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Phytoplankton and Bacterial Community Structures and Their Interaction during Red-tide Phenomena

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Abstract – Phytoplankton and bacteria diversity were studied before, during and after red tide phenomena during spring season 2015 in the Eastern Harbour (E.H.) of Alexandria, Egypt. Fifty five species of phytoplankton were identified and represented different distinct classes "Bacillariophyceae; Dinophyceae, Chlorophyceae, Cyanophyceae and Eugelenophyceae". Also, Diatom formed the most dominant group. The average number of the phytoplankton density varied from 4.8×10^4 to 1.1×10^6 cell l^{-1} during the study period and *Skeletonema costatum* was the agent causing the red tide. The existence percentages of bacteria ranged from 2.6 to 17.9% on all media tested. The bacterial isolates on the nutrient agar medium represented the highest existence with a total percentage of 43.6%, followed by MSA medium (25.7%), while the lowest percentage was for the AA medium at 7.8%. However, twelve isolates were selected as representative for bacterial community during study interval. Based on the morphological, biochemical, physiological and enzymatic characteristics, the bacterial strains were described. Depending on the 16S rDNA gene sequence, three common antagonists were aligned as: *Vibrio toranzoniae* strain Vb 10.8, *Ruegeria pelagia* strain NBRC 102038 and *Psychrobacter adeliensis* strain DSM 15333. The interaction between these bacteria and *S. costatum* was studied. The growth of *S. costatum* was significantly lower whenever each bacterium was present as compared to axenic culture. More specifically, 30% (v/v) of the all tested bacteria showed the strongest algicidal activities, as all *S. costatum* cells were killed after two days. 10% of *R. pelagia* and *P. adeliensis* also showed significant algicidal activities within six days.

Keywords – bacterial, biodiversity, phytoplankton, red tide, interaction

1. Introduction

Bacteria and phytoplankton are major components of marine ecosystems (Azam 1998; Doucette et al. 1998). There are specific interactions between them and these interactions influence the composition of both communities (Rooney-Varga et al. 2003). Algal-bacterial interactions can be classified into four types; (1) symbiotic, where both partners benefit from each other. Green et al. (2004) and Jasti et al. (2005) detected that some bacteria are known to live inside red tide (R.T.) dinoflagellates in a symbiotic relationship; (2) parasitic, where bacteria can lyse algae and algal antibiosis can inhibit bacterial growth; (3) commensalistic, where the bacteria have no actual negative effects on the host; and (4) bacteria are competitors for limited nutrients like phosphate by being loosely associated with algae (Grossart 1999). Interactions between algae and bacteria are commonly observed in both freshwater and marine ecosystems with bacteria increasingly cited as being responsible for the regulation of the growth and dynamics of phytoplankton blooms (Mayali and Azam 2004). Some marine bacteria are capable of stimulating or inhibiting phytoplankton growth (Ferrier et al. 2002), killing or lysis of phytoplankton (Mayali and Azam 2004), or altering phytoplankton physiology (Gallacher et al. 1997). Hulot and Huisman (2004) reported that heterotrophic bacteria may degrade harmful substances and in this way counteract the development of allelopathic phytoplankton populations. On the other hand, algae represent the primary source of organic nutrients for heterotrophic microbes in the mixed layer of the ocean and abundance of the bacteria has shown a positive correlation with algal concentrations (Kjelleberg et al. 1993). During phytoplankton bloom development the organic carbon is made available for bacteria through different pathways, while healthy and dying algal cells can release large amounts of dissolved organic matter into their surroundings (Myklestad 1995). The red tide phenomenon naturally occurs

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world-wide and its seasonal occurrence depends on environmental conditions (e.g. increase in nutrients, water temperature and pH) and meteorological factors (air temperature, precipitation, sunshine and winds). In the Eastern Harbour, the first red tide was recorded by Halim (1960) who detected bloom from dinoflagellate *Alexandarium minutum*. In October 1994, *A. minutum* Halim caused a heavy bloom in E. H. which then extended outside along the coastline for about 20 km in both east and west directions (Labib and Halim 1995). Mikhail (2003) recorded that *A. catenella*, *A. ostenfeldii*, *Chattonella antique*, *Gymnodinium mikimotoi* and *Prorocentrum sigmoides* were the chief causal agents for the red tide phenomena during 1998–2000. She also reported that *Ch. antique* and *G. mikimotoi* occurred together with the bloom of *Skeletonema costatum*. *S. costatum* is a common red-tide causing genus and a toxigenic that is harmful to fisheries when it blooms in Jiaozhou Bay and to the human health (Huo et al. 2001) and potentially influences plankton community structure (Badylak et al. 2007). *S. costatum* may be considered as a pollution and eutrophication indicator species (El-Sherif and Gharib 1994; Nassar 2000).

Several researchers have reported that bacteria can play a major role in controlling phytoplankton dynamics (Liu et al. 2008). For example, Fukami et al. (1991) found that natural bacterial communities collected during a *Gymnodinium nagasakiense* bloom inhibited *Skeletonema costatum,* but stimulated *G. nagasakiense*. Fukami et al. (1992) isolated *Flavobacterium* sp. str. 5N-3, which was found to have algicidal properties against *G. nagasakiense* but to have no effect on *Chattonella antiqua, Heterosigma akashiwo,* or *S. costatum*. Skerratt et al. (2002) stated that lysis of algae by algicidal bacteria is known to play an important role in terminating red tides.

The aim of our study was to detect the biodiversity of phytoplankton and bacteria community before, during and after red tide phenomena in Eastern Harbour, Alexandria, Egypt during spring 2015 and also to determine the interaction between the most common bacterial spp. and *Skeletonema costatum* the causal agent of red tide. Interestingly, this is the first report which investigates the interactions between the bacterial and phytoplankton community before, during and after the red tide in Eastern Harbour, Alexandria, Egypt.

2. Material and Methods

Study area

The Eastern Harbour of Alexandria (Fig. 1) is situated between longitudes 29°53' to 29°54'E and latitudes from 31°12' to 31°13'N; the surface area is about 2.53×10^6 m^2 with an average depth of 6 m. The Harbour is shallow along its southern margin, and deeper toward the openings. Its bottom slopes down gradually northwards reaching maximum depth at the central area behind the El-Boughaz opening.

Identification of phytoplankton species

Phytoplankton samples were collected every 2 days throughout the period from 5 to 19 April 2015 from one station at Eastern

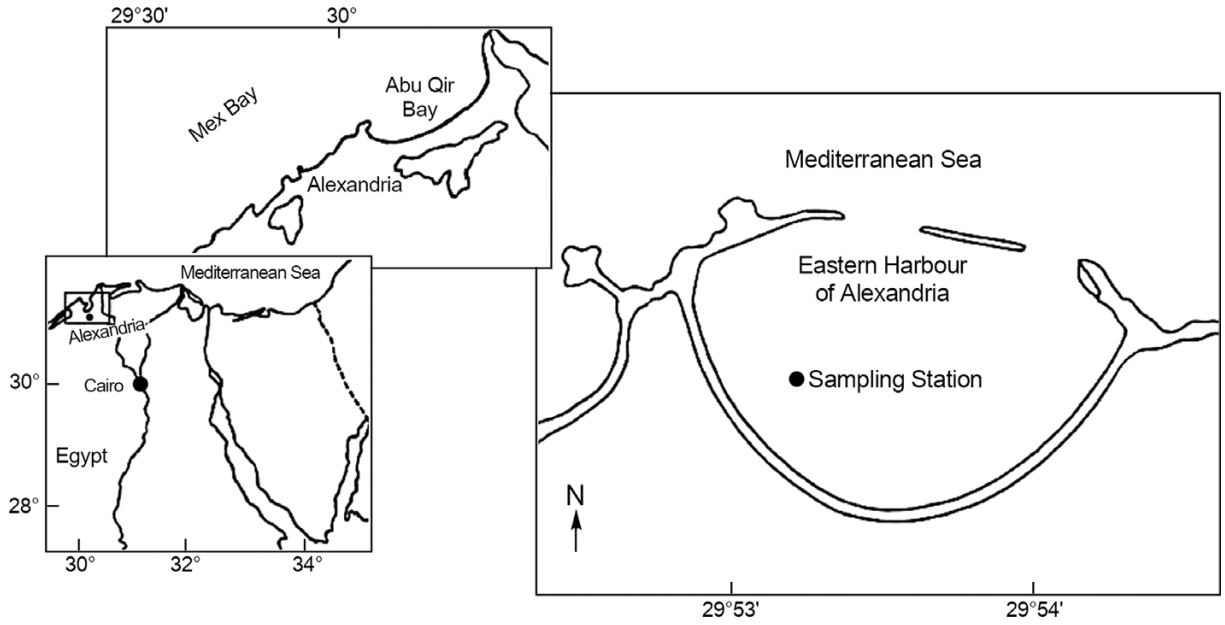

Fig. 1. Study area of the collected samples

Harbour (red tide continued for 7 days from 9 to 15 April) with a 20 μm mesh size standard plankton net held against the surface current of the ebbing tide for 10 min. The net was then hauled in and samples were transferred to a 250 ml well labeled plastic container with a screw cap each time. Samples were preserved with 4% formalin with the addition of a few drops of Lugol's solution (Nwankwo 1996) and stored in the laboratory prior to microscopic analysis in the laboratory. Phytoplankton were counted and identified using the sedge wick-Rafter counting cell with a Nikon TS 100 inverted microscope at 400x magnification using sedimentation technique as reported in the slandered method (APHA 2005) and expressed in cell l^{-1}. Counting was repeated three times and the average determined. Identification of algal taxa followed Dodge (1982), Sournia (1968, 1986), Tikkanen (1986), Mizuno (1990) and were confirmed using the Algae Base website.

Isolation of marine bacteria species

Water samples were collected in 500 ml sterile screw-caped bottles as previously described by Austin (1988). Serial dilutions (10^{-2} to 10^{-6}) were made using filtered sterilized seawater. A portion (0.1 ml) from each appropriately diluted sample was used to inoculate plates prepared with sea water agar for counting aerobic heterotrophs onto solid nutrient, Aeromonas, Mannitol salt and Thiosulfate citrate bile salt sucrose media. Plates were incubated at 30°C for 24–48 h. Purification of bacterial colonies was carried out by streaking on agar plates of the same medium. The pure colonies obtained were transferred to fresh slants. Sub-cultures were kept under refrigeration for further investigations.

Morphological and biochemical characterization of marine bacteria

According to differences in colony shape and color, the marine bacteria were isolated and purified using the streak plate technique. The shape of the cells and the reaction to Gram staining were determined according to the method described by Hendrickson and Krenz (1991), and a series of biochemical tests was made by standard bacteriological methods. All of the bacterial strains were examined for several characteristics. Most of the tests were carried out in Petri-plates: VP, Methyl red, Indole production, H_2S precipitation, nitrate reduction and acid production were performed in test tubes. Unless otherwise stated, pH of all media was adjusted to 7.0 with potassium hydroxide and incubation at 28°C was carried out. The pH range of growth was determined on nutrient agar media, initially adjusted to different pH values (4–12) with hydrochloric acid or potassium hydroxide. After sterilization, pH was checked and readjusted when necessary. Temperature range (10–60°C) was determined on nutrient agar medium. Results were considered positive if there was visible growth after 24–48 h. Sodium chloride requirement was examined in liquid cultures of nutrient broth supplemented with different sodium chloride concentrations (0–13%). Carbon source utilization was investigated using agar plates of mineral salts agar medium containing (g L^{-1}) ammonium sulfate; 2.64, dipotassium hydrogen orthrophosphate; 2.38, hydrated potassium dihydrogen orthrophosphate; 5.65, hydrated magnesium sulfate; 1.0 and agar; 15. The pH was adjusted to 7.0. Finally, these isolates were identified according to tests in Bergy's manual (Garrity et al. 2005).

Cross antagonism between the bacterial community isolates

Tooth picking technique was used to test the ability of isolated bacteria to inhibit the growth of each other as the indicator strains. The 12 bacterial isolates from the Eastern Harbour were used as test isolates and crossed against themselves as indicators by tooth picking technique. Tooth picking is considered as a type of patching. Two NA plates were used in this test. One plate was freshly inoculated with 0.5% (v/v) of 24 h old culture (10^8 cell m^{-1}) of the indicator strain. The second plate was used as a template without being seeded with the indicator strain. The NA plates were stabbed consequently using a sterile clean tooth pick each time with a single colony of each of the tested bacteria. The un-inoculated NA plate was stabbed first, and then the seeded NA plate with the indicator strain was stabbed with the same tooth pick. After stabbing all the tested bacterial strains, the NA seeded and control plates were incubated at 30°C for 24–48 h. The clear zone around the tooth picked isolates indicates a positive result in the antagonistic action.

Molecular characterization of the bacterial antagonists

The genomic DNA of different strains was isolated as described by Ausubel et al. (1999) using the GFX genomic DNA purification kit (Amersham Bioscience) according to the manufacturer's instructions. The DNA was analyzed using 0.7% agarose gel electrophoresis. The 16S rDNA gene was amplified by polymerase chain reaction (PCR) using the primers: 16S 357 F; ACT CCT ACG GGA GGC AGC AG and 16S 907R; CCG TCA ATT CAT TTG AGT TT. The three selected bacterial isolates from the previous test were

identified based on sequence analysis of their PCR amplified 16S rRNA genes. Database matching of the 16S rRNA sequences was performed using the Ribosomal Database Project. Biology workbench was used for computational analyses of DNA sequence, multisequence alignment, and construction of the phylogenetic tree. The phylogenic tree of each isolate and its closely related relatives were analyzed by using the PHYLIP (maximum-likelihood) program in Biology Work Bench software (http:biology.ncsa.edu/).

Interactions between the most common bacterial strains and *Skeletonema costatum*

Axenic culture of *Skeletonema costatum* was obtained after several attempts putting the subculture of the red tide samples on sterilized Guillard seawater medium (f/2) (Guillard and Ryther 1962). Then it was grown at 26°C in a 16:8 h light:dark cycle and a photon flux of 85 $\mu Em^{-2}s^{-1}$. Three samples were taken from the culture medium every 2 days and stained with Lugal's solution for cell-count measurements under an inverted microscope. However, the most common three bacterial strains were inoculated into sterilized fresh nutrient liquid medium made with seawater at pH 7.5, separated and incubated at 26°C overnight with shaking at 110 rpm/min. Mixed cultures were obtained by adding 10, 20 and 30 ml of each culture broth to 90, 80 and 70 ml algal culture f/2 seawater media. The density of *S. costatum* was (0.85 to 1) × 10^6 cell l^{-1} and that of bacteria was 1 × 10^8 cells l^{-1}. All treatments were co-cultured for 10 days under the same conditions. Bacterial and algal growth in the tested culture was measured twice by counting every 2 days and the mean value of the count obtained was used to calculate the growth rate. The growth of *S. costatum* was measured by the counting method as described previously (APHA 2005). The bacteria were counted on nutrient agar plates by pouring plate technique as mentioned before regarding the isolation process.

Statistical analysis

The Pearson correlation (r) was used to evaluate the interactions between *Skeletonema costatum* and the most common bacterial species *in vitro* (N = 80) with the SPSS 15.0 Statistical Package Program.

3. Results

Phytoplankton diversity

From microscopic examination of the phytoplankton it was revealed that there were visible variations in the phytoplankton community with regard to numerical abundance and species composition during the study period. The analysis of the phytoplankton community is summarized in Table 1. A total of 57 taxa (43 Bacillariophyceae; 8 Dinophyceae, 3 Chlorophyceae, 2 Cyanophyceae and 1 Eugelenophyceae species) were identified at the genus and species level. The total numbers of phytoplankton in the water column fluctuated between (177824, 1100681 and 47964 cells l^{-1}) before, during and after red tide, respectively (Table 1). The highest biodiversity of phytoplankton was recorded before the red tide interval with 57 species as compared with 46 & 32 spp. during and after R.T. phenomena, respectively (Table 2). However, the high occurrence of phytoplankton during R.T. interval was associated with the lowest number of species (46 spp.). Diatoms were the most dominant group of phytoplankton in the studied area, with the dominancy of *Skeletonema costatum* (1.06 × 10^6 cell l^{-1}) contributing 96.849% to total diatoms and 96.304% to total phytoplankton counts during red tide interval and 46.838 and 58.403% to total diatoms before and after red tide, respectively. Also, *Actioptychus senarius* Ehrenberg, *Coscinodiscus centralis* Ehrenberg, *Leptocylindrus danicus* Cleve, *Licmophora lyngbyei* Kũtz. Grunow., *Navicula* longa (Gregory) Ralfs ex Pritchard and Rhizosolenia setigera Brightwell were dominant species during all intervals. *Prorocentrum micans* was the most dominant dinoflagellates during study interval and represented 33.12%, 24.11% and 47.58% of total dinoflagellates before, after and during red tide, respectively. Three species of toxic dinoflagellates were persistent during the study period: *Ostreopsis ovata* Fukuyo, *Pyrophacus steinii* (Schiller) Wall et Dale, *Protoperidinium minutum* (Kofoid) Balech. On the other hand, Chlorophyceae and Euglenophyceae species were not recorded during and after the red tide interval, but Cyanophyceae spp. appeared throughout the study period. The freshwater taxa e.g. Chlorophyta, Cyanophyceae and Euglenophyceae showed a very low count, constituting collectively 0.041, 0.001 and 0.025% of total phytoplankton before, during and after R.T.

Bacterial community structure

Thirty eight bacterial isolates were isolated and purified during the study period on different tested media. The percentages of bacterial existence on the different isolation media used varied according to the duration of marine bacteria isolation; before, during and after the red tide (Spring 2015) in the Eastern Harbour, Alexandria, Egypt (Table 3). Clearly,

Table 1. A checklist and percentage (%) of the recorded species before; during and after red tide (cell/l)

Species	Before R.T.		During R.T.		After R.T.	
	(cell/l)	%	(cell/l)	%	(cell/l)	%
Bacillariophyceae						
Actinastrum hantzschi Lagerheim	64	0.040	ND	0.000	ND	0.000
Actinocyclus octonarius Ehrenberg	456	0.285	119	0.011	133	0.294
Actioptychus senarius Ehrenberg	12360	7.713	6117	0.559	2091	4.623
Actinoptychus splendens (Shad.) Ralfs.	3255	2.031	778	0.071	ND	0.000
Amphiprora paludosa W. Smith	218	0.136	56	0.005	ND	0.000
Amphora cymbifera Greg	325	0.203	42	0.004	ND	0.000
Amphora marina Smith	3000	1.872	1344	0.123	564	1.247
Amphora ovalis (Kützing) Kützing	86	0.054	ND	0.000	ND	0.000
Bellerochea malleus (Brightwell) Van Heurick	2298	1.434	ND	0.000	227	0.502
Chaetoceros atlanticus Clevé	681	0.425	315	0.029	285	0.630
Chaetoceros decipience Clevé	1555	0.970	815	0.074	157	0.347
Chaetoceros muelleri Lemm.	978	0.610	545	0.050	ND	0.000
Cocconeis placentula Ehrenberg	89	0.056	ND	0.000	ND	0.000
Coscinodiscus centralis Ehrenberg	11780	7.351	5114	0.467	2420	5.350
Coscinodiscus granii L.F. Gough	6666	4.160	3406	0.311	1233	2.726
Coscinodiscus radiates Ehrenberg	7427	4.635	4717	0.431	2450	5.416
Cyclotella mengheniana Kütz	1333	0.832	800	0.073	1215	2.686
Cyclotella ocellata Pantocsek	208	0.130	198	0.018	201	0.444
Diploneis crabro (Ehrenberg) Ehrenberg	600	0.374	265	0.024	283	0.626
Diploneis didyma (Ehrenberg) Ehrenberg	245	0.153	22	0.002	46	0.102
Gramatophora oceanica (Her.) Grunow	187	0.117	133	0.012	156	0.345
Gramatophora marina (Lyngb.) Kütz	750	0.468	36	0.003	256	0.566
Gyrosigma attenuatum (Kz.) Clevé	1456	0.909	568	0.052	606	1.340
Gyrosigma balticum Ehrenberg	1500	0.936	490	0.045	485	1.072
Fragilaria oceanica Clevé	254	0.159	205	0.019	ND	0.000
Leptocylindrus danicus Clevé	800	0.499	406	0.037	227	0.502
Licmophora lyngbyei Kũtz. Grunow	4650	2.902	1824	0.167	1352	2.989
Licmophora gracilis Ehrenberg	354	0.221	ND	0.000	ND	0.000
Melosira granulate var. angustissima Mũller	335	0.209	48	0.004	ND	0.000
Navicula lyra Ehrenberg	136	0.085	48	0.004	ND	0.000
Navicula longa (Gregory) Ralfs ex Pritchard	2000	1.248	46	0.004	995	2.200
Nitzschia longissima (Brébisson) Ralfs	146	0.091	86	0.008	ND	0.000
Nitzschia sigma Kütz	198	0.124	153	0.014	ND	0.000
Paralia sulcata (Ehrenberg) Clevé	1897	1.184	780	0.071	529	1.169
Pinnularia viridis (Nitzsch) Ehrenberg	570	0.356	283	0.026	ND	0.000
Pleurosigma rigidum W.Smith	298	0.186	235	0.021	54	0.119
Pleurosigma decorum W. Smith	106	0.066	65	0.006	ND	0.000
Pleurosigma elongatum W. Smith	2860	1.785	1323	0.121	1107	2.447
Rhaphoneis amphiceros Ehrenberg	5750	3.588	2096	0.192	1304	2.883
Rhizosolenia alata Brightwell	3380	2.109	166	0.015	ND	0.000
Rhizosolenia setigera Brightwell	3750	2.340	666	0.061	355	0.785
Skeletonema costatum (Greville) Clevé	75055	46.839	1060000	96.849	26419	58.404
Thalassiosira rotula Meunier	120	0.075	112	0.010	85	0.188
Thalassionema nitschoides Grun	65	0.041	65	0.006	ND	0.000
Total Bacillariophyceae	160241		1094487		45235	

Table 1. Continued

Species	Before R.T.		During R.T.		After R.T.	
	(cell/l)	%	(cell/l)	%	(cell/l)	%
Dinophyceae						
Gyrodenium fusiform Kof. and Swezy	598	3.415	287	4.639	ND	0.000
Ostreopsis ovata Fukuyo	3360	19.188	703	11.363	551	20.191
Prorocentrum micans Ehrenberg	5800	33.122	2944	47.584	658	24.111
Prorocentrum triastinum Schiller	634	3.621	476	7.694	378	13.851
Protoperidinium cerasus Paulsen	1105	6.310	105	1.697	ND	0.000
Protopenidinium depressum Bailey	1654	9.445	762	12.316	525	19.238
Pyrophacus steinii (Schiller) Wall et Dale	3320	18.960	567	9.164	304	11.140
Scrippsiella trochoidea (Stein) Balechex	1040	5.939	343	5.544	313	11.469
Total Dinophyceae	17511		6187		2729	
Chlorophyceae						
Ankistrodismus falcatus (Corda) Ralfs	11	34.375	ND	0	ND	0
Pediastrum duplex Meyen	7	21.875	ND	0	ND	0
Pediastrum simplex Meyen	14	43.75	ND	0	ND	0
Total Chlorophyceae	32		0		0	
Cyanophyceae						
Lyngbya limnetica lemm	24	64.865	7	100	ND	0
Spirulina platensis (Nordst) Geitler	13	35.135	ND	0	ND	0
Total Cyanophyceae	37		7		0	
Euglenophyceae						
Euglena gracilis (Klebs)	3	100	ND	0	ND	0
Total Euglenophyceae	3		0		0	

Table 2. Taxonomic composition and proportional representation of phytoplankton groups during study periods

Class	Before R.T				During R.T				After R.T			
	Genus	%	Spp.	%	Genus	%	Spp.	%	Genus	%	Spp.	%
Bacillariophyceae	23	67.647	43	75.439	17	68	36	78.261	10	71.429	26	81.25
Dinophyceae	6	17.647	8	14.035	6	24	8	17.391	4	28.571	6	18.75
Chlorophyceae	2	5.882	3	5.263	0	0	0	0.000	0	0	0	0
Cyanophyceae	2	5.882	2	3.509	1	4	1	2.174	0	0	0	0
Euglenophyceae	1	2.941	1	1.754	1	4	1	2.174	0	0	0	0
Total	34	100	57	100	25	100	46	100	14	100	32	100

there percentages ranged from 2.63 to 18.42%. However, seven bacterial isolates were common both during the red tide on nutrient agar medium (DRT/N) and before and after the red tide on TCBS medium (B/ART/V) with the existence percentage (18.42%), while the existence percentage of bacteria before and during the red tide on NA medium (B/DRT/N) reached 13.15% with five isolates, followed by 10.52% after the red tide on NA medium (ART/N) with four isolates. However, the other existence percentages declined to 7.89%.

From these data, the bacterial isolates on the nutrient agar medium represented the highest existence with a total percentage of 44.74%, followed by both Mannitol salt agar medium and TCBS medium (23.68%), while the lowest was for the Aeromonas medium (7.89%). However, twelve isolates were selected as representative for the bacterial community associated with the interval studied in the Eastern Harbour.

Morphological, physiological, biochemical and enzymatic characteristics of the selected bacterial isolates were examined (Table 4). Twenty seven properties were detected for all bacterial isolates. Variations in the results of these properties were also noticeable. All isolates revealed the marine origin and/or adaptation. There were 5 bacterial isolates that showed negative Gram stain and 7 that showed positive Gram stain. Nine isolates were characterized by irregular shaped cells,

Table 3. Duration of marine bacteria isolation; before, during and after the red tide (Spring 2015) in the Eastern Harbor, Alexandria, Egypt

Number of isolates	Sum of isolates	Duration of isolation	Percentage of existence (%)
1, 8, 10, 11, and 12	5	Before and during red tide on NA medium	13.20
2, 3, 4, 5, 6, 7, and 9	7	During red tide on NA medium	18.42
13, 14, 15, 16 and 17	4	After red tide on NA medium	13.14
18	1	Before, during and after red tide on AA medium	2.63
19	1	During and after red tide on AA medium	2.63
20	1	After red tide on AA medium	2.63
21	1	After red tide on MSA medium	2.63
22, 25, 27 and 28	1	Before and after red tide on MSA medium	10.52
23, 24 and 26	3	During and after red tide on MSA medium	7.89
29	1	Before, during and after red tide on MSA agar medium	2.63
30, 31, 32, 33, 34, 35 and 36	7	Before and after red tide on TCBS medium	18.42
37 and 38	2	Before, during and after red tide on TCBS medium	5.26
Total sum of isolates	38		100

NA = Nutrient agar; AA = Aeromonas agar; MSA = Mannitol salt agar; TCBS = Thiosulfate citrate bile salt sucrose agar

Table 4. Morphological, physiological, biochemical and enzymatic characteristics of the bacterial isolates; before, during and after the red tide (Spring 2015) in the Eastern Harbor, Alexandria, Egypt

Test	Bacterial isolate code											
	MH1	MH2	MH3	MH4	MH5	MH6	MH7	MH8	MH9	MH10	MH11	MH12
Isolation time	B/DRT	DRT	ART	B/ART	B/D/ART	D/ART	ART	D/ART	B/ART	B/D/ART	B/ART	B/D/ART
Isolation medium	NA	NA	NA	NA	AA	AA	AA	MSA	MSA	MSA	TCBS	TCBS
Color	Buff	White	Buff	Orange	White	yellow	White	Buff	White	Colorless	White	Orange
Shape	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Puncitform	Irregular	Circular	Irregular	Irregular	Circular
Elevation	Raised	Raised	Raised	Raised	Flat	Flat	Raised	Raised	Convex	Raised	Raised	Raised
Margin	Undulate	Entire	Wave	Undulate	Filamentous	Wave	Filamentous	Undulate	Entire	Undulate	Curled	Entire
Surface	Smooth	Smooth	Rough	Smooth	Powdery	Smooth	Powdery	Wrinkle	Smooth	Rough	Wrinkle	Smooth
Gram stain	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve
Cell shape	Rods	Spherical	Spherical	Spherical	Spherical	Rods	Rods	Rods	Spherical	Spherical	Rods	Spherical
pH range	5–10	6–10	5–10	5–10	5–10	6–10	6–12	6–10	6–11	6–10	6–12	5–10
Temp. range (°C)	10–50	10–50	10–40	10–40	20–40	10–50	20–40	10–50	10–40	20–50	20–40	10–40
Salinity range (‰)	0–13	0–13	0–13	0–10	0–10	0–13	0–10	0–5	0–10	0–3	0–7	0–7
Catalase test	+	+	+	-	+	+	+	+	-	-	+	-
Oxidase test	+	+	+	-	-	-	+	+	-	-	+	-
Indole test	-	ND	ND	+	-	-	-	-	+	ND	-	ND
Aeroic/anerobic	+f	+r	+r	-r	+f	+r	+r	+f	+f	+f	+r	+f
Glucose (acid)	-	+	-	+	ND	ND	ND	+	-	+	-	+
Carboh. [F/O/-]	F	-	O/-	F/-	ND	ND	ND	F	F	F	-	F
H_2S production	+	ND	ND	ND	-	-	+	ND	ND	ND	-	ND
V-P test	+	ND	-	+	-	-	+	+	+	+	+	-
Methyl red test	-	ND	ND	ND	+	+	-	ND	ND	ND	-	ND
Nitrate reductase	+	-	+	-	+	+	ND	ND	-	ND	+	-
Gelatinase	-	+	+	-	-	-	-	-	+	-	-	+
Lipase	+	+	+	+	+	+	+	+	+	-	-	-
Protease	+	+	+	+	+	+	+	+	+	+	+	+
Cellulase	+	-	-	+	+	+	+	-	+	-	-	-
Amylase	+	+	+	+	+	+	+	+	-	+	+	+
Chitinase	-	-	-	-	-	-	-	+	-	-	-	-
Agarase	-	-	-	-	-	-	-	-	-	-	-	-

BRT = before red tide; DRT = during red tide; ART = after red tide; NA = nutrient agar; AA = Aeromonas agar; MSA = Mannitol salt agar; TCBS = Thiosulfate citrate bile salt sucrose agar; Temp. = Temperature; Carboh. = Carbohydrates; ND = not detected

while 2 isolates were characterized spherical shaped cells and 1 isolate was puncitiform (Table 4). There were 5 isolates capable of growing in restricted aerobic conditions and 6 isolates were facultative anaerobe under anaerobic conditions facultative, while only one isolate (MH4) was restricted anaerobe. The pH ranged between 5 and 12, while the growth temperature for all isolates ranged from 10 to 50. The salinity sustainability (%) range was raised from 0 to 13%. There was a positive Catalase result recorded in 8 isolates and negative in 4 isolates. Oxidase detection was shown in 5 isolates, while it not present in 7 isolates. The production of an indole ring was detected in only 2 isolates and absent in 6 isolates. In 4 other isolates the test was not carried out and, hence, it did not detected. The enzymatic activities of bacterial isolates were detected. A package of hydrolytic enzymes (gelatinase, lipase, protease, cellulase, amylase, chitinase and agarase) was produced. However, the most detectable enzyme was protease, while the least detectable was chitinase. Also, agarase was not detected in any isolate at all. Moreover, there were clear variations in the other tests (such as: acid from glucose, carbohydrates fermentation/oxidation, H_2S production, V-P, methyl red, and nitrate reductase) between positive, negative and not detected records.

However, the 12 isolates were characterized at the genus level as follows: MH1; *Vibrio* sp. 1, MH2; *Neisseria* sp., MH3; *Micrococcus* sp., MH4; *Streptococcus* sp., MH5; *Vibrio* sp. 2, MH6; *Bacillus* sp., MH7; *Pseudomonas* sp., MH8; *Aeromonas* sp., MH9; *Pediococcus* sp., MH10; *Lactococcus* sp., MH11; *Alcaligenes* sp. and MH12; *Aerococcus* sp. (Table 5).

Table 5. Identification of bacterial isolates depending on data in Table 2

Isolate code	Possible genus
MH1	*Vibrio* sp.1
MH2	*Neisseria* sp.
MH3	*Micrococcus* sp.
MH4	*Streptococcus* sp.
MH5	*Vibrio* sp.2
MH6	*Bacillus* sp.
MH7	*Pseudomonas* sp.
MH8	*Aeromonas* sp.
MH9	*Pediococcus* sp.
MH10	*Lactococcus* sp.
MH11	*Alcaligenes* sp.
MH12	*Aerococcus* sp.

Cross antagonism between dominant bacterial isolates

The interaction between the 12 bacterial isolates themselves was estimated to determine their ability to inhibit or suppress each other. Thus, in this experiment, a 12 × 12 array of tests (144) was performed using the tooth pick technique. The result shown in Table 6 confirmed the occurrence of antagonistic interactions among the experimental bacteria. *Vibrio* sp.2 was the most powerful antagonist against the rest, since it recorded positive activity against four bacterial species (*Micrococcus* sp., *Alcaligenes* sp. *Pseudomonas* sp., and *Streptococcus* sp.). Both *Aeromonas* sp. and *Neisseria* sp. exhibited antagonistic activity against two isolates; the former was against *Alcaligenes* sp. and *Streptococcus* sp., while the latter was against *Alcaligenes* sp. and *Aerococcus* sp., respectively. On the other hand , the most affected indicator isolate was *Streptococcus* sp. by *Aeromonas* sp.

Molecular characterization of the bacterial antagonists

Based on morphological observations, biochemical tests and homology comparisons by 16S rDNA sequences, the selected strains MH2, 5 and 8 were identified as: *Psychrobacter adeliensis* strain DSM 15333, *Vibrio toranzoniae* strain Vb 10.8 and *Ruegeria pelagia* strain NBRC 102038, respectively (Fig. 2; Table 7).

Table 6. Positive cross antagonism between bacterial isolates during and after the red tide (Spring 2015) in the Eastern Harbor, Alexandria, Egypt

Antagonistic isolate	Indicator isolate						
	Micrococcus sp.	*Vibrio* sp.1	*Alcaligenes* sp.	*Pseudomonas* sp.	*Streptococcus* sp.	*Aerococcus* sp.	*Streptococcus* sp.
Neisseria sp.			x		xx		
Pediococcus sp.						x	
Vibrio sp.1			x				
Lactococcus sp.				x			
Aeromonas sp.			x				xxx
Bacillus sp.			xx				
Vibrio sp.2	xx	xxx		xx			x

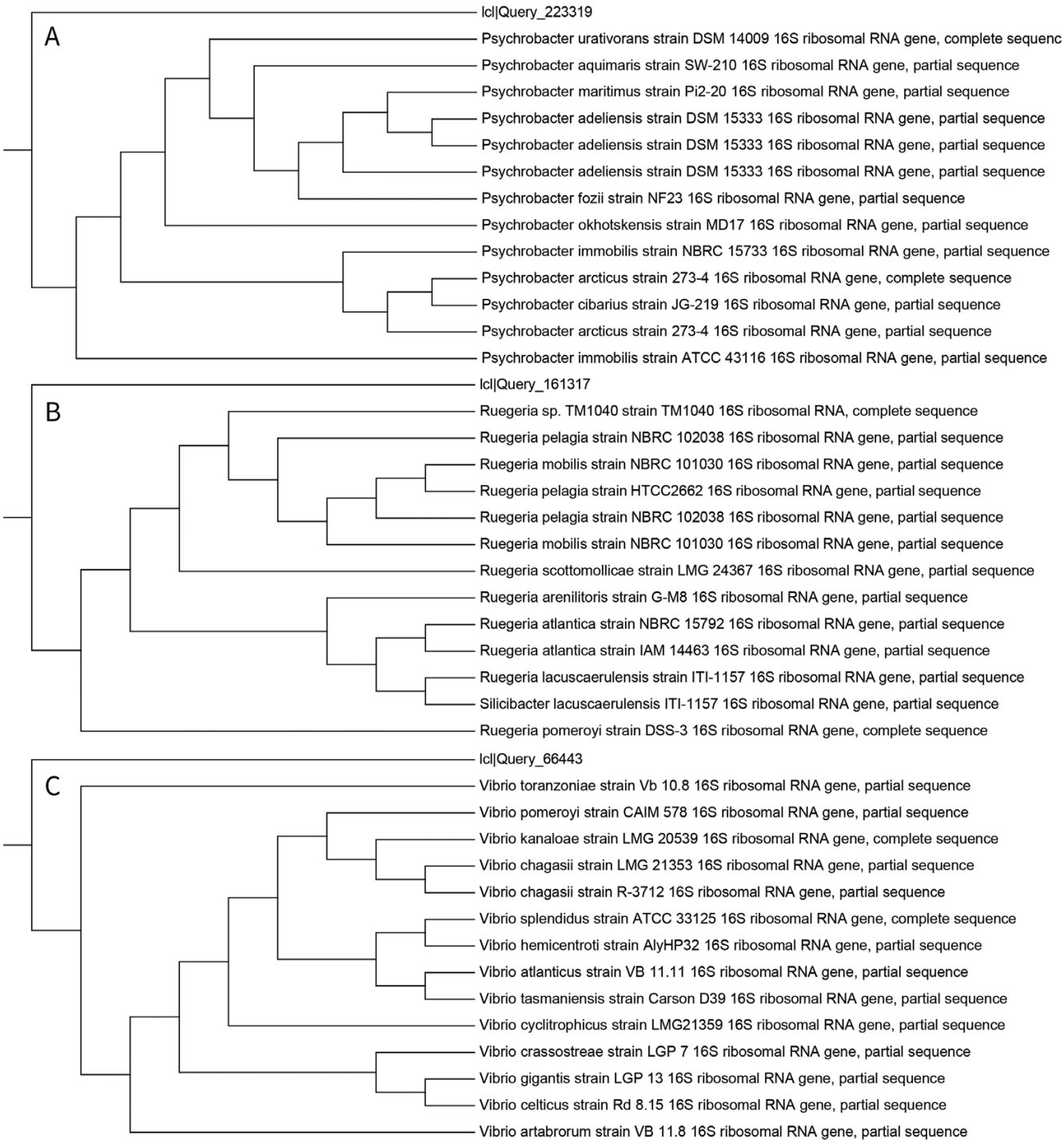

Fig. 2. Phylogenic analysis of 3 marine isolates based on partial sequencing of 16Sr DNA. *P. adeliesis* (A), *V. toranzoniae* (B) and *R. apelagia* (C)

Table 7. Accession number of the experimental 16S rDNA sequence and similarity percentage to the closest known species

Antagonist code	Accession no.	Most related species	Similarity[1] (%)
MH2	NR_117634_1	*Psychrobacter adeliensis* strain DSM 15333	79
MH5	NR_117680_1	*Vibrio toranzoniae* strain Vb 10.8	98
MH8	NR_114024_1	*Ruegeri apelagia* strain NBRC 102038	97

[1]Aligned ribosomal DNA fragments were obtained by PCR using primers F27 and R149

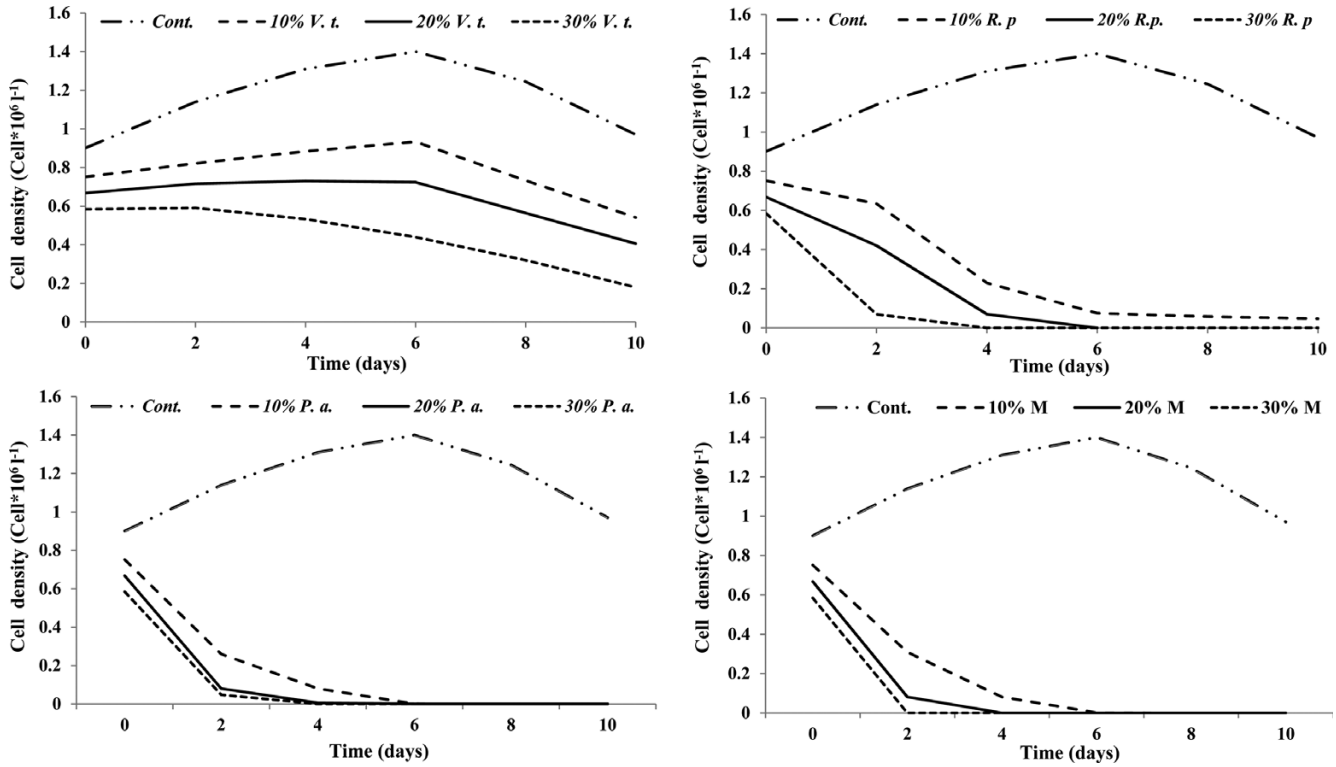

Fig. 3. Impact of different concentration of bacterial strains (*V.t.*; *V. toranzoniae*, *R. p.*; *R. apelagia*, *P.a.*; *P. adeliensis* and their mixture) on growth of *S. costatum*

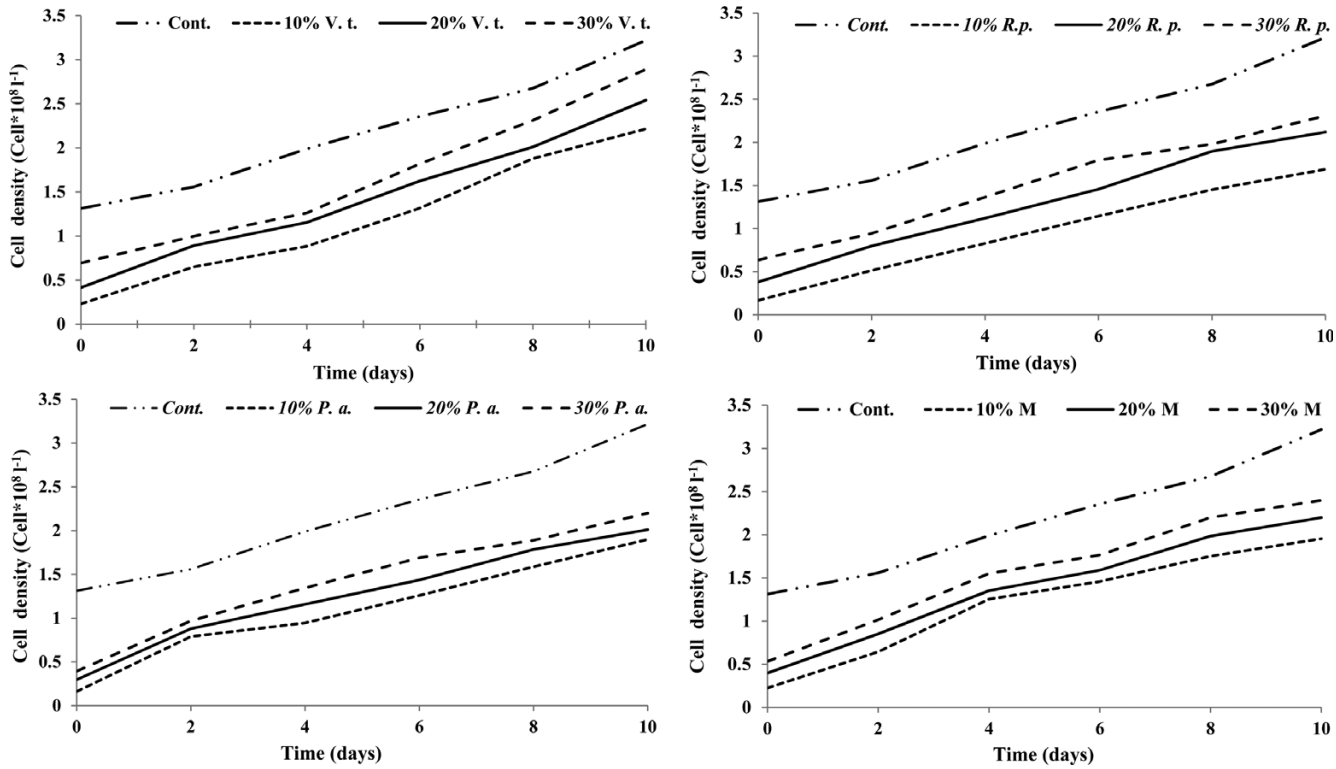

Fig. 4. Comparison of growth curves of bacterial strains (*V.t.*; *V. toranzoniae*, *R. p.*; *R. apelagia*, *P.a.*; *P. Adeliensis* and their mixture) and their mixture with *S. costatum*

Interaction between the most common bacterial species and *S. costatum*

The most common three bacterial strains and their mixture (10, 20 and 30% (v/v)) were added separately to *S. costatum* (causal agent of the red tide) culture before the stationary growth phase to study the relationship between them. The cell number of *S. costatum* significantly increased with time and reached a maximum after six days in f/2 seawater medium (Fig. 3). The growth of *S. costatum* was significantly lower whenever each bacterium was present as compared to axenic culture. More specifically, 30% (v/v) of the *R. pelagia* (r = -0.97) and *P. adeliensis* (r = -0.96) showed the strongest algicidal activities against *S. costatum* cells after two days of incubation period as compared with *V. toranzoniae* (r = -0.86). 10% of *R. pelagia* (r = -0.76) and *P. adeliensis* (r = 0.80) also showed significant algicidal activities and negative impact on the growth of *S. costatum* within six days. The mixture of common bacteria species had the most lethal effect as compared with each one separately. Regarding the impact of the different concentrations of *S. costatum* on bacterial growth, there was a significant effect on bacterial growth. however, bacterial numbers of each strains and their mixture increased until the end of the experiment as compared to control (Fig. 4).

4. Discussion

This study attempted to study the biodiversity of phytoplankton and bacteria community before, during and after red tide phenomena in Eastern Harbour, Alexandria, Egypt and also to determine the interaction between the most common bacterial spp. and *Skeletonema costatum,* the causal agent of the red tide.

Phytoplankton reflects water quality trough changes in its community structure, patterns of distribution and the proportion of sensitive species. During the study, Bacillariophyceae dominated the taxa and *S. costatum* was the most common species during the study period. This observation is similar to the observation of Khairy et al. (2014) who detected that Bacillariophyceae flourished during spring season. Dango et al. (2015) also recorded that Bacillariophyceae comprised the highest number of taxa during spring and autumn seasons along the coastal area of Alexandria. The results reflect differences in phytoplankton diversity and abundance during and after red tide phenomena. The total count of phytoplankton increased during the red tide period due to the high densities of *Skeletonema costatum* which was the main causal agent of the bloom phenomena. In an earlier study, Labib (1994) stated that the centric diatom "*Skeletonema costatum*" 6.27 $\times$ 10^6 cell l^{-1} (90.37% of community) was the causative species of bloom. Red tide phenomena occur regularly in warm seasons in Eastern Harbour (Labib and Halim 1995). Mikhail (2003) recorded the count of *S. costatum* bloom in Eastern Harbour with a fluctuation count (1.5, 1.37, 8.34 and 2.94) $\times$ 10^6 during July 1999, May, September and October 2000. Similar findings were reported previously by Wawrik (1961) who stated that *S. costatum* was widely distributed in the Mediterranean Sea with a maximum occurrence in spring. *S. costatum* was detected quantitatively as the most common species during 1972–1973 (Sultan 1975) and in 1986–1987 (Zaghloul and Halim 1992) and later in March 2007 in Eastern Harbour (Abd El-Haliem and Khairy 2007).

In marine environments, competition among microbes for space and nutrients is a selective force that has led to the evolution of a variety of effective strategies for colonizing and growing on surfaces (Burgess et al. 1999). It is hypothesized that bacteria use chemically mediated defenses to compete for space and nutrients in these micro environments (Long and Azam 2001). Grossart and Colleagues (2003) have further suggested that between species antagonistic interactions are micro-scale factors that can influence particle colonization rates (Grossart et al. 2003). Efficient methods for isolation of microorganisms from the oceans are required, since only a small percentage (< 1%) of the bacteria of sea water can be cultured (Park et al. 2002). Selective media were used to enumerate specific groups of bacteria. Although used in many studies, several have concluded that selective enrichment cultivation is not a suitable tool for characterizing microbial communities (Pace 1997; Muyzer and Smalla 1998). It is well known that marine microbial communities are influenced by environmental variables such as temperature, nutrient availability, water salinity and other factors (Thingstad and Passoutzadegan 1995; Uphoff et al. 2001).

The occurrence of antagonistic interactions among experimental bacterial isolates obtained in this study was proven. Eight isolates from twelve showed a prominent antimicrobial effect against 75% of the total. Data showed that *Vibrio* sp.2 was the most effective antagonist, while the most affected indicator isolate was *Streptococcus* sp. by *Aeromonas* sp. The production of inhibitory substances is a common phenomenon among bacteria isolated from bacterial bio-film, giving them a competitive advantage over other bacteria (Avendano-Herrera and Riquelme 2007). A large fraction (25%) of the examined

bacterial isolates exhibited antagonistic properties against other pelagic bacteria. Much lower percentages of bacterial antagonism (5 to 8%) (Nair and Simidu 1987) and much higher percentage (35–53.5%) (Long and Azam 2001) were reported in previous studies.

The three bacterial antagonists submitted to identification by 16S rDNA belonged to the order Proteobacteria. The isolate MH2 was characterized phenotypically as *Neisserias* sp. which belongs to Moraxellaceae and near to *Psychrobacter* sp. so, further work needs to be done to investigate genetically similar species before the true generic position of this organism can be determined. However, *Psychrobacter* sp. is a genus of Gram negative, osmotolerant, oxidase positive, psychrophilic orpsychrotolerant, aerobic bacteria which belong to the family of Moraxellaceae and the class of Gammaproteobacteria. The shape is typically cocci or coccobacilli. Some of those bacteria were isolated from humans and can cause infections in humans like endocarditis and peritonitis. This genus of bacteria is capable of growing at temperatures between -10°C and 42°C. *Psychrobacter* occur in wide range of moist, cold saline habitats. But they also occur in warm and slightly saline habitats (Garrity et al. 2005; Kim et al. 2012; Azevedo et al. 2013). *Psychrobacter adeliensis* is a gram-negative bacterium from the genus of *Psychrobacter* which was isolated from fast ice in the middle of Geologie Archipelago in Adelie Land in the Antarctica (Shivaji et al. 2004).

The isolate MH5 was characterized phenotypically as *Vibrio* sp. which belongs to Vibrionaceae and genotypically identified as *Vibrio toranzoniae*. However, Lasa et al. (2013) obtained four motile facultative anaerobic marine isolates (Vb 10.8(T) [CECT 7225(T), CAIM 1869(T)], CMJ 9.4 [CECT 8091, CAIM 1870], CMJ 9.11 and Cmf 13.9) from cultured clams (*Venerupis philippinarum* and *Venerupis decussata*) in Galicia (NW Spain). These isolates were studied by a polyphasic approach, including a phylogenetic analysis based on sequences of 16S rRNA and results demonstrated that the strains constitute a novel specie of the genus for which the name *Vibrio toranzoniae* sp. nov. was proposed.

The isolate MH8 was characterized phenotypically as *Aeromonas* sp. which belongs to Enterobacteriaceae but genotypically was identified closely to *Ruegeria* sp. that belongs to Rhodobacteraceae. However, Lee et al. (2007) isolated Gram-negative, facultative aerobic, chemoheterotrophic, short rod-shaped marine bacterial strains HTCC2662 (T) and HTCC2663, isolated from the Sargasso Sea. Characterization of the two strains by phenotypic and phylogenetic analyses revealed that they belonged to the same species. Phylogenetic analysis of the 16S rRNA gene sequences showed that the strains represented a distinct line of descent within the genus *Ruegeria*, with the highest sequence similarities to *Ruegeria atlantica* DSM 5823(T) (97.2%), *Ruegeria lacuscaerulensis* DSM 11314 (T) (96.5%) and *Ruegeria pomeroyi* DSM 15171(T) (95.6%).

Interactions between bacteria and HAB-causing species are potentially important factors affecting both the population dynamics and the toxicity of these algae. Many studies on harmful algal blooms have focused on the effects of environmental, including physical and chemical, parameters but very little data show close links between bulk parameters of bacteria and phytoplankton communities in coastal marine environments. Nevertheless, literature is available on the interaction between phytoplankton and heterotrophic bacteria and their diversity. These analyses are helpful to understand the relation between bacteria and *S. costatum* which is one of the Harmful Algal Blooms (HABs) and also have attempted to define the roles of bacteria in bloom termination and to determine whether various bacteria could inhibit or stimulate the growth of phytoplankton. The results indicate that specific interactions between phytoplankton and bacteria may occur and that such interactions could be important factors affecting both the population dynamics and the toxicity of these algae. In this connection, Mullins and Priddle (1987) stated that it is logical to expect bacteria to respond to variation in phytoplankton growth and abundance, and Grossart and Simon (2007) reported that the bacteria populations have distinct effects on the growth and organic matter release of marine diatoms and other algae.

In our study, the most common three bacterial species show negative relationships with *S. costatum*. This may be related to the competition for nutrients, mainly N and P compounds or these bacteria may produce bioactive secondary metabolites such as biotoxins which inhibited the algal growth or/and *S. costatum* may produce secondary metabolites with stimulative effects on these bacterial species. The taxa of marine algicidal bacteria (*Vibrio, Flavobacterium*, *Acinetobacter*, *Alteromonas* and *Pseudomonas*) which belong to the γ proteobacterial group are thought to kill microalgae by means of algicidal substances (Yoshinaga et al. 1998). The algicidal bacteria control or kill *S. costatum* using extracellular proteases (Lee et al. 2000), aminophenol (Yoshikawa et al. 2000), hydroxylamine (Berger et al. 1979), exopolymers (Decho 1990) and antibiotics (Dakhama et al. 1993). These enzymes or bioactive metabolites

could be used to control the growth of HAB-causing species (Fukami et al. 1992).

The results of the present study showed that the different concentrations of bacterial strain *V. toranzoniae* revealed moderate inhibition activity against *S. costatum*. On the other hand, all concentrations of *R. pelagia* and *P. adeliensis* exhibited lethal effects on *S. costatum* growth; these may be related to their species and /or concentration. These results were in agreement with Lee et al. (2008) who stated that the concentrations of algicidal bacteria required to kill target red tide organisms (LCBK) differ depending on the species of bacteria and red tide organisms. Seong and Jeong (2011) reported that the harmful effect of pathogenic bacterium *V. parahaemolyticus* depended on bacterial concentration and incubation time.

In all bacteria-*S. costatum* cultures, the bacterial numbers increased during all incubation periods. This may be related to stimulation substance release during interaction between *S. costatum* and bacterial sp.. Grossart (1999) stated that phytoplankton enhanced bacterial growth by stimulatory substances that are not of direct bacterial origin can be released during microbial colonization and degradation of senescent algae.

5. Conclusion

Our results show that shifts in the community composition of eukaryotic phytoplankton and bacteria attached to them are correlated, suggesting that the dynamics of these two communities are linked. In contrast, patterns of community composition of free-living bacteria were not correlated with those of the phytoplankton. To our knowledge, this is the first study showing links between phytoplankton attached bacterial community dynamics in a natural coastal environment. The results indicate that specific interactions between phytoplankton and attached bacteria may occur and that such interactions could be important in controlling the composition of both communities. The biological factor is one of the most important factors which mainly control algal blooms in marine environments. *V. parahaemolyticus* can be simultaneously a predator or prey for all red tide dinoflagellates tested in the present study; *V. parahaemolyticus* induces the most harmful effects on *C. polykrikoies*; Bacterial concentration and incubation time were important factors; With increasing *V. parahaemolyticus* concentration, ingestion rates of *P. minimum*, *P. micans*, and *A. carterae* on the prey increased, whereas ingestion rates on *C. polykrikoides* decreased. The associated bacterial community acts as potential regulators by enhancing or decreasing algal blooms through direct or indirect modes of action depending on the causal agent of red tide, bacterial species, and their concentration and incubation time.

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