C); c. pH; d. salinity; e. dissolved oxygen (μmol/L); f. primary productivity (mg C/(m^3·d))

Table 1 Culturable bacterial load during the March 2007 bloom of green *Noctiluca miliaris* in the northern Arabian Sea

Sampling stations	Total MPN index (cells/mL)				Plate counts (CFU/mL)						
	Tryptone Yeast Extract broth (TYE)				Tryptone-Yeast Extract agar (TYE)			Zobell's 1/10 strength marine agar			
	25%NaCl[a]	(Pe:C:S)[d]	3.5%NaCl[b]	(Pe-C-S)[d]	25%NaCl[a]	3.5%NaCl[b]	0%NaCl[c]	pH8	pH7	pH6	pH5
*DWK	9.49×10^2	(14.8:14.4:920)	$>3.08\times10^3$	(300:>2 200:580)	7×10^4	1.28×10^6	1.12×10^6	7.5×10^5	5.8×10^5	2.5×10^5	6×10^4
*PRB	2.82×10^2	(14.4:28:240)	$>4.41\times10^3$	(>2200:6:>2 200)	4×10^3	1.18×10^6	8.5×10^5	3.58×10^5	4.2×10^5	2.94×10^5	5×10^4
CAS	6.92×10^1	(22:7.2:40)	$>4.5\times10^3$	(150:>2 200:>2 200)	1×10^3	7.9×10^5	5.9×10^5				
KTW	0.72×10^2	(12.2:6:54)	2.25×10^3	(186:40:2 020)	1.2×10^4	6.6×10^5	3.9×10^5				
CHD	1.18×10^2	(6: 6:106)	3.74×10^2	(6:128:240)							
SMN	4.33×10^2	(6:7.2:420)	$>4.5\times10^3$	(18.4:>2 200:>2 200)							
DPL	-				-	3.9×10^4	4×10^3	3×10^4	2.16×10^4	1.8×10^4	9×10^3

*: Bloom stations; -: no growth detected; [a] TYE with 25% NaCl (NTYE); [b] TYW with 3.5% NaCl (3.5%TYE); [c] TYE with 0% NaCl (TYE); [d] MPN index (cells/mL) of Pe - Pellicle, C - Column, and S- Submerged bacterial growth detected in MPN tubes.

respectively, while a maximum of 9.49×10^2 cells/mL were found at the bottom. The MPN index for submerged growth was highest at all stations in NTYE, while stations CAS, *PRB, and SMN showed submerged growth in excess of 2.2×10^3 cells/mL in 3.5% TYE. Interestingly, while the column growth was highest at bloom station *DWK (>2.2×10^3 cells/mL) and was undetectable at *PRB, the pellicle growth was highest at *PRB (>2.2×10^3 cells/mL) and dropped to 3×10^2 cells/mL for bloom station *DWK in 3.5% TYE.

Simultaneous estimates of total retrievable bacteria, carried out in terms of viable counts on NTYE medium (Table 1), decreased from 7×10^4 CFU/mL at *DWK to 1×10^3 CFU/mL at CAS. Viable counts on TYE and on 3.5% TYE were also highest at *DWK and corresponded to 1.12×10^6 CFU/mL and 1.28×10^6 CFU/mL, respectively. Counts for *PRB, the second station with bloom, were similar in range to those of *DWK, and varied from 8.5×10^5 CFU/mL on TYE to 1.18×10^6 CFU/mL on 3.5% TYE. Compared to the bloom stations *DWK and *PRB, viable counts for DPL, a station without bloom, were 2-fold lower on 3.5% TYE (3.9×10^4 CFU/mL) and 3-fold lower (4×10^3 CFU/mL) on TYE.

As shown in Table 1, at stations *DWK and *PRB,

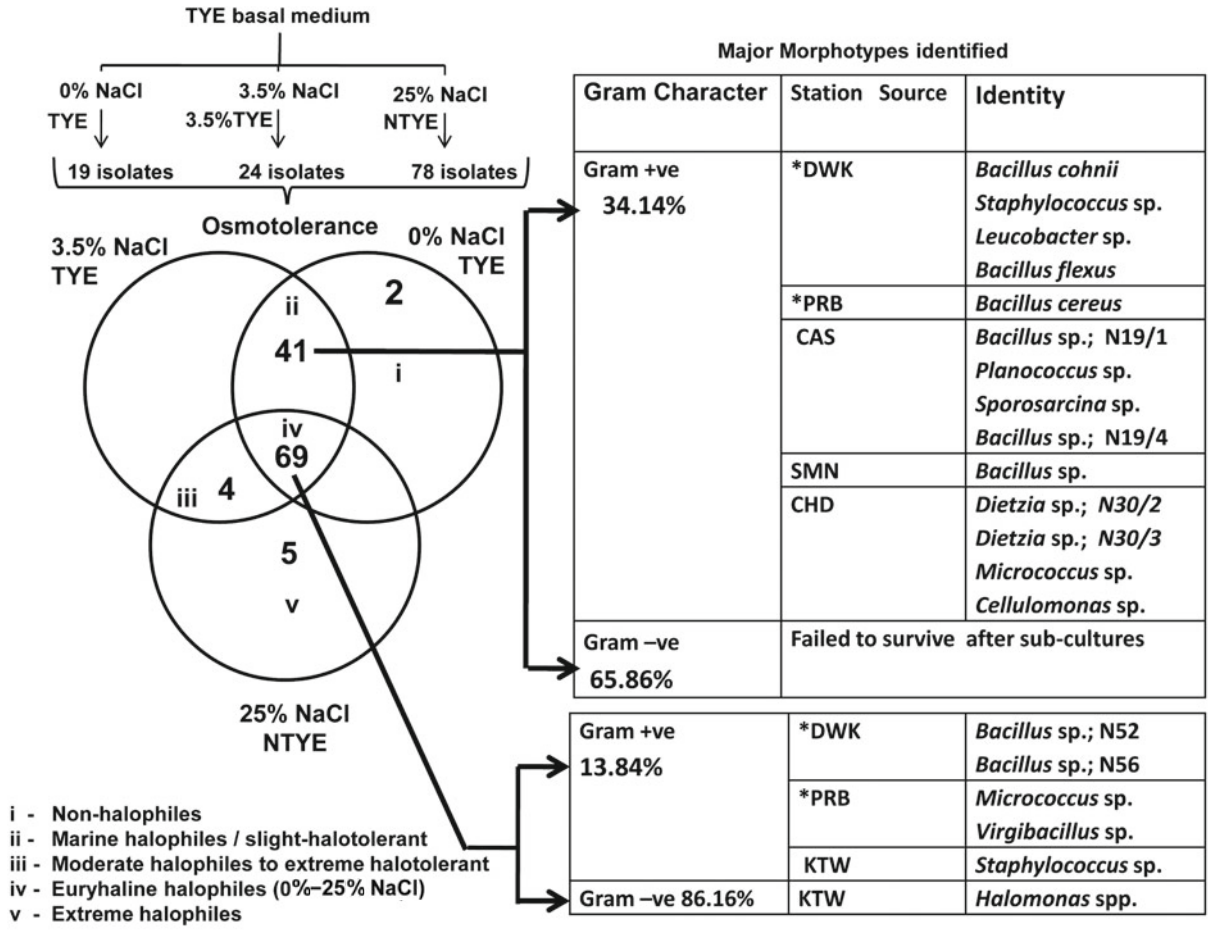

Fig.3 Osmotolerance of bacteria isolated from different stations and bloom-patch (*DWK and *PRB)

viable counts on ZB0.1 with pH 5, 6, 7, and 8 were (5–6)×10^4 CFU/mL, (2.5–2.94)×10^5 CFU/mL, (4.2–5.8)×10^5 CFU/mL, and (3.58–7.5)×10^5 CFU/mL, respectively. Comparatively, the non-bloom station DPL recorded ~10 times lower counts of 9×10^3 CFU/mL, 1.8×10^4 CFU/mL, 2.16×10^4 CFU/mL, and 3×10^4 CFU/mL on ZB pH 5, 6, 7, and 8, respectively. The unfiltered *Noctiluca* patch from *PRB (designated PRB-1) gave high counts of 3.74×10^5 CFU/mL on ZB pH 5. Two out of nine colonies picked from the pH 5 plate failed to grow at pH>6, showing their true acidophilic nature, while the remaining grew at pH 6–8. Further, out of a total of 21 colonies picked from the pH 6, 7, and 8 plates, 17 colonies grew at pH 5.

3.5 Osmotolerance of retrieved bacteria

A total of 19 colonies were isolated from TYE, 24 from 3.5% TYE and 78 from NTYE, and all these isolates were further checked for their osmotolerance (Fig.3). A majority of 69 isolates grew in 0%–25% NaCl and showed extreme halotolerance, whereas 41 isolates only grew between 0%–3.5% NaCl (marine halophiles or slight halotolerance). It was interesting to note that out of nine isolates that showed growth on NTYE with 700 IU/mL of benzyl-penicillin, five isolates failed to grow on MacConkey's medium. The remaining four grew in TYE without NaCl. Further, eight isolates among these extreme halotolerant/euryhaline forms developed a chalky, white, fibrous, rough surface with a yellow base when subjected to increased incubation, and microscopically resembled actinomycetes.

3.6 Phenotypic characterization and identification of isolates

Gram negative rods dominated the bloom (57.3%), followed by Gram negative coccobacilli (22.4%), Gram positive rods (18.2%), and Gram positive cocci (2.09%). Pigmented forms included orange translucent (23.7%), orange opaque (15.8%), translucent blue (28.9%), matt blue (10.5%), yellowish-green (7.89%), rose pink (7.89%), brown (2.63%), and colorless

translucent (2.63%). Following the generic keys from Bergey's Manual of Determinative Biology (Holt et al., 1994; Reva et al., 2001), 20 bacterial isolates (recorded in Table 2 and Fig.3) were identified based on their individual phenotypic and biochemical characteristics. Isolates were thus referred to as:
GUFBSS253N10—*Leucobacter* sp.;
GUFBSS253N3—*Halomonas* sp.;
GUFBSS253N19/2—*Planococcus* sp.;
GUFBSS253N19/3—*Sporosarcinae* sp.;
GUFBSS253N32—*Cellulomonas* sp.;
GUFBSS253N78—*Virgibacillus* sp.,
GUFBSS253N30/3 & GUFBSS253N30/2—*Dietzia* spp.;
GUFBSS253N1 & GUFBSS253N4—*Staphylococcus* spp.;
GUFBSS253 N30/1 & GUFBSS253N75—*Micrococcus* spp.;
GUFBSS253N2,GUFBSS253N19/1,GUFBSS253N19/4, GUFBSS253N30, GUFBSS253N35, GUFBSS253N52, GUFBSS253N56, and GUFBSS253N84—*Bacillus* spp. Further, isolates GUFBSS2532, GUFBSS25330, and GUFBSS25384 were confirmed as *Bacillus cohnii*, *Bacillus flexus*, and *Bacillus cereus* through 16S rDNA sequence homology. Although the Gram negative isolates dominated over Gram positive isolates by 20.3% and mostly included oligotrophs growing at low pH, they did not survive repeated sub-culturing. Four *Bacillus* spp. were isolated from bloom waters of *DWK and one from *PRB, while the remaining four were isolated from stations CAS, SMN, and CHD in the non-bloom area.

4 DISCUSSION

Heterotrophic bacteria are drivers of the microbial loop of the marine food web (Azam et al., 1994), and hence expected to be attracted to econiches with impending or decaying algal blooms to capitalize on the available particulate and dissolved organic matter. A significant ~2–3 fold increase was observed in the culturable bacterial load under different conditions (salinity, pH, and nutrient media) from the bloom area of the NAS, compared to the non-bloom waters of station DPL (Table 1). This finding indicates a natural enrichment of these bacteria in the high biomass organic matter of *Noctiluca* (green). Although bacterial growth on TYE, 3.5% TYE, and NTYE media was detected at all stations, retrieved counts clearly show the dominance of marine halophiles, which grow best with 3.5% (w/v) NaCl supplementation. Interestingly, the majority of isolates obtained on NTYE medium could also grow in 0%–25% (w/v) NaCl, despite the surface salinity varying between 35.5–36. As shown in Table 2 and Fig.3, these isolates were found to be *Bacillus* sp., *Virgibacillus* sp., *Halomonas* sp., *Micrococcus* sp., and *Staphylococcus* sp. Recent studies have shown that such euryhaline *Halomonas* are abundant in extreme deep-sea niches (Kaye et al., 2011). The presence of these euryhaline bacteria (capable of growth in hyper-saline environment as well as in the absence of NaCl) in the high organic matter open-ocean bloom environment of *Noctiluca* (green) speaks of the remarkable physiological flexibility of these isolates (Ventosa et al., 1998), which can adapt to the changing osmolar conditions during the bloom. Further, the ability of reported euryhaline bacteria to degrade a range of organic pollutants and hydrocarbons (Diaz et al., 2000; Al-Awadhi et al., 2007) by expressing extracellular enzymes (Margesin et al., 2001) is also likely to be important during a bloom. The identification of isolated marine halophiles (Fig.3) revealed that the majority were *Bacillus* spp., followed by the red-pigmented forms of *Dietzia*. The ubiquity and important role of *Bacillus* in hydrocarbon and organic matter degradation in marine-sediments are well documented (Diaz et al., 2000). Slightly halophilic strains of *Dietzia* have been also identified as important degraders of hydrocarbons (Szvetnik et al., 2010), and along with strains of *Micrococcus*, *Cellulomonas*, and *Bacillus* are known to be important hydrocarbon degraders in the oil-spill areas of the Persian-Gulf (Al-Awadhi et al., 2007). As several species of the isolated Gram-negative bacteria failed to grow during further sub-culturing for identification, we refrain from further conclusions on the relative abundance of the retrieved flora. However, we suggest the presence of a cohabitating bacterial consortium in the *Noctiluca* (green) bloom that can be differentiated into non-halophiles, marine-halophiles, extreme halophiles, and halotolerant/euryhaline halophilic bacteria, based on NaCl requirements (Fig.3). Further, the ability of four of the isolates growing on NTYE to also grow in the presence of 700 IU benzyl-penicillin, and their inability to grow in MacConkey's medium and TYE, is a preliminary indicator of the possible archaeal nature of these isolates (Raghavan and Furtado, 2004).

Because the bacterial growth in several MPN tubes could be visibly detected as pellicle (surface), column (homogenous), and submerged (bottom), we scored the presence or absence of such growth in each MPN tube directly, along with the total MPN estimates of culturable bacteria (Table 1). Such an approach has been previously used for enumerating halophiles from salt-pans (Carolene, 2006) and should also be

Table 2 Biochemical potentials and tentative identification of major isolates obtained from *N. miliaris* bloom and non-bloom areas

Biochemical character	*Isolates																			
	N1	#N2	N3	N4	N10	N19/1	N19/2	N19/3	N19/4	#N30	N30/1	N30/2	N30/3	N32	N35	N52	N56	N75	N78	#N84
Source station	KTW	DWK	KTW	DWK	DWK	CAS	CAS	CAS	CAS	DWK	CHD	CHD	CHD	CHD	SMN	DWK	DWK	PRB	PRB-1	PRB
Morphology	Cocci	Rods	Rods	Cocci	Rods	Rods	Cocci	Rods	Rods	Rods	Cocci	Rods	Rods	Rods	Rods	Rods	Rods	Cocci	Rods	Rods
Pigmentation	W	LY	W	CW	W	PC	LO	C	C	C	CW	B	BR	PY	B	W	W	CW	W	W
Gram character	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anaerobic growth	±	+	+	+	±	+	-	-	+	+	-	±	+	+	-	±	-	-	+	-
Endospores	-	T	-	-	-	C	-	T	Ts	Ts	-	-	-	-	T	T	T	-	T	C
Oxidase	-	+	+	-	-	-	-	+	-	+	+	+	+	+	+	-	-	+	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility	-	-	+	-	-	+	+	+	-	+	-	-	-	+	-	+	-	-	+	-
Growth at:																				
10ºC	-	-	-	-	-	-	-	-	-	-	-	-	-	±	-	-	±	-	-	-
25ºC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40ºC	+	+	+	+	±	+	+	+	+	+	+	±	-	+	+	+	-	+	+	+
50ºC	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	+	-	-	-	+
Growth with NaCl																				
0%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.5%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25%	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-
Urease	-	±	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	-	+
Nitrate reduction	+	-	+	-	-	+	+	-	+	+	+	+	+	+	-	+	-	+	-	-
H_2S production	-	-	-	-	-	-	+	-	-	-	-	-	-	-	±	-	-	+	-	-
Utilization of Citrate	-	-	-	+	+	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+
Gelatin	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	-	-	-	+
Starch	-	+	+	+	+	+	-	-	+	+	-	+	-	+	-	-	+	-	-	+
Tributyrin	-	-	+	-	+	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-
Casein	-	+	+	-	-	+	-	-	+	+		-	-	-	-	-	+	+	+	+
ONPG	nd	+	nd	-	+	nd	nd	nd	nd	-	nd	nd	nd	nd	nd	+	nd	+	nd	+
Esculin	nd	-	nd	-	+	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	-	nd	+	+	+
Acid from:																				
Glucose	+	+	+	+	-	+	-	+	+	+	-	-	-	+	+	+	+	-	+	+
Adonitol	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-
Lactose	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-
Arabinose	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	+	+	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+
Fructose	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	+	+	+
Galactose	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-
Trehalose	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
Mannose	+	+	-	-	-	-	+	-	+	+	-	+	+	-	+	+	-	+	-	+
Ribose	-	+	-	+	+	+	-	-	+	+	-	-	-	-	+	+	+	+	+	-
Cellobiose	-	+	-	+	-	+	-	+	+	-	-	-	-	+	+	-	+	-	-	-
Tentative identification	*Staphylococcus* sp.	*Bacillus cohnii*	*Halomonas* sp.	*Staphylococcus* sp.	*Leucobacter* sp.	*Bacillus* sp.	*Planococcus* sp.	*Sporosarcina* sp.	*Bacillus* sp.	*Bacillus flexus*	*Micrococcus* sp.	*Dietzia* sp.	*Dietzia* sp.	*Cellulomona* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.	*Micrococcus* sp.	*Virgibacillus* sp.	*Bacillus cereus*

*Isolates code: GUFB-SS253-isolate number. # Identification supported by 16S rDNA sequence homology having accession numbers JN315891 – JN315893. Pigmentations are denoted by W: White, LY: Lime yellow, PC: pale cream, B: Beige, BR: Brick red, LO: Light orange; Endospores as T: terminal, Ts: sub-terminal, C: central, and 'nd' as not determined.

useful for future studies involving characterization and retrieval of bacterial cultures using MPN.

In consideration of this, we feel justified in adding the counts of the emerging minute colonies to the total counts of the corresponding station; particularly as the counts of emerging colonies on nutrient rich media are similar to those obtained on 1/10 Zobell's medium. Cohabitation of nutrient competitors in the same environments may occur as a result of generation of nutrient micro-niches, wherein extracellular metabolites play a role in the cellular sensing mechanism - quorum-sensing (Miller and Bassler, 2001).

As stated in the results section, plating of bloom-patch PRB-1 on ZB0.1 pH 5 revealed 10-fold higher bacterial counts than those found in non-bloom waters. While only two isolates showed obligate requirement of pH 5, the majority could grow from pH 5–8. Thus, high-plate counts of such bacteria from the bloom waters of the NAS, which has a pH level varying from 8.29–8.31 (Fig.2c), indicated the ability of these species to also grow in acidophilic conditions. As such conditions exist in the cytoplasm of *Noctiluca* (Nawata and Sibaoka, 1976; Furuya et al., 2006); these may be important physiological species for further study.

The surface DO values are in agreement with previous estimates from this region (DeSousa et al., 1996), and the high primary productivity observed for bloom stations was expected. The only previous bacteriological studies were conducted in a red *Noctiluca* bloom, observed off Mangalore during May 1993 (Nayak et al., 2000). Plate counts from this red *Noctiluca* bloom varied between 3.3×10^2–6.5×10^3 CFU/mL on luminescent agar, while acridine orange direct counts (AODC) ranged from 4.2×10^4–3.7×10^6 cells/mL. In comparison to this coastal bloom, our counts from the visible bloom stations (*DWK and *PRB) in the open waters of the NAS were 3-fold higher.

The overall high culturable bacterial counts in bloom waters from stations *DWK and *PRB in comparison to station DPL, located farthest away from the bloom site, are likely an indication of the role of these heterotrophs in utilization and/or remineralization of organic matter. Further ecological studies pertaining to these green *N. miliaris* blooms are therefore needed to determine the role of bacteria in the context of the monsoon-driven Arabian Sea biogeochemistry (Azam et al., 1994; Wiggert et al., 2005) and intensifying seasonal anoxia along the western continental shelf of India (Naqvi et al., 2000).

In summary, our results showed: (i) Culturing of heterotrophic bacteria from bloom and non-bloom waters of the NAS was dependent on specific NaCl concentration and/or pH, either singly or in combination with low inputs of nutrients. These cohabitating bacteria are referred to as non-halophiles (failing to grow on 3.5% NaCl), marine halophiles (3.5% NaCl), extreme halophiles (25% NaCl), and halotolerant or euryhaline halophiles (0%–25% NaCl), supporting that the strategy devised in the study has a potential to retrieve diverse community from marine environment. (ii) The higher bacterial counts found at stations with bloom compared to those without bloom cannot be merely related to abundance of nutrients. This can possibly be explained as a consequence of development of a niche, wherein the product of one microbe becomes the substrate of another. (iii) The occurrence and isolation of bacteria capable of growth in medium with 25% (w/v) NaCl, and unable to grow in the absence of NaCl, from waters with salinities of ~35–36. This raises the question as to how do these bacteria switch on and off the mechanism to cope with low and high osmolar conditions, to adapt and successfully survive in the changing environment.

These observations, along with the isolation of several *Bacillus* spp., *Cellulomonas* sp., *Staphylococcus* spp., *Planococcus* sp., *Dietzia* spp., *Virgibacillus* sp., *Micrococcus* spp., *Sporosarcinae* sp., *Halomonas* sp., and *Leucobacter* sp. will help in developing strategies for retrieval of culturable bacteria associated with *Noctiluca* blooms in particular, and algal blooms in general. The study is also the first report of extremely euryhaline bacteria occurring in association with an open-ocean high-biomass harmful algal bloom.

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