

Molecular diversity of marine pigmented bacteria in the central Arabian Sea with special reference to antioxidant properties

**Ravindra PAWAR¹, Chellandi MOHANDASS^{1*}, Raju RAJASABAPATHY^{1,2}
and Ram Murti MEENA¹**

¹ Biological Oceanography Division, CSIR-National Institute of Oceanography, Dona Paula - 403004, Goa, India

² Department of Marine Science, Bharathidasan University, Tiruchirappalli – 620024, Tamilnadu, India

***Correspondence:** Dr. C. Mohandass, Biological Oceanography Division,
CSIR-National Institute of Oceanography, Dona Paula - 403004,
Goa, India. E-mail: cmohan@nio.org; Phone: +91-832-2450414; Fax: +91-832-2450606

Abstract

Pigmented bacteria (PIGB) from marine environment are recognized to impart protective action against oxidative stress-induced cell damages. Since very little is known about their diversity and antioxidant potential, we attempted to study the molecular diversity of the culturable PIGB with respect to antioxidant properties. In this study, we isolated 190 pigmented colonies of bacteria (yellow, orange, creamy, pink-red, brown and violet) from central west coast of India (CWCI). The ratio of pigmented and total viable counts of bacteria revealed that the CWCI possess 26.9 to 33.3 % of pigmented bacteria (PIGB). Antioxidant evaluation by a DPPH-radical decolourization assay was revealed that 44.2 % of pigmented bacteria exhibited radical scavenging property. Molecular identification by 16S rRNA gene sequence of the antioxidant potent bacteria showed a wide genetic diversity covering 22 genera affiliated to 4 major phyla, *γ-Proteobacteria*, *α-Proteobacteria*, *Actinobacteria*, and *Firmicutes*. Members of genera *Pseudomonas*, *Serratia*, *Psychrobacter*, *Bacillus*, and *Vibrio* had the highest proportion of antioxidant activity. Sequencing analysis of the PIGB revealed that some strains had <97.0 % similarity with their nearest type strains in GenBank database indicating that they may be novel taxa. This work forms first comprehensive report on the phylogenetic structuring of antioxidative PIGB in CWCI.

Keywords: Pigmented bacteria, antioxidant, 16S rRNA gene, phylogeny, Arabian Sea.

Introduction

The absorption of solar light by seawater organic matter initiates various photochemical reactions and ends up with the formation of radicals, most commonly the reactive oxygen species (ROS). The formation of such reactive radicals in the marine environments is very common and can have adverse effects on living organisms including the tiny bacteria (Horta et al., 2014). In addition to ROS exposure, marine bacteria continuously experience varying environmental conditions that induce secretion of stress relieving metabolites (e.g. peptides, enzymes, pigments, polysaccharides etc.) to adapt the vicinity parameters. The pigment, an eye-catching universal trait plays an important function in the physiology of living organisms including bacteria. It acts as a defensive agent, scavenges harmful free radicals and helps the host to withstand stressful conditions such as UV radiation, heavy metals, anthropogenic pollutants and extreme environments (Cardona-Cardona et al., 2010).

The interest in aquatic heterotrophic pigmented microbes is increasing the attention of researchers due to their potential roles in host physiology, biological and industrial processes (Stafsnes et al., 2010; Pawar et al., 2015). However, the essential prerequisite towards utilization of particular organisms from any location needs to decipher the available diversity of active flora. Culture based investigation of marine bacteria has shown that pigmented bacteria are very diverse and widely distributed (Du et al., 2006). Among the pigmented bacteria, yellow and orange pigments are reported to be higher on the surface of the microalgae (Hollants et al., 2013). Most recently, Leiva et al. (2015) explored pigmented bacteria which belong to the classes *γ-Proteobacteria*, *α-Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Flavobacteria* and *Sphingobacteria*. On the other hand, marine pigmented bacteria have been underexplored with respect to biotechnological applications. Till date, only a few research works have reported the biotechnological potential of marine pigmented bacteria (e.g. Egan et al., 2002; Leiva et al., 2015; Alvarado & Leiva, 2017) with respect to antifouling, antimicrobial and enzymes.

To our knowledge, though studies have documented the vastness of bacterial flora in the Arabian Sea (Singh et al., 2011; Rajasabapathy et al., 2013; Jain et al., 2014), hitherto there is no detailed information exclusively on the availability, types and bioactive potential of pigmented bacteria (PIGB) along the Indian coasts. Therefore, in this investigation, we explored quantitative estimation and the ratio of pigmented bacteria with non-vivid bacteria, antioxidant properties of pigmented bacteria and their molecular based phylogenetic relationship.

Materials and Methods

Description of sampling sites and collection strategy

Water, sediment and seaweeds were sampled along the central west coast of India (CWCI) for pigmented bacteria covering 270 km² area (Figure 1). The sampling stations include Kunkeshwar (16° 20' 02.4" N; 73° 23' 31.3" E), Malwan (16° 03' 47.5" N; 73° 27' 22.5" E) from Maharashtra state and Vagator (15° 35' 54.4" N; 73° 44' 14.6" E) and Cabo De Rama (15° 05' 25.37" N; 73° 55' 15.15" E) from Goa. These coasts are composed of an open coastal ecosystem with dominated sedimentary rocky outcrops over long stretches. It represents natural variations in seashore properties like water, rock beds and sediment texture. They are rich in nutrients, salts, minerals and are known to harbor abundant prokaryotic and eukaryotic organisms (Das et al., 2006). One of the key interests in assessing the chosen locations along CWCI for the present study is to bring out the data on the PIGB diversity from the central Arabian Sea which is not updated or investigated in recent past. Further, this paper also indicates the bio-resources of antioxidants from the central Arabian Sea.

The west coast of India experiences variable environmental conditions due to the southwest monsoon. This results in high wave conditions during raining season whereas, the rest of the period coast remains calm throughout the year (Kumar et al., 2006). This, in turn, brings out variation in ecological parameters and affects the living organisms as well. Thus, to interpret the effect of such changing environment the sampling was carried out during different seasons. The seawater, sediment and seaweeds were collected seasonally during March-2010 to January-2011 and March 2012 to January- 2013 from selected sites. The water samples were collected straight from the above coastal sites using sterile plastic bottles. Sediment samples were collected with the help of core while the seaweed samples as attached healthy plants (4-5 dominant species/ each site/ season) picked with sterile forceps from the intertidal region during ebb tide. The seaweed samples were added to the little amount of seawater from the same vicinity to maintain the viability of associated flora. All collected samples were stored in cold condition and transported to the laboratory at the earliest for further analysis.

Isolation of pigmented bacteria

The seawater and sediment samples were serially diluted and plated preferably by spread plate method. The isolation of epiphytic bacteria from 28 seaweed samples (9 Chlorophyta, 12 Phaeophyta and 7 Rhodophyta) was carried out by shake flask method (Lakshmanaperumalsamy & Purushothaman, 1982). In detail, each seaweed sample was rinsed with sterile seawater to remove debris and unattached bacteria. Approximately 10 g (wet weight) of seaweed suspended in 100 ml of

sterile seawater in a sterile conical flask and consequently kept for shaking at 120 rpm for 20 min in a shaker incubator (MaxQ 4000, Thermo Scientific). Finally, the supernatant was serially diluted and spread plated in duplicates on Zobell Marine Agar (ZMA) and 50 % seawater nutrient agar (SWNA). Plates incubated at 28 ± 2 °C for 24 – 72 hrs and observed for the appearance of pigmented colonies. The viable bacterial count (VBC) and viable pigmented counts (VPC) were recorded in terms of colony forming units (CFU). PIGB cultures were purified individually by streak plate method and stored in 25 % glycerol vials at -80°C.

Antioxidant evaluation

A DPPH-radical decolourization screening assay of Takao et al. (1994) was followed for initial antioxidant testing of pigmented bacteria. Pure culture suspension broth (2 µL) of individual pigmented bacteria (PIGB 1-190) was drop inoculated on marine agar plates and incubated at temperature 28 ± 2 °C for 24 h. Later, a sterile filter paper (Whatman no. 1) was then placed on the agar plate carefully, so that colony and its extracellular metabolites get replicated on the paper. Incubation was further continued for another 24 h. Then the filter paper was taken out with the help of sterile forceps, dried and consequently sprayed with DPPH solution (80 µg ml⁻¹ in methanol). Strains showing a yellowish/white zone on the purple background considered as antioxidant positive. Colony size and the antioxidant-DPPH decolorization zones were recorded for each of the analyzed cultures.

16S rRNA gene sequencing and phylogenetic analysis

Each pure isolate of antioxidant potent PIGB was inoculated in 10 ml of Zobell Marine broth and incubated at 28 °C for 24 h or until the cell density reached 10⁶ cells ml⁻¹. The bacterial cells were obtained by centrifugation (10000 rpm/10 min) and the genomic DNA was isolated using DNeasy Blood & Tissue kit (Qiagen) following the manufacturer's instructions. PCR amplification of 16S rRNA gene was carried out using the universal 27F and 1492R bacterial primers (Lane, 1991). The amplified products (~1500 bp) were purified and gel eluted using Qiaquick gel extraction kit according to the manufacturer's protocol. 16S rDNA amplicons were sequenced on an automatic sequencer (Applied Biosystems 3130xl Genetic Analyzer) with the bacterial primers 27F, 518F and 1492R.

The 16S rRNA gene sequences obtained during this study were compared with the NCBI nucleotide database using BLASTn (www.ncbi.nlm.nih.gov) and EzTaxon tool (Kim et al., 2012). The 16S rDNA Sequence similarity of ≥ 99 % was considered as a phylotype. The PINTAIL program 1.0 and Decipher were used to check chimera and no chimeric sequences were detected

from our sequences (Ashelford et al., 2005). Sequence alignment and comparison was performed using multiple sequence alignment program Clustal W (Thompson et al., 1994). The gene sequences were submitted to the GenBank with the accession numbers KU363987-KU364059, JX915781-JX915783, KJ396267-KJ396269, KJ651245, KJ651248, KJ651250, KJ651252 and KU375126. Identified bacterial genera and affiliated phyla were clubbed based on their percentage contributions to the overall diversity of pigmented bacteria. Neighbor-joining method was applied to construct the Phylogenetic tree using the MEGA5 software. The evolutionary distance was calculated by maximum likelihood method (Tamura et al., 2011). The topology of the phylogenetic tree was statistically evaluated by bootstrap analysis with 1,000 replications and branches supporting >50 % node values were considered significant.

Statistical analysis

(a) Rarefaction analysis for species richness was performed by plotting the number of phylotypes against total number of isolates using EcoSim 700 software (Gotelli & Entsminger, 2004). Shannon diversity index and evenness were calculated using online program (<https://www.easycalculation.com/statistics/shannon-wiener-diversity.php>). Good's coverage of bacterial isolates was calculated using the formula $C = [1 - \left(\frac{n1}{N}\right)] * 100$ where C is the homologous coverage, n1 is the number of species appearing only once, and N is the total number of isolates observed. (b) The bacterial community structure was analyzed by clubbing majorly occurred bacterial genera using hierarchical agglomerative clustering as well as nonmetric multidimensional scaling (nMDS) using the Bray-Curtis similarity matrix (Clarke et al., 2001). (c) The formed clusters/ groups were further analyzed using the similarity percentage (SIMPER) programme to identify the species contributing to the similarity within the group (indicator species) and those species responsible for dissimilarity between groups (discriminating species). The average similarity (dissimilarity)/standard deviation ratio and percentage similarity/dissimilarity contribution data was used to find the Indicator and discriminating species. It was based on the statistical assumption that species with large similarity/dissimilarity percentage and small standard deviation (SD) value will have large similarity (dissimilarity)/standard deviation ratio.

Results

Counts and retrievable of pigmented bacteria

The pigmented counts were enumerated separately from seawater, sediment and seaweed samples and their ratio with non-vivid viable bacteria summarized in Table 1. Among total viable bacteria, the chromogenic population was retrieved 2-3 orders lower (10^2 - 10^3) than the non-pigmented ones

(10^4 - 10^6). Seawater and sediments evidenced to hold the maximum number of PIGB up to 10^4 CFU during monsoon which consequently reduced to 10^2 CFU ml⁻¹ in late post-monsoon (January) and early pre-monsoon (March) months. Though the sediments hold higher viable counts ($\sim 10^6$ gm⁻¹), fewer numbers of pigment producers were observed (0.02 - 36.3×10^3 gm⁻¹) than seawater samples. In case of seaweeds, species like *Ulva*, *Caulerpa* (Chlorophyta) and *Sargassum* sp. (Phaeophyta) observed to hold more number of bacteria (VBC = 10^5 - 10^6 ; VPC = $\sim 10^4$ CFU g⁻¹) whereas *Gracilaria*, *Hypnea* and *Porphyra* (Rhodophyta) harbored a low population of PIGB (i.e. 10^2 cells g⁻¹). Seaweeds collected during post-monsoon months (Sept.-Jan.) were lodged with a greater number of epibiotic bacteria as compared to monsoon and pre-monsoon months.

The overall bacterial populations categorized based on the colour property showed a distinct range of chromogens (Figure 2). Bacteria with yellow pigment (40 %) dominated the overall population of PIGB in the CWCI followed by orange (26.32 %), creamy (21.05 %), pink (5.26 %), brown (5 %), red (1.58 %) and violet (1 %). Seasonal wise grouping showed greater abundance of yellow PIGB during pre-monsoon (59 %) which consequently decreased during monsoon (46 %) and post-monsoon (24 %) seasons (Figure 3 a). The orange colored colonies appeared during pre-monsoon and monsoon months with 21 %, was elevated in the post-monsoon season by 13 % (i.e. 34 %). In contrast, as demonstrated in Figure 3 b, the creamy isolates showed exact opposite trend, where the monsoon and post-monsoon seasons supported in retrieving 24-25 % got suppressed by 12 % during pre-monsoon. Notably, rare eye-catching red, pink and violet pigmented bacterial strains showed their considerable appearance during the late monsoon to early pre-monsoon seasons and were mostly associated with seaweed samples (Figure 3 a, b).

Antioxidant properties of PIGB

The antioxidant screening results on pigmented bacteria are depicted in Table 2. It demonstrated 44.2 % of the PIGB secrete antioxidant metabolites in their extracellular environment. Among which, 7.4 % potent isolates showed greater than 2 cm while 3.9 % isolates retained with > 3 cm antioxidant zones. Organisms with 0-1 cm AO activity zones were considered as less active. Strains isolated from seaweeds (36 isolates) and seawater (34 isolates) contributed three fourth of the population with positive AO activity. PIGB isolates with AO properties were very less in the sediment samples and could contribute only 7.4 %.

16S rRNA based phylogenetic analysis of antioxidant potent PIGB

Out of 190 pigmented bacteria, we observed that 44.21 % (n=84) of PIGB are antioxidant producing isolates. Phylogenetic relationships of these 84 isolates were studied through 16S rRNA gene

sequencing technique. Identification and phylogenetic affiliations are summarized in Table 2, Figure 4 and Figure 5. Phylogenetic analysis revealed 22 distinct PIGB genera affiliating to four phyla (γ -*Proteobacteria*, α -*Proteobacteria*, *Actinobacteria* and *Firmicutes*). Bacteria belonging to *Pseudomonas* (14.3 %), *Halomonas* (10.7 %), *Serratia* (17.9 %) and *Psychrobacter* (13.1 %) jointly contributed 56 % of AO potent PIGB. Looking at higher order classification, the phyla *Proteobacteria* alone contributed 72.6 % of pigmented isolates followed by *Firmicutes* with 23.8 % (Figure 5). Remaining 3.6 % of the AO potent PIGB was belonging to the group *Actinobacteria*.

Species richness and similarity index

The diversity indices like Shannon, Evenness and Coverage values are 3.17, 0.87 and 69.04 respectively for the overall PIGB in CWCI. Species richness of PIGB is higher which is given as rarefaction curve in Figure 6 a. The diversity of PIGB as discussed earlier was influenced by seasons and source of isolation. The nMDS and SIMPER tests demonstrated considerable differences among the clubbed bacterial groups especially those collected from different seasons (Figure 6 b). The cluster-I clubbed *Halomonas* and *Pseudomonas* with 80 % similarity in abundance during pre-monsoon and monsoon season whereas, the *Psychrobacter* and *Vibrio* sp. showed near about similar occurrence during monsoon and post-monsoon seasons (Cluster-II). It hallmarks the above bacteria as an indicator species that endures and represent dominant flora of a particular season. Organisms clubbed in cluster-III demonstrated 60 % chances that the *Halomonas*, *Pseudomonas*, *Psychrobacter*, *Vibrio*, *Serratia*, *Planococcus* sp. may occur during all the seasons along CWCI.

Discussion

Pigment ‘the colouring substance’ is considered to be one of the most conspicuous traits in live forms. Their diverse nature helps them to take part in various physiological and molecular processes like colouration, photosynthesis and protection against cell damage (Cardona-Cardona et al., 2010). During last decade, researchers are keenly engaged in drug screening programs to demonstrate the bio-pharmaceutical potential of marine organisms for the betterment of human health (e.g. ‘Drugs from Sea’) (Kijjoa & Sawangwong 2004). In this study, we isolated diverse pigmented bacteria from various sources (seawater, sediments and seaweeds from CWCI) and explored their diversity in terms of antioxidant potent of PIGB.

Earlier studies by Du et al. (2006) documented the diversity of heterotrophic pigmented bacteria from the Pacific Ocean and China Sea at the surface, subsurface and depth water samples. They found surface waters have the higher number of pigmented bacteria (VPC/VBC = 31.4-35%) and the counts keep decreasing with increasing depth samples. In the current study, the PIGB in terms of the

VPC/VBC ratio were retrieved higher from seawater (33.3%) followed by intertidal seaweeds and sediment samples. This is a clear indication that most of the PIGB are surface dwellers and prefers the light reaching areas for the production of pigment.

Previous reports were documented 15 to 50 % of the PIGB to the overall population of bacteria in the marine environments (Zobell & Feltham 1934; ZoBell & Upham, 1944; Du et al., 2006). The distinct number of PIGB experienced from algal species can be attributed towards the morphology and seasonal occurrence of individual host algae species, as the responsible factors that determine the overall population of associated bacteria (Burke et al., 2011). For example, we observed *Chaetomorpha*, *Sphacelaria* and *Gelidium* with less surface area supported a small number of PIGB than *Padina* and *Sargassum*. Notably, some algal species (e.g. *Sargassum* sp.) collected from different locations had varied numbers of viable (9.3 to 94.3×10^4 CFU g⁻¹) and pigmented bacteria (5.34 to 86.7×10^2 CFU g⁻¹). It may be emphasized that the survival of PIGB is not only affected by the season and sample source but possibly influenced by the environmental conditions of a particular location where the host resides.

The pure culturing of PIGB led consolidated collection of 190 distinct isolates from the CWCI waters (Table 1). Among them, 87 PIGB from seaweeds, 64 PIGB from seawater and 39 PIGB from sediments were retrieved. From this observation, it may be inferred that the existence and distribution of chromogenic bacteria in this ecosystem is highly influenced by the amount of light and nutrients. Du et al. (2006) studied pigmented heterotrophic bacteria from oceanic environments and realized that such bacteria endure more in surface waters than sub-surface levels. They further observed decreased frequencies of PIGB with increasing depth samples. Our observations were also in supportive of that where we could recover more pigment producers from seawater and intertidal seaweeds than the bottom sediment.

Yellow pigmented bacteria dominated the overall population of PIGB followed by orange, creamy, pink, brown, red and violet. These results matched with an early investigation of Zobell & Feltham (1934) who showed the occurrence of yellow, orange, brown and red coloured bacteria in the order of $31.3 > 15 > 9.9 > 5.5$ % respectively. Likewise, a more recent study by Jafarzade et al. (2013) also reported a similar trend in the occurrence of coloured bacteria in marine ecosystems of Malaysian coast. In their study, 55 pigmented isolates from diverse resources (seawater, sediment, sponge, mangrove associates) were discussed and demonstrated that the yellow and orange bacteria could cumulatively dominate 72 % of the coloured population. However, very few studies have been conducted globally on diversity and distribution of PIGB, and this forms the first report from the Indian Ocean region.

The CWCI water in pre-monsoon exposes to higher temperatures (~28-33 °C) and evaporation increases the salinity (~30-36 PSU). Researchers have revealed that the bacteria utilize pigment as a light-driven energy pumps and also act as a physiological metabolite in response to halotolerant conditions (De Carvalho & Fernandes, 2010; Soliev et al., 2011). During the present study, the higher number of pigment producers, in particular, the yellow ones in the surface waters is an indication that such pigments help the bacteria to adapt high temperature and salinity. Conversely, Stafsnes et al. (2010) documented the role of bacterial pigments (carotenoids) in scavenging the UV light. Although we did not record the UV radiation along the CWCI in particular during the sampling time, interestingly the high retrieval of yellow pigmented bacteria shows the possible contribution of UV protective carotenoid producers in this region. Considering the effect of the seasonal switch, PIGB showed a linearly increasing trend from pre-monsoon < monsoon < post-monsoon in their abundance. In this study, we would like to highlight that the isolation of maximum and distinct chromogens can be achieved during the month of September to January and especially from the seaweed surfaces. Jayant et al. (2002) isolated 162 pigmented strains from different source samples (east coast of India) for studying antimicrobial properties. However, there was no detailed information on the colour property as well as its variation with seasons and source of isolation.

The AO potent strains when looked in terms of their pigment property, the antioxidant zones followed the order pink/red (1.9-4.9 cm) > yellow/brown (1.6-4.3 cm) > orange (2.8 cm) > creamy (0.2-1.9). Some of the above pigmented bacteria are reported in our recent communications where we concluded that the brown/ yellow/ pink coloured bacteria ascertain greater AO potentials in comparison to other colour forms (Pawar et al., 2015 & 2016). In addition, recent studies on individual pigmented bacteria with red (Horta et al., 2014), yellow (Mohana et al. 2013) and orange (Shindo & Misawa, 2014) form documented with higher radical scavenging effects which are further support to the present study. Interestingly, another important observation during this study was, most of the potent bacteria produced diffusible pigments in culture plate (data not shown) which indicates that such pigmented compounds may be of polar in nature and contribute higher order reactivity than the non-diffusible ones.

Also, as shown in Table 2 the AO property recorded with reference to bacterial identity made us to realize that *Pseudomonas*, *Vibrio* (yellow/brown), *Serratia* (Red/pink), *Bacillus/Salinicoccus/Staphylococcus* (orange) with >2.5 cm AO zones express considerable quantity of antioxidants and contributed more positive AO strains. *Halomonas* and *Psychrobacter* had lower AO activity. Among these PIGB 46, 77, 88 and 184 have confirmed their potential over synthetic antioxidants like Butylated hydroxytoluene (BHT), quercetin and ascorbic acid (Pawar et al., 2015 &

2016). Horta et al. (2014) observed similar findings on the marine *Vibrio* and *Serratia* species isolated from Portugal coast which recorded considerable AO properties. Thus, more comprehensive studies on antioxidant bacteria mentioned in Table 1 could give promising resource of natural antioxidant compounds.

Various studies on bioactive compounds from marine bacteria explored various antioxidant compounds. Pigment types like astaxanthin (Pink) from *Agrobacterium aurantiacum* (Misawa et al., 1995; Konzen et al., 2006), Violacein (Violet) from *Chromobacterium violaceum* and *Pseudoalteromonas luteoviolacea* (Duran & Menck, 2001; Yang et al., 2007), Carotenoid (Red) from *Planococcus maritimus* (Shindo et al., 2008), Deinoxanthin (Red) from *Dienococcus* spp. (Lavy et al., 2005; Ji, 2010), Zeaxanthin (Yellow) from *Flavobacter* sp. (Prabhu et al., 2013), Carotenoids (Yellow/ Red) from *Rubritalea*, *Planococcus*, *Flavobacter* sp. (Shindo and Misawa, 2014), and many unknown pigment types (Shindo & Misawa, 2014; Nongkhlaw & Joshi, 2015) were proved to have antioxidant compounds with various clinical interest. In addition, further research activities of our own pigmented bacteria are under progress especially PIGB with >2 cm AO zone in the plate assay. One of them designated PIGB184 (*Vibrio* sp.) was already reported as a good candidate for the production of antioxidant compounds (Pawar et al., 2016).

A study on marine pigmented bacteria from Kinko Bay (Japan) had shown the predominance of the γ -*Proteobacteria* group (Kakimoto et al. 1980). Du et al. (2006) reported supremacy of γ -*proteobacteria* (78.7 %) in tropical North Pacific Oceanic waters. Our study from Indian coastal locations also reports similar findings on the distribution of pigmented bacteria on this particular group. Contrastingly, literature evidenced the occurrence of *Actinobacteria* and *Firmicutes* groups in specified environments like oxygen minimum zones, bays and estuaries (Nithya & Pandian, 2010; Divya et al., 2010). A more relevant investigation by Du et al. (2006) explored the diversity of pigmented bacteria with 37 *Actinobacteria* and 62 *Firmicutes* strains from Yangtze River Estuary, South China Sea, East China Sea and Eastern Tropical North Pacific Ocean. Fascinatingly, among these two groups, they retrieved 16 *Actinobacteria* and 56 *Firmicutes* from estuary alone. Whereas in this investigation, out of 190 pigmented isolates 15 of them belongs to *Actinobacteria* and 32 belong to *Firmicutes*. This may be due to the limitation of this study because we didn't focus to isolate Gram-negative bacteria alone.

The antioxidative pigmented bacteria were clubbed the active flora into 4 phylogenetic classes (γ -*Proteobacteria*, α -*Proteobacteria*, *Actinobacteria* and *Firmicutes*), 22 genera and 39 phylotypes. Studies by Du et al. (2006) revealed 25 genera of six phylogenetic classes, Flavobacteria and Sphingobacteria were additions to this study. Neighbor-joining phylogenetic tree well supported this

result (Figure 4). It was clearly indicated that *Serratia* contributes plethora (15 out of 84) of AO chromogenic population. Conversely, the isolates branched together in the tree may be secreting similar kind of metabolites. The gene level difference might intern translate novel antioxidant metabolites and hence these particular organisms are having greater prospects for antioxidant research. Based on 16S rRNA gene sequence of the antioxidant positive strains, isolates like PIGB76 and PIGB156 may form novel genera, whereas PIGB117, PIGB60, PIGB87, PIGB88 and PIGB46 may form novel species. However, additional experiments on biochemical and chemotaxonomic properties may give a clear picture of these novel strains.

The SIMPER analyses made us realize that *Serratia*, *Psychrobacter* and *Planococcus* sp. have 72.04 % average similarity during monsoon (Sim/SD (δ) = 9) and post-monsoon (Sim/SD (δ) = 23.36) seasons. Likewise, the major types of bacteria isolated from pre-monsoon (*Bacillus*, *Pseudomonas* and *Halomonas*) and monsoon/post-monsoon (*Vibrio*, *Serratia*, *Psychrobacter* and *Planococcus*) gave 49.16 % average dissimilarity during monsoon (Dis/SD (δ) = 3.42). Conversely, the statistical analyses concerning sample source had no considerable variations among the above dominant bacterial groups and the only exception was *Staphylococcus* sp. which formed a separate cluster away from all the other bacteria (data not shown). It might be due to its common endurance in sediment samples. Overall statistical analyses supported our views on the season-specific switch by particular bacteria and established significant δ values.

In conclusion, this study highlights the importance of pigmented bacteria and their antioxidant metabolites in the area of marine microbiology and blue biotechnology. The diversity of PIGB revealed that the yellow and orange pigmented bacteria dominate the chromogenic population in CWCI waters throughout the seasons. Our results suggest that violet and red-coloured organisms are more abundant during post-monsoon months, especially from seaweed surfaces. Considerable radical scavenging responses, particularly by the *Pseudomonas*, *Psychrobacter*, *Bacillus*, *Serratia* and *Vibrio* spp. suggests future work which will be directed towards investigating the chemical nature of the pigmented compounds and their physiological and ecological functions.

Acknowledgements

Authors would like to thank the Director of NIO, Dr. TG. Jagtap, Dr. N. Ramaiah and Dr. P. Vethamony for providing lab facility support. Ms. Renu Bankar is acknowledged for her technical help. This work was supported by the CSIR grant projects NWP0018 and PSC0206. This is NIO contribution number ----.

References

- Alvarado R. & Leiva S. (2017). Agar-degrading bacteria isolated from Antarctic macroalgae. *Folia Microbiologica*, **62**: 409-416.
- Ashelford K.E., Chuzhanova N.A., Fry J. C., Jones A.J. & Weightman A.J. 2005. At least 1 in 20 16S rRNA sequence records currently held in public repositories is estimated to contain substantial anomalies. *Applied and Environmental Microbiology*, **71**: 7724–7736.
- Burke C.M., Thomas T., Lewis M., Steinberg P.D. & Kjelleberg S. 2011. Composition, uniqueness and variability of the epiphytic bacterial community of the green alga *Ulva australis*. *ISME Journal*, **5**: 590–600.
- Cardona-Cardona V., Arroyo A., Scellekens J. & Rios-velazquez C. 2010. Characterization of blue pigmented bacteria isolated from Puerto Rico. In: *Current research, technology and education topics in applied microbiology and microbial biotechnology* (ed. Mendez-vilas A.), pp. 117-123. Formatex Research Center.
- Clarke K.R. & Warwick R.M. 2001. Change in marine communities: An approach to statistical analysis and interpretation, PRIMER-E. Plymouth UK, 2nd edn.
- Das S., Lyla P.S. & Khan A.S. 2006. Marine microbial diversity and ecology: importance and future perspectives. *Current Science*, **90**: 1325–1335.
- De Carvalho C.C.C.R. & Fernandes P. 2010. Production of metabolites as bacterial responses to the marine environment. *Marine Drugs*, **8**: 705-727.
- Divya B., Soumya K.V. & Nair, S. 2010. 16S rRNA and enzymatic diversity of culturable bacteria from the sediments of oxygen minimum zone in the Arabian Sea. *Antonie van Leeuwenhoek*, **98**: 9–18.
- Du H.L., Jiao N.Z., Hu Y.H. & Zeng Y.H. 2006. Diversity and distribution of pigmented heterotrophic bacteria in marine environments. *FEMS Microbiology Ecology*, **57**: 92–105.
- Duran N. & Menck C.F.M. 2001. *Chromobacterium violaceum*: a review of pharmacological and industrial perspectives. *Critical Reviews in Microbiology*, **27**: 201-222.
- Egan S., James S., Holmstrom C. & Kjelleberg S. 2002. Correlation between pigmentation and antifouling compounds produced by *Pseudoalteromonas tunicata*. *Environmental Microbiology*, **4**: 433–442.
- Gotelli N.J. & Entsminger G.L. 2004. Ecosim: Mull models software for ecology, version 7. Acquired Intelligence Inc. and Kesey-Bear, Burlington, Vermont, USA.
- Hollants J., Leliaert F., De Clerck O. & Willems A. 2013. What we can learn from sushi: a review on seaweed-bacterial associations. *FEMS Microbiology Ecology*, **83**: 1–16.
- Horta A., Pinteus S., Alves C., Fino N., Silva J., Fernandez S., Rodrigues A. & Pedrosa R. 2014. Antioxidant and antimicrobial potential of the *Bifurcaria bifurcata* epiphytic bacteria. *Marine Drugs*, **12**: 1676–1689.

- Jafarzade M., Yahya N.A., Mohamad S., Usup G. & Ahmad A. 2013. Isolation and characterization of pigmented bacteria showing antimicrobial activity from Malaysian marine environment. *Malaysian Journal of Microbiology*, **9**: 152–160.
- Jain A., Bandekar M., Gomes J., Shenoy D., Meena R.M., Naik H., Khandeparkar R. & Ramaiah N. 2014. Temporally invariable bacterial community structure in the Arabian Sea oxygen minimum zone. *Aquatic Microbial Ecology*, **73**: 51–67.
- Jayanth K., Jeyasekaran G. & Jeya Shakila R. 2002. Isolation of marine bacteria, antagonistic to human pathogens. *Indian Journal of Marine Science*, **31**: 39–44.
- Ji H.F. 2010. Insight into the strong antioxidant activity of deinoxanthin, a unique carotenoid in *Deinococcus radiodurans*. *International journal of Molecular Sciences*, **11**: 4506–4510.
- Kakimoto D., Maeda H., Sakata T., Sharp W. & Johnson R.M. 1980. Marine pigmented bacteria-I Distribution and characteristics of pigmented bacteria. *Memoirs of Faculty of Fisheries Kagoshima Univ*, **29**: 339–347.
- Kijjoa A. & Sawangwong P. 2004. Drugs and Cosmetics from the Sea. *Marine Drugs*, **2**: 73–82.
- Kim O.S., Cho Y.J., Lee K., Yoon S.H., Kim M., Na H., Park S. C., Jeon Y.S., Lee J.H., Yi H., Won S. & Chun J. 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. *International Journal of Systematic and Evolutionary Microbiology*, **62**: 716–721.
- Konzen M., Marco D.D., Cordova C.A.S., Vieira T.O., Antonio R.V., & Creczynski-Pasa T.B. 2006. Antioxidant properties of violacein: possible relation on its biological function. *Bioorganic and Medicinal Chemistry*, **14**: 8307–8313.
- Kumar V.S., Pathak K.C., Pednekar P., Raju N.S.N. & Gowthaman R. 2006. Coastal processes along the Indian coastline. *Current Science*, **91**: 530–536.
- Lakshmanaperumalsamy P. & Purushothaman A. 1982. Heterotrophic bacteria associated with seaweed. *Proceedings of the Indian Academy of Sciences*, **91**: 487–493.
- Lane D.J. 1991. 16S/23S rRNA sequencing. In *Nucleic acid techniques in bacterial systematics* (eds. Stackebrandt, E. & Goodfellow, M.). pp. 115–175. John Wiley and Sons Inc, New York.
- Lavy A., Neeman Y., & Fuhrman B. 2005. The antioxidative effect of the bacteria *Dienococcus radiophilus* against LDL lipid peroxidation. *European Journal of Nutrition*, **44**: 281–284.
- Leiva S., Alvarado P., Huang Y., Wang J. & Garrido I. 2015. Diversity of pigmented Gram-positive bacteria associated with marine macroalgae from Antarctica. *FEMS Microbiology Letters*, **362**: fnv206.
- Misawa N., Satomi Y., Kondo K., Yokoyama A., Kajiwara S., Saito T., Ohtani T. & Miki W. 1995. Structure and functional analysis of a marine bacterial carotenoid biosynthesis gene cluster and astaxanthin biosynthetic pathway proposed at the gene level. *Journal of Bacteriology*, **177**: 6575–6584.

- Mohana D.C., Thippeswamy S. & Abhishek R.U. 2013. Antioxidant, antibacterial, and ultraviolet-protective properties of carotenoids isolated from *Micrococcus* spp. *Radiation Protection and Environment*, **36**: 168–174.
- Nithya C. & Pandian S.K. 2010. Isolation of heterotrophic bacteria from Palk Bay sediments showing heavy metal tolerance and antibiotic production. *Microbiological Research*, **7**: 578-593.
- Nongkhlaw F.M.W. & Joshi S.R. 2015. L-asparaginase and antioxidant activity of endophytic bacteria associated with ethnomedicinal plants. *Indian Journal of Biotechnology*, **14**: 59-64.
- Pawar R.T., Mohandass C., Sivaperumal E., Sabu E., Rajasabapathy R. & Jagtap T.G. 2015. Epiphytic marine pigmented bacteria: a prospective source for natural antioxidants. *Braz. J. Microbiol.*, **46**: 29–39.
- Pawar R., Mohandass C., Dastager S.G., Kolekar Y.M. & Malwankar R. 2016. Antioxidative metabolites synthesized by marine pigmented *Vibrio* sp. and its protection on oxidative deterioration of membrane lipids. *Applied Biochemistry and Biotechnology*, **179**: 155–167.
- Prabhu S., Rekha P.D. Young C.C., Hameed A., Lin S.Y. & Arun A.B. 2013. Zeaxanthin production by novel marine isolates from coastal sand of India and its antioxidant properties. *Applied Biochemistry and Biotechnology*, **171**: 817-831.
- Rajasabapathy R., Mohandass C., Vijayaraj A.S., Madival V.V. & Meena R.M. 2013. Iron bacterial phylogeny and their execution towards iron availability in Equatorial Indian Ocean and coastal Arabian Sea. *Polish Journal of Microbiology*, **62**: 391–400.
- Shindo K., Endo M., Miyake Y., Wakasugi K., Morrith D., Bramley P.M., Fraser P.D., Kasai H., & Misawa N. 2008. Methyl glucosyl-3,4-dehydro-apo-8'-lycopenoate, a novel antioxidative glyco-C30-carotenoic acid produced by a marine bacterium *Planococcus maritimus*. *Journal of Antibiotics*, **61**: 729-735.
- Shindo K. & Misawa N. 2014. New and rare carotenoids isolated from marine bacteria and their antioxidant activities. *Marine Drugs*, **12**: 1690–1698.
- Singh S.K. & Ramaiah N. 2011. Denaturing gradient gel electrophoresis profiling of bacterial communities composition in Arabian Sea. *Journal of Environmental Biology*, **32**: 339–346.
- Soliev A.B., Hosokawa, K. & Enomoto K. 2011. Bioactive Pigments from Marine Bacteria: Applications and Physiological Roles. *Evidence-Based Complementary and Alternative Medicine*, 670349.
- Stafsnes M.H., Josefsen K.D., Kildahl-Andersen G., Valla S., Ellingsen T.E. & Bruheim P. 2010. Isolation and characterization of marine pigmented bacteria from Norwegian coastal waters and screening for carotenoids with UVA-Blue light absorbing properties. *Journal of Microbiology*, **48**: 16–23.
- Takao T., Kitatani F., Watanabe N., Yagi A. & Sakata K. 1994. A simple screening method for antioxidants and isolation of several antioxidant produced by marine bacteria from fish and shellfish. *Bioscience Biotechnology and Biochemistry*, **58**: 1780–1783.

- Tamura K., Peterson D., Peterson N., Stecher G., Nei M. & Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology Evolution*, **28**: 2731–2739.
- Thompson J.D., Higgins D.G. & Gibson T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**: 4673–4680.
- Yang L.H., Xiong H., Lee O.O., Qi S.H. & Qian P.Y. 2007. Effect of agitation on violacein production in *Pseudoalteromonas luteoviolacea* isolated from a marine sponge. *Letters in Applied Microbiology*, **44**: 625-630.
- Zobell C.E. & Feltham C.B. 1934. Preliminary studies on the distribution and characteristics of marine bacteria. *Bulletin of the Scripps Institution of Oceanography Technical Series*, **3**: 279–296.
- ZoBell C.E. & Upham H.C. 1944. A list of marine bacteria including descriptions of sixty new species. *Bulletin of the Scripps Institution of Oceanography University of California*, **5**: 239–292.

Table 1. Abundance and number of pigmented bacteria isolated from different sample sources and seasons along CWCI

Source	Viable bacterial count (VBC) (CFU × 10 ⁴ ml ⁻¹ or g ⁻¹)			Viable pigmented count (VPC) (CFU × 10 ³ ml ⁻¹ or g ⁻¹)			Ratio VPC/VBC (%)			No. of PIGB isolated and percentage contribution ^a	Antioxida nt producers ^b	
	Pre-M	M	Post-M	Pre-M	M	Post-M	Pre-M	M	Post-M			
Seawater	0.03-2.6	0.8-35	0.02-38	0.1-7.0	0.06- 11.5	0.05-1.5	26.9- 33.3	0.75- 3.28	0.39- 27.5	64 (33.7%)	34 (40.4%)	
Sediment	0.9-20	1.5- 155	0.12- 25.4	0.27-9.5	0.15- 36.3	0.02-3.5	3-4.75	1-2.33	1.37- 1.67	39 (20.5%)	14 (16.7%)	
Seaweeds	Chlorophyt a	0.61-7.7	0.2-1	5.1-238	0.19- 0.36	0.11- 1.13	6.33- 15.6	0.46- 14.9	5.5-11.3	0.66- 12.4	87 (45.8%)	36 (42.9%)
	Phaeophyta	5-10.3	9.3- 12.6	14.7- 36.3	0.53- 2.63	0.73- 1.03	1.1-8.67	1.06- 2.55	0.78- 0.81	0.75- 2.38		
	Rhodophyt a	1-4.7	1.5- 17.6	3.2-59	0.4-1.5	1.5-8.3	0.8-1.95	3.19-4	4.71-10	0.33-2.5		

Pre-M: Pre-monsoon, M: Monsoon, Post-M: Post-monsoon. PIGB: Pigmented bacteria. CFU: Colony forming unit values are presented in terms of ml⁻¹ for seawater and g⁻¹ for sediment and seaweed samples. All the values are normalized to the decimal unit. The VBC, VPC, VPC/VBC ratio and PIGB in case of seaweed samples varied from species to species and values presented here are based on the minimum and maximum counts of a particular group. Percentage contribution calculated based on the total number of PIGB = 190^a and antioxidant producers = 84^b.

Table 2. Antioxidant analysis and 16S rRNA based molecular identification of AO potent PIGB

Culture code	Source	Pigment type	AO zone (cm)*			16S rRNA based identity	Culture code	Source	Pigment type	AO zone (cm)*			16S rRNA based identity
			a	b	b-a					a	b	b-a	
PIGB1	<i>G. micropterum</i>	Orange	0.4	3.1	2.7	<i>S. roseus</i>	PIGB87	<i>Ulva</i> sp.	Creamy	0.5	1.3	0.8	<i>S. marcescens</i>
PIGB4	Seawater	Yellow	0.4	1.5	1.1	<i>P. azotoformans</i>	PIGB88	<i>Ulva</i> sp.	Pink	0.5	5.4	4.9	<i>S. rubidaea</i>
PIGB5	Seawater	Pale yellow	0.4	1.7	1.3	<i>Pseudomonas</i> sp.	PIGB91	Seawater	Pale orange	0.8	1.2	0.4	<i>S. marcescens</i>
PIGB6	Seawater	Red	0.4	1.0	0.6	<i>M. vinifirmus</i>	PIGB92	<i>G. corticata</i>	Orange	0.6	1.8	1.2	<i>S. nematodiphila</i>
PIGB10	<i>G. corticata</i>	Yellow	0.3	0.9	0.6	<i>B. flexus</i>	PIGB93	<i>Sargassum</i> sp.	Pale yellow	0.4	1.5	1.1	<i>S. arlettae</i>
PIGB17	<i>Ceramium</i> sp.	Pale yellow	0.5	0.7	0.2	<i>P. gessardii</i>	PIGB96	Seawater	Red	0.5	1.0	0.5	<i>S. nematodiphila</i>
PIGB18	<i>E. clathrata</i>	Red	0.6	2.5	1.9	<i>H. titanicae</i>	PIGB99	Sediment	Orange	0.6	1.2	0.6	<i>S. nematodiphila</i>
PIGB22	Seawater	Creamy	0.5	0.7	0.2	<i>P. fluorescens</i>	PIGB100	Seawater	Creamy	0.5	0.7	0.2	<i>S. nematodiphila</i>
PIGB23	Seawater	Yellow	0.4	1.0	0.6	<i>H. titanicae</i>	PIGB101	Seawater	Yellow	0.5	0.7	0.2	<i>P. fluorescens</i>
PIGB28	<i>Chaetomorpha</i> sp.	Pale yellow	0.3	0.9	0.6	<i>H. titanicae</i>	PIGB102	Seawater	Orange	0.6	1.0	0.4	<i>P. celer</i>
PIGB2	<i>Sargassum</i>	Pale	0.	1.1	0.	<i>P. celer</i>	PIGB1	Sediment	Yellow	0.	1.	0.	<i>S. marcescens</i>

9	sp.	yellow	2		9		03		w	5	0	5	
PIGB3 0	<i>Gelidium</i> sp.	Pale yellow	0. 4	0.1 3	0. 9	<i>V. harveyi</i>	PIGB1 09	Seawater	Cream y	0. 5	0. 8	0. 3	<i>P. maritimus</i>
PIGB3 1	Seawater	Orange	0. 4	0.6 6	0. 2	<i>P. azotoformans</i>	PIGB1 14	Sediment	Pale orange	0. 6	0. 8	0. 2	<i>P. maritimus</i>
PIGB3 2	Sediment	Pale brown	0. 4	0.6 6	0. 2	<i>P. angustum</i>	PIGB1 17	<i>S. vulgare</i>	Cream y	0. 4	2. 3	1. 9	<i>H. profundus</i>
PIGB3 3	<i>S. ilicifolium</i>	Pale yellow	0. 4	0.9 9	0. 5	<i>H. titanicae</i>	PIGB1 19	<i>C. racemosa</i>	Brown	0. 2	2. 8	2. 6	<i>B. marisflavi</i>
PIGB3 4	Sediment	Orange	0. 5	0.7 7	0. 2	<i>S. roseus</i>	PIGB1 22	Sediment	Orang e	0. 8	2. 4	1. 6	<i>B. infantis</i>
PIGB3 5	Seawater	Creamy	0. 5	0.7 7	0. 2	<i>H. titanicae</i>	PIGB1 25	<i>S. marginatum</i>	Pale orange	0. 4	0. 7	0. 3	<i>C. xerosis</i>
PIGB3 7	Seawater	Pale yellow	0. 6	1.2 12	0. 6	<i>Planococcus</i> <i>sp.</i>	PIGB1 32	Seawater	Orang e	0. 5	1. 0	0. 5	<i>B. vietnamensis</i>
PIGB4 4	<i>U. Fasciata</i>	Pale yellow	1. 0	2.6 26	1. 6	<i>P. okeanokoites</i>	PIGB1 40		Pale orange	0. 5	1. 0	0. 5	<i>P. pulmonis</i>
PIGB4 5	<i>U. Fasciata</i>	Yellow	0. 3	0.8 8	0. 5	<i>H. profundus</i>	PIGB1 49	Seawater	Brown	0. 4	0. 6	0. 2	<i>P. faecalis</i>
PIGB4 6	<i>C. media</i>	Pale yellow	0. 5	4.0 40	3. 5	<i>P. argentinensis</i>	PIGB1 50	Seawater	Pale yellow	0. 3	0. 7	0. 4	<i>P. faecalis</i>
PIGB4 7	<i>C. media</i>	Red/oran ge	0. 5	2.8 28	2. 3	<i>S. cohnii</i>	PIGB1 52	<i>P. tetrastromati</i> <i>ca</i>	Cream y	0. 4	0. 8	0. 4	<i>S. cohnii</i>
PIGB5 0	Seawater	Pale yellow	0. 5	1.0 10	0. 5	<i>H. titanicae</i>	PIGB1 53	Seawater	Orang e	0. 4	0. 8	0. 4	<i>B. bullata</i>
PIGB5	Seawater	Pale	0.	1.2	0.	<i>P. punonensis</i>	PIGB1	<i>C. peltata</i>	Orang	0.	0.	0.	<i>M.</i>

1		yellow	6		6		55		e	3	6	3	<i>esteraromaticum</i>
PIGB58	Seawater	Pale yellow	0.8	2.9	2.1	<i>V. fluvialis</i>	PIGB156	<i>C. peltata</i>	Brown	0.4	0.7	0.3	<i>B. oryzaecorticis</i>
PIGB60	Seawater	Creamy	0.9	2.0	1.1	<i>A. caviae</i>	PIGB159	Seawater	Yellow	0.3	0.6	0.3	<i>E. aquaticum</i>
PIGB61	<i>G. corticata</i>	Creamy	0.7	1.3	0.6	<i>P. stutzeri</i>	PIGB160	Sediment	Pale orange	0.4	1.0	0.6	<i>L. defluvii</i>
PIGB64	Seawater	Orange	0.4	0.7	0.3	<i>H. titanicae</i>	PIGB162	<i>Gelidium</i> sp.	Pale orange	0.2	0.3	0.3	<i>B. marisflavi</i>
PIGB68	Seawater	Pale orange	0.7	1.0	0.3	<i>Exiguobacterium</i> sp.	PIGB163	Seawater	Pink	0.7	0.6	0.9	<i>S. nematodiphila</i>
PIGB69	Seawater	Yellow	0.5	1.9	1.4	<i>Arthrobacter</i> sp.	PIGB166	<i>Gelidium</i> sp.	Yellow	1.3	0.5	0.7	<i>V. harveyi</i>
PIGB70	Seawater	Creamy	0.5	2.3	1.8	<i>P. faecalis</i>	PIGB167	<i>C. peltata</i>	Orange	0.6	0.3	0.2	<i>P. pulmonis</i>
PIGB71	Sediment	Orange	0.4	1.0	0.6	<i>S. marcescens</i>	PIGB168	<i>C. peltata</i>	Orange	0.3	0.6	0.3	<i>P. celer</i>
PIGB73	Sediment	Pale yellow	0.3	0.6	0.3	<i>P. faecalis</i>	PIGB169	Sediment	Pink	0.5	1.0	0.5	<i>Zooshikella</i> sp.
PIGB75	Sediment	Yellow	0.4	0.6	0.2	<i>S. nematodiphila</i>	PIGB174	<i>P. tetrastromatica</i>	Pink	0.3	0.5	0.2	<i>B. agri</i>
PIGB76	<i>S. vulgare</i>	Creamy	0.4	1.5	1.1	<i>P. brassicacearum</i>	PIGB177	<i>U. fasciata</i>	Pink	1.0	1.2	0.2	<i>V. harveyi</i>
PIGB77	<i>S. vulgare</i>	Brown	0.7	5.0	4.3	<i>P. koreensis</i>	PIGB179	Seawater	Yellow	0.4	0.8	0.4	<i>A. media</i>

PIGB79	<i>P. boergesinii</i>	Orange	0.7	3.5	2.8	<i>S. nematodiphila</i>	PIGB180	Seawater	Pale brown	0.5	2.0	1.5	<i>F. marina</i>
PIGB80	<i>P. boergesinii</i>	Orange/Red	0.3	1.7	1.4	<i>P. faecalis</i>	PIGB181	Seawater	Yellow	0.3	0.6	0.3	<i>B. firmus</i>
PIGB81	Sediment	Yellow	0.4	0.9	0.5	<i>S. marcescens</i>	PIGB183	Seawater	Brown	0.4	3.2	2.8	<i>P. aeruginosa</i>
PIGB82	Seawater	Orange	0.6	1.0	0.4	<i>S. nematodiphila</i>	PIGB184	Seawater	Pale yellow	0.5	3.7	3.2	<i>Vibrio sp.</i>
PIGB83	Sediment	Creamy	0.5	0.8	0.3	<i>S. nematodiphila</i>	PIGB186	<i>P. tetrastromatica</i>	Orange	0.5	1.0	0.5	<i>P. pulmonis</i>
PIGB84	Sediment	Yellow	0.6	1.1	0.5	<i>P. maritimus</i>	PIGB190	<i>S. tenerrimum</i>	Pale brown	0.5	1.2	0.7	<i>V. harveyi</i>

*a: Colony size, b: DPPH decolorization zone, b-a: Final antioxidant zone. Among 190 PIGB, bacteria exhibiting positive AO activity (84 isolates) are only summarized.

Figure captions

Figure 1. Map depicting sampling locations along the central west coast of India.

Figure 2. Representative pigmented isolates and their closest bacterial identity. Asterisk indicates that the purple pigmented colony was not grown after subsequent sub-culturing hence it was unidentified

Figure 3. Percentage contribution of pigmented bacteria retrieved from different source (a) and seasons (b) of isolation.

Figure 4. Neighbor-joining phylogenetic tree based on 16S rRNA genes of antioxidative pigmented bacteria (Nodes with Bootstrap values $\geq 50\%$ are shown). Short length sequences (PIGB50, PIGB69, PIGB71, PIGB87, PIGB132 and PIGB169) were not included in the tree. Bar 0.02 substitutions per nucleotide position. *Aquifex pyrophilus* M83548 was used as an out-group.

Figure 5. Relative abundance of bacterial phyla and families in CWCI waters with antioxidant activities

Figure 6. Rarefaction curve based on 16S rRNA gene sequence of antioxidant producing pigmented bacteria (a). The ordination illustration for species contribution and similarity occurrence based on the Bray-Curtis square root transformed data of the major bacterial groups isolated from different seasons (b).

Figure 1.

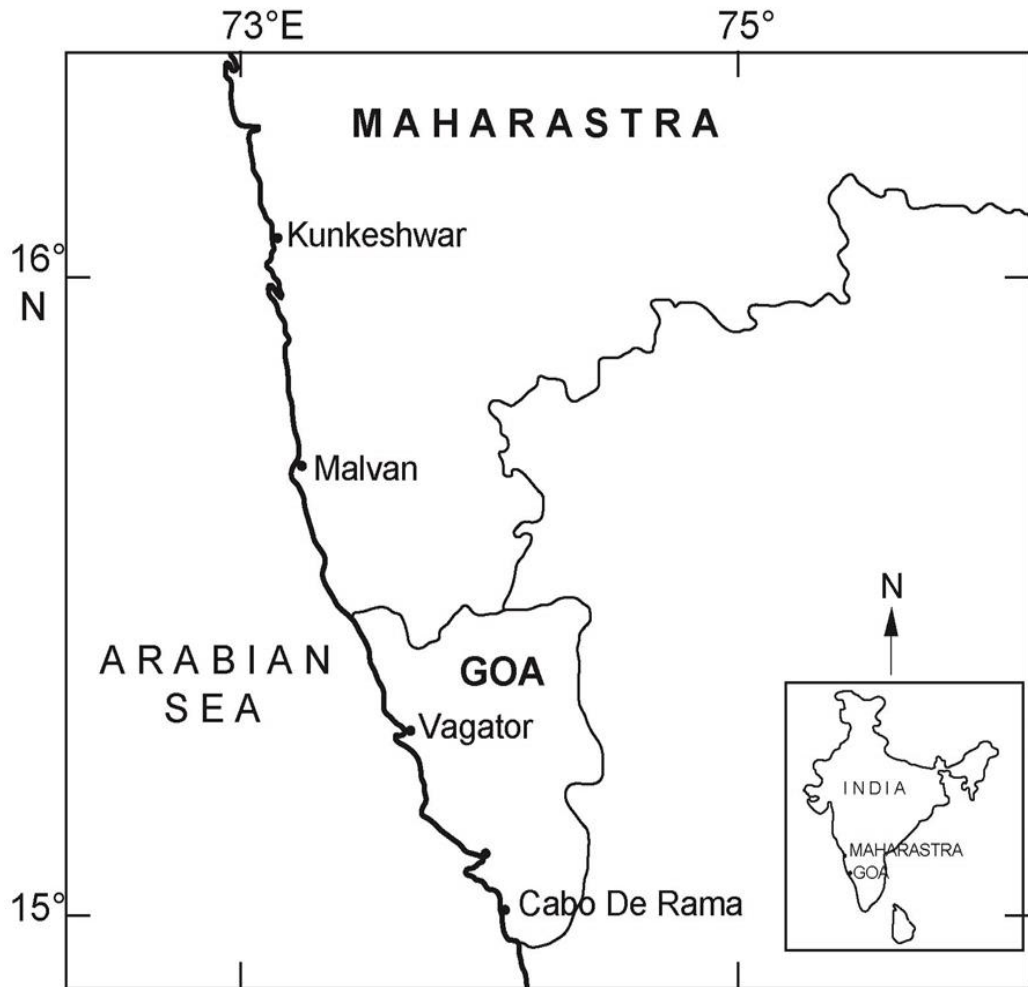


Figure 2.

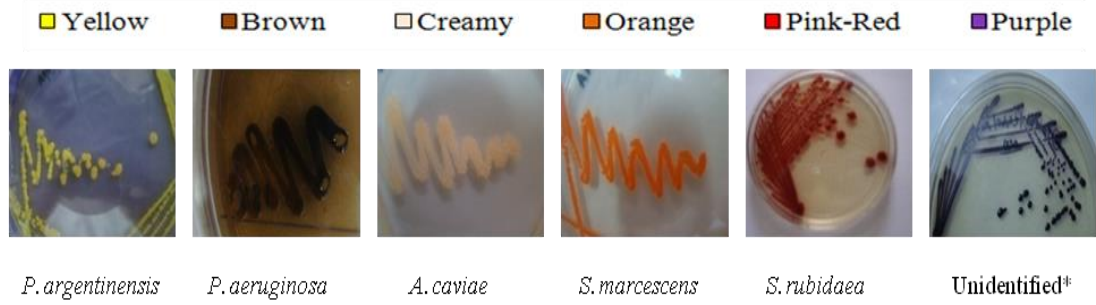
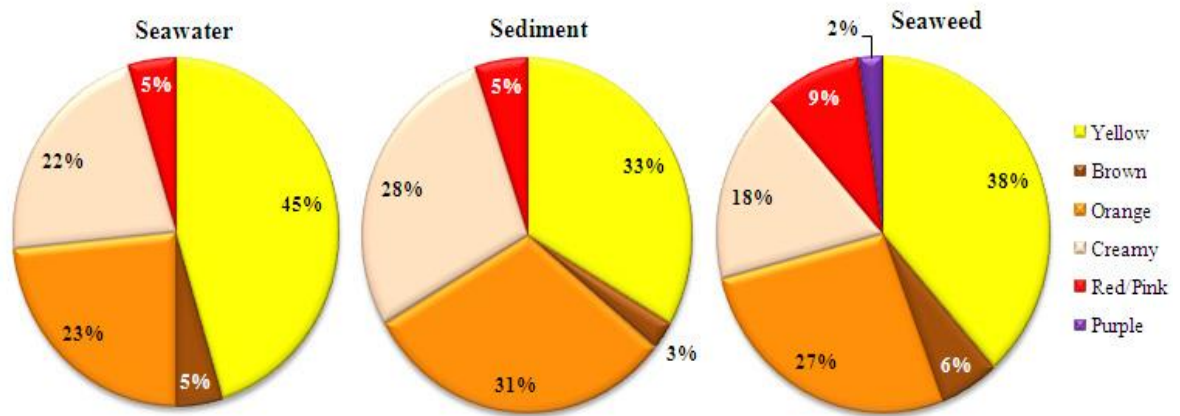


Figure 3.

a)



b)

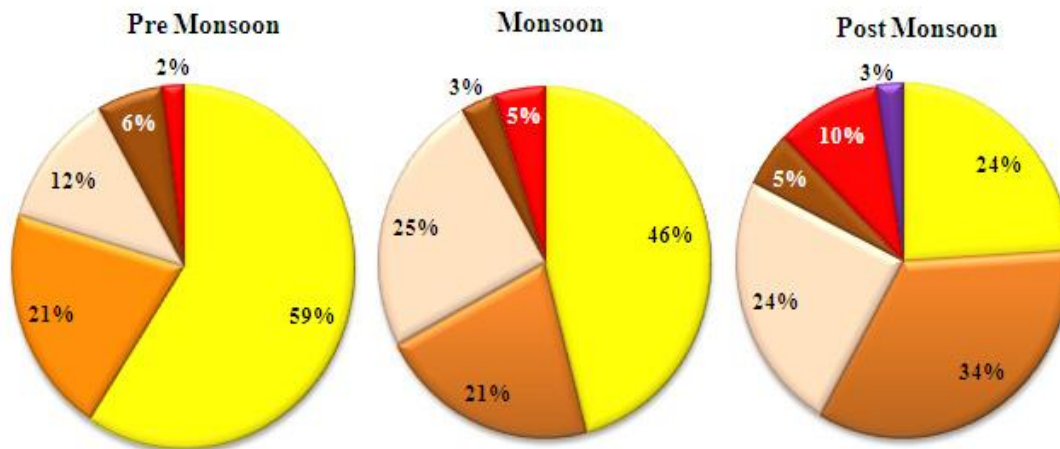


Figure 4.

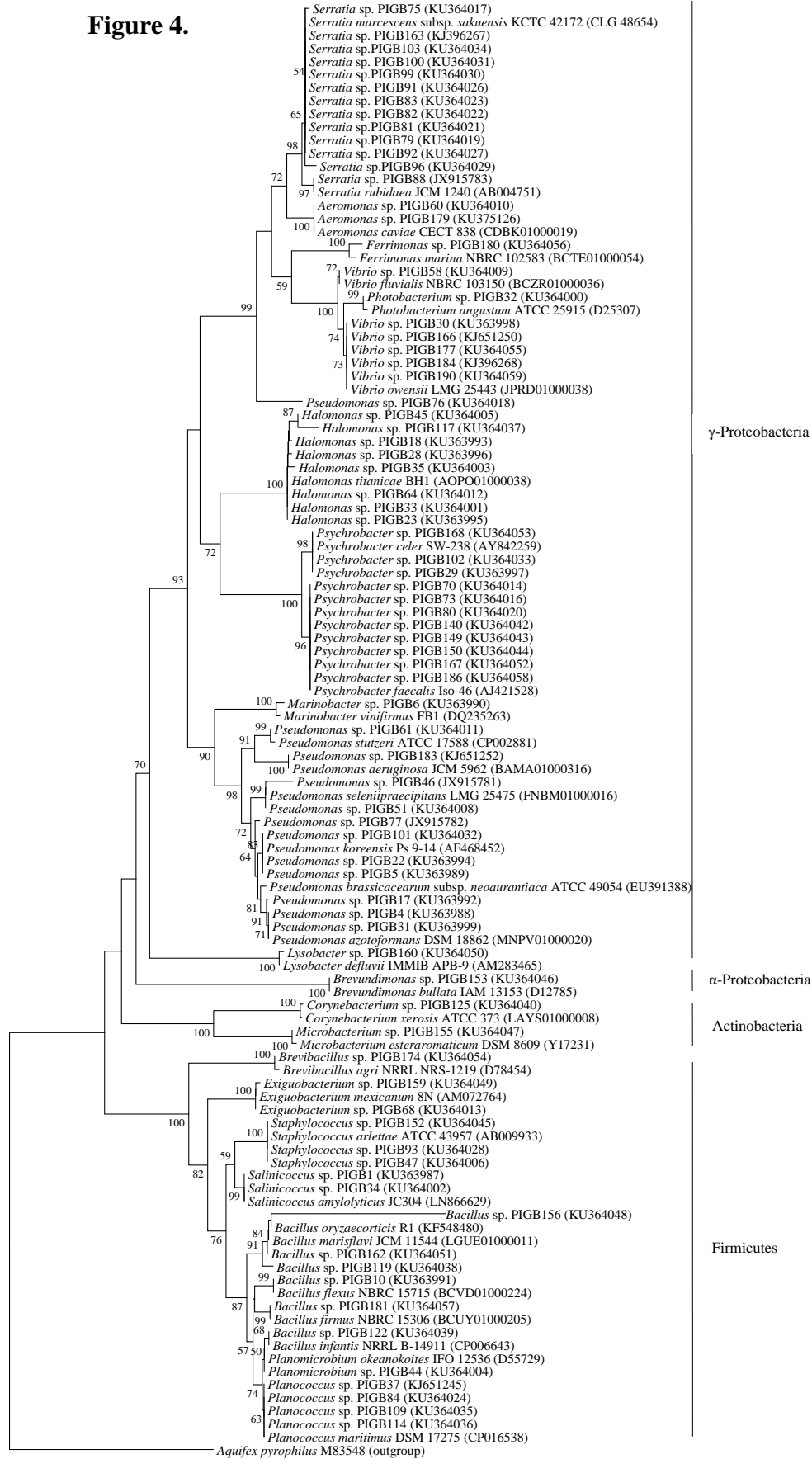


Figure 5.

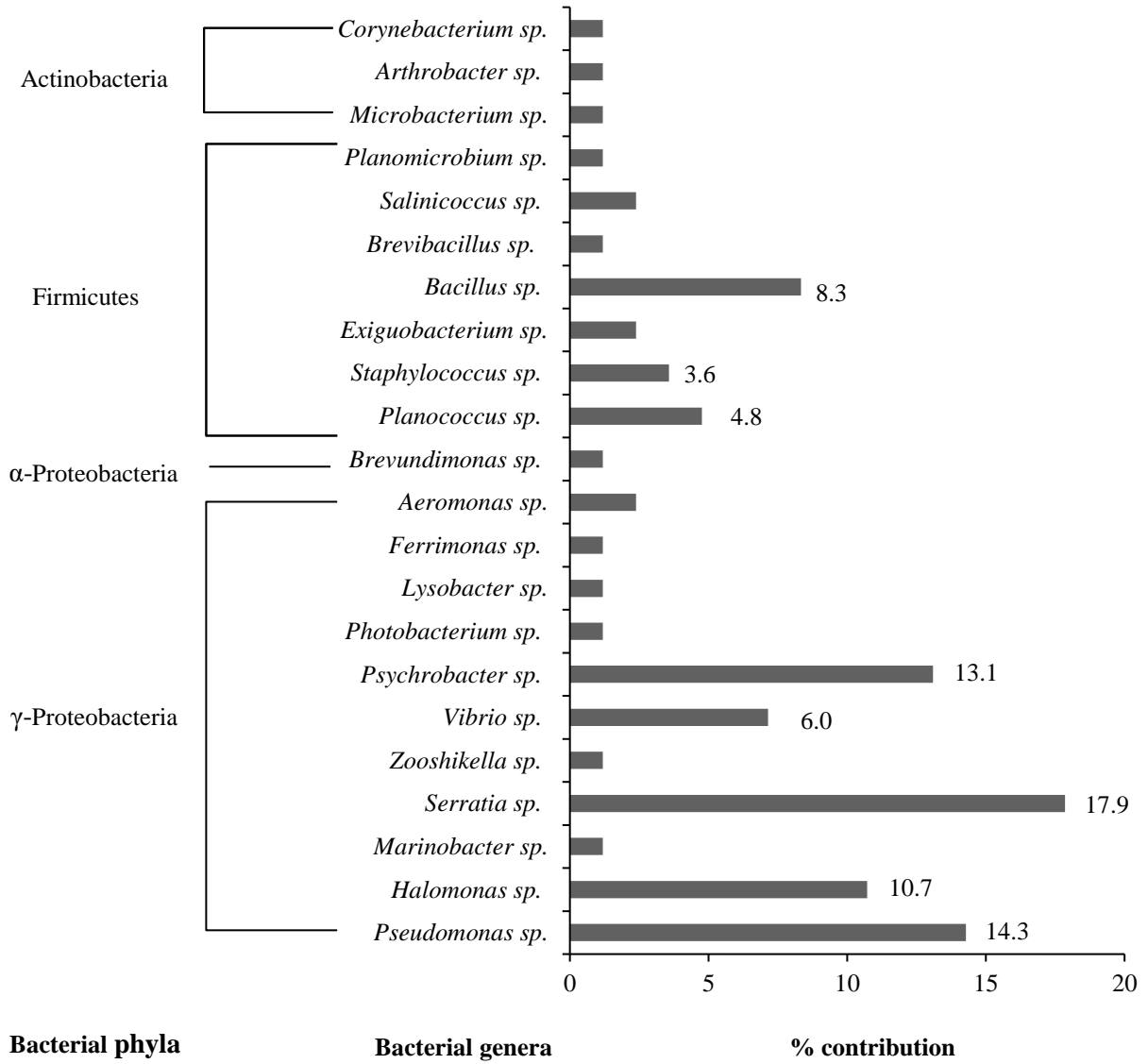
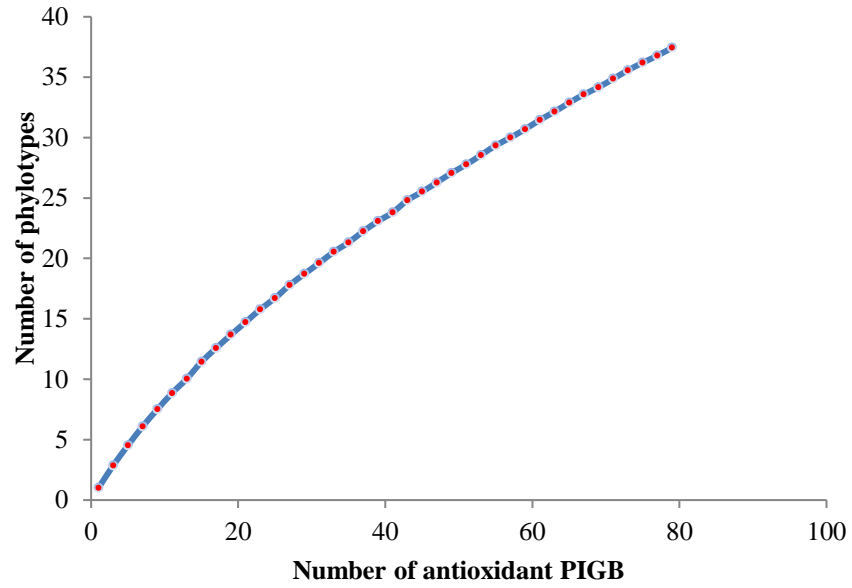
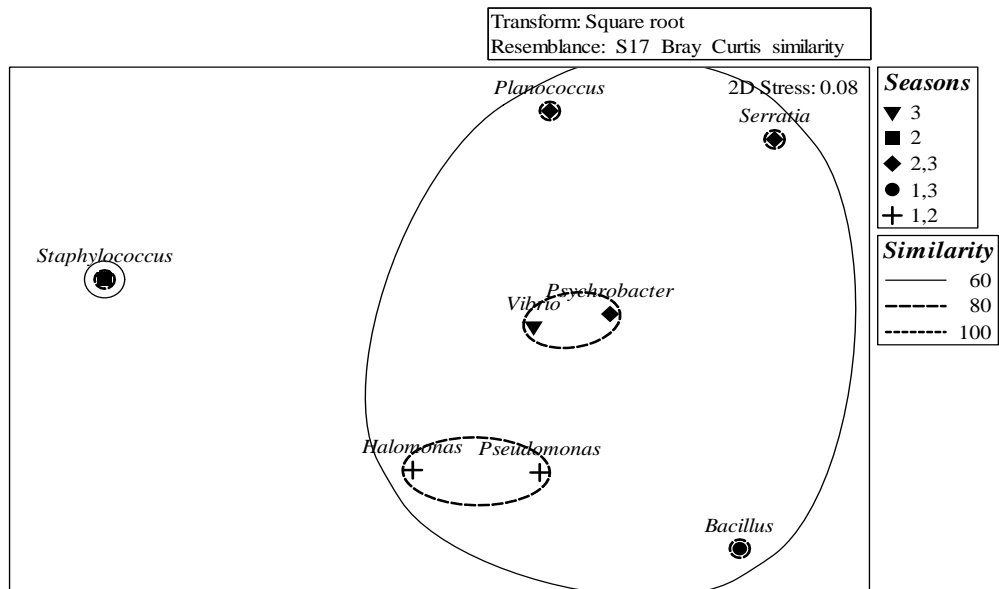


Figure 6.

(a)



(b)



Seasons: 1-Premonsoon, 2- Monsoon, 3-Postmonsoon.