Effects of different pCO_2 concentrations on marine bacterial community structure, Eastern Harbor, Alexandria, Egypt

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Abstract: The direct effect of an elevated CO_2 concentration range (280, 385, 550, 750 and 1050 µatm) on the marine bacterial counts and dominancy of species were examined. Our results demonstrated that the variation in glucose consumption corresponding to the incubation period (h) of bacterial community structure showed that glucose degradation as a carbon source for bacteria is in good consistency with the total bacterial count pattern. Glucose uptake and oxygen consumption are increased by increasing the temperature from 28 to 35°C and also by increasing pCO_2 from nowadays pCO_2 (385µatm) to (1050 µatm). The highest consumption of glucose and oxygen was recorded in consistence with the dominancy of glucose degrading bacteria. The dominant bacterial species isolated from the Eastern Harbor, Alexandria, Egypt were counted and genetically identified. The total bacterial count (CFU/ml) increased linearly with increasing different pCO_2 at 35°C from 280 and 1050, respectively. Total bacterial count (CFU/ml) at different pCO_2 increased linearly with the incubation temperature (28-35°C). There were seven bacterial isolates from the Eastern Harbor with codes (HW1-HW7). They were affiliated according to their 16S rDNA to *Bacillus cereus* HW1, *Psychrobacter maritimus* HW2, *Shima marina* HW3, *Pseudoalteromonas atlantica* HW4, *Bacillus horikoshii* HW5, *Oceanicola marinus* HW6 and *Oceanicola nanhaiensis* HW7, respectively. *Oceanicola nanhaiensis* HW7 exhibited fluctuation in hydrolytic activities against several carbon sources. The highest activity was for lipase followed by agarase, while the lowest activity was for cellulase. This It is also concluded that ocean acidification will impact bacterial organic matter degradation by changing reaction velocities of extracellular enzymes.

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1. Introduction

Since the beginning of the industrial period (ca. 1750), the oceans have taken up one-quarter to one-third of anthropogenic CO_2 emissions was around 280 µatm [1, 2]. Since that time, a period of only 250 years, this has increased to more than 380 ppm. This increase, caused primarily by the burning of fossil fuels and the manufacture of cement, is implicated as a primary driver of rising global temperatures and the environmental impacts associated with this 'climate change'. However, as far as Earth's oceans and the organisms therein are concerned, the biggest threat could come from another impact associated with atmospheric CO_2 increase; ocean acidification and the reduction in carbonate concentrations [3]. Surface ocean pH has been maintained between 8.0 and 8.3 pH units for the last 25 million years. However, the current rates of increase in CO_2 atmospheric concentration—approximately 100 times greater than previous naturally induced increases are causing seawater pH to decrease [4]. This has already led to a reduction in the surface ocean pH of 0.1 units, which may reach up to 0.7 units assuming the depletion of all fossil fuel reserves during the next three centuries [4]. In contrast, pH has constantly remained above 8.1 for the last 23 million years [5]. By the year 2100, atmospheric pCO_2 values of 700 or 1000 µatm may lower the mean surface pH in the southern North Sea to 7.82 or 7.67, respectively [6]. The effects of the anticipated rapid reduction in pH on marine organisms, and their ability to adapt, will determine future marine biodiversity and ecosystem functions; yet the impact of ocean acidification on different groups of marine organisms remains under debate [7, 8], especially regarding heterotrophic bacteria as important players in marine biogeochemical cycles. Recently it has been argued that microbe-dependent processes will not substantially change in a more acidic ocean, as marine microbes already experience large regional, temporal and depth-dependent pH variability. Also, greater pH ranges are observed in freshwater lakes [9]. In the case of marine microbes, the experimental results are inconsistent and occasionally contradictory [9, 10]. Compared to phytoplankton, much less laboratory or field experiments have been assessed on the effects of changes in CO_2 concentrations on heterotrophic microbes [9], despite playing a major role in the

marine carbon cycle, mineralizing organic carbon in the oceans to CO_2 [11]. A few mesocosms experiments have tested the effect of high CO_2 concentrations on the abundance and/or production of natural bacterioplankton populations [12-14]. These studies have found either no or an indirect (linked to phytoplankton dynamics) effect of elevated pCO_2 on bacterial production. Only a few studies have demonstrated a direct effect of CO_2 on natural prokaryotic plankton from the deep ocean [15, 16] or on marine bacterial isolates [17, 18]. The latter studies found a decrease in the production and growth rates at pH < 7; values are, nevertheless, far from the usual pH observed in ocean waters under present or future scenarios of elevated pCO_2. Most microorganisms, particularly heterotrophic bacteria, are able to assimilate CO_2 as part of their metabolism through anaplerotic reactions [19].

In order to investigate the direct effect of pCO_2 corresponding to different incubation temperature on bacterioplankton community structure, it is essential to count, isolate and then identify the dominant strains using 16S rDNA technique. Moreover, the biodegradability of the most abundant bacterial species for organic matter was examined.

2. Material and Methods

Experimental design:

In current marine research, the biological response to elevated seawater pCO_2 and biogeochemical consequences are mainly investigated by perturbation experiments, in which different approaches are used to manipulate the seawater carbonate chemistry [20]. In our experiments, to reference incubations representing present-day pH conditions were compared to acidified incubations that exhibited pH values projected for the future ocean compared to preindustrial pH value . Manipulation was carried out by acid base addition.

Our treatments for bacterial culture were Control/Natural seawater. The pCO_2 (in µatm) treatments levels were maintained using acid–base addition with (±0.02 pH units). These treatments were (pCO_2 = 280, pH_T = 8.21), (pCO_2 = 380, pH total = 8.11), (pCO_2 = 550, pH_T = 7.99), (pCO_2 = 750, pH_T = 7.87), (pCO_2 = 1050, pH_T = 7.75). TA was kept constant at 2700µatm. Bacterial acidification treatments were carried at four different temperatures, 25, 28, 32 and 35°C. Seawater Salinity was measured using a Beckman Induction Salinometer (model RS-7C). Dissolved oxygen was measured using a highly accurate modified Winkler method [21].

Sample collection:

Seawater samples were collected using the Nansen water sampler according to the method described by Grasshoff (1976) [22]. Samples were collected from a depth of 1.5 meter at the Eastern Harbor, Alexandria, Egypt taking all the required prequotions.

Culture medium:

The medium used for culturing bacterial community contained filtered seawater that was amended with 0.1% glucose. **Seawater agar** was used for the enumeration and the isolation viable aerobic heterotrophic bacteria. It contained peptone, 5g; ferric phosphate, 0.1 g; agar, 15 g; seawater 1L (in one liter seawater) [23].

Dissolved inorganic carbon and total alkalinity:

Culture aliquots were sampled for pH and measured with a Metrohm (827 pH) pH electrode calibrated with TRIS buffer on total scale (pH_T) following Dickson and Millero, 2007 [24]. Total Alkalinity (TA) was measured following Sarazin et al, 1999 [25]. Certified reference materials (CRM batch 19) that used to calibrate and establish correction factors for TA measurements were obtained from Professor Andrew Dickson at the Marine Physics Laboratory of the Scripps Institute of Oceanography, University of California San Diego. The carbonate system speciation (pCO_2, CO_3 and HCO_3) was calculated for pH and AT using CO_2SYS [26] with dissociation constants from the study done by Mehrbach et al, 1973 [27] and refitted by Dickson and Millero, 1987 [28]. The acidity constant of the ion H_2SO_4 in seawater was taken into account. Glucose was measured at the beginning of the experiment and at time intervals every two hours in consistency with optical density according to Strickland and Parsons, 1972 [29].

Isolation and counting of bacterial isolates:

Serial dilutions from 10^{-2} through 10^{-4} were made using sterilized seawater. A portion (0.1 ml) from each appropriately diluted sample was used to inoculate plates prepared with seawater agar for counting aerobic heterotrophs. Plates were incubated at 30^{o}C for 24 h and then counted. The pure dominant colonies obtained were transferred to fresh slants. Sub-cultures were kept at 4^{o}C for further investigations.

Molecular characterization of bacterial isolates:

The genomic DNA of different strains was isolated using the GFX genomic DNA purification kit (Amersham Bioscience) according to the manufacturer instruction. The DNA was analyzed using 0.7% agarose gel electrophoresis. The 16S rDNA gene was amplified by the polymerase chain reaction (PCR) using the primers; the 16S rDNA gene was amplified by the polymerase chain reaction (PCR) using the primers which are: 16S 357 F; ACT CCT ACG GGA GGC AGC AG and 16S 907R; CCG TCA ATT CAT TTG AGT TT. The selected bacterial isolates were identified based on the sequence analysis of their PCR amplified 16S rRNA genes. The PCR

primers (F357 and R907) were designed using the Primer 3 software to amplify approximately a 550-base pair fragment of the 16S rDNA region according to the *Escherichia coli* genomic DNA sequence. The PCR reaction mixture contained 200 µM of each dNTP, .5 µM primers, 10mM Tris-HCl pH 8.3, 1.5 mM magnesium chloride, 50 mM potassium chloride, 2.5 units Tag polymerase, m and 1 µl of template DNA. Amplicons were obtained with a PCR cycling program of 94°C for 1 min followed by 35 cycles of denaturing at 94°C for 30 sec, annealing at 55°C for 30 sec, and polymerization at 72°C for 2 min. At the end of thermocycling, the PCR reaction was incubated at 72°C for 7 more min. As described by Ausubel et al, 1999 [30], amplicons were visualized by electrophoretic separation on 1% agarose gels stained with ethidium bromide. PCR fragments were purified from amplification reactions with QlAquick PCR purification reagents (QlAGEN) according to the kit manual. DNA sequence was determined using the ABI Prism™ DNA automated sequencer and dye terminator cycle sequencing kit with AmpliTaq DNA polymerase (Applied BioSystems). These primers were separately used for sequencing of the amplified 16S rDNA fragments. The PCR sequencing program used has three steps: denaturation at 96°C for 15 sec, annealing at 60°C for 15 sec and extension at 72°C for 240 sec. The DNAstar was used for sequence editing. The database matching of the 16S rRNA sequences was performed using the Ribosomal Database Project. Biology workbench was used for the computational analyses of DNA sequence, multisequence alignment, and construction of the phylogenetic trees.

Activities of extracellular enzymes:

Extracellular enzymatic activities of the most dominant species were determined on solid media as the hydrolysis rate of specific substrates (cellulase and amylase according to Fritze et al, 1990 [31], lipase according to Karnetova et al, 1984 [32], protease according to Cowan and Steel (1993) [33] and agarase according to Hu et al, 2009 [34] in order to test bacterial potential to degrade proteins, carbohydrates and lipids.

3. Results

The effect of different concentrations pCO_2 (280, 385, 550, 750 and 1050 µatm) in relation to the incubation temperature (25, 28, 32 and 35°C) was investigated.

The variation in glucose consumption corresponding to the incubation period (h) of bacterial community structure was illustrated in Figure 1. It shows that glucose degradation as a carbon source for bacteria is in good consistency with the total bacterial count pattern. Glucose uptake is also increased by increasing the temperature from 28 to 35°C. It is also increased by increasing pCO_2 from now a day pCO_2 (385µatm) to (1050 µatm). The same trend was found for oxygen consumption (Figure 2).

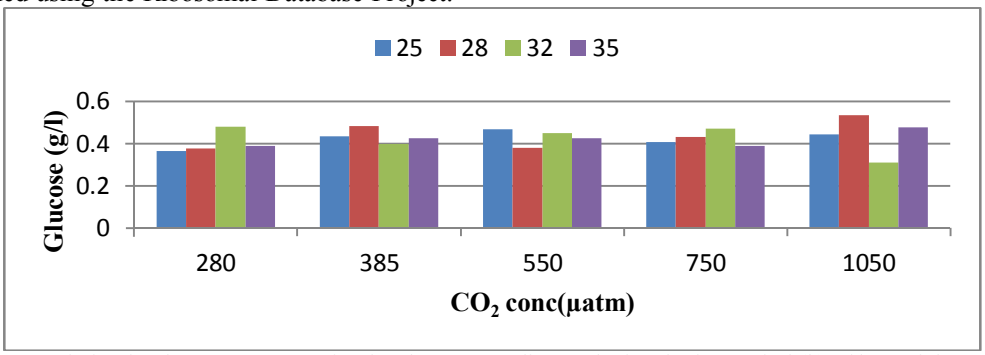

Figure 1: Variation in glucose concentration level corresponding to the incubation period (h) of bacterial community structure in the Eastern Harbor, Alexandria, Egypt

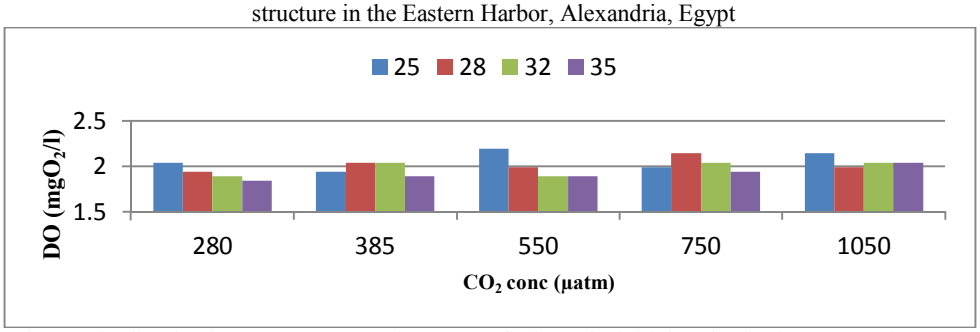

Figure 2: Changes in dissolved oxygen concentration (mgO2/l) from the initial to the final concentrations corresponding to different pCO2 (280, 385, 550, 750 and 1050)

The dominant bacterial species isolated from the Eastern Harbor, Alexandria, Egypt were counted and genetically identified. Results in Figure 3 showed that the total bacterial count (CFU/ml) increased linearly with increasing different pCO_2 at 35°C from 280 and 1050, respectively. Total bacterial count (CFU/ml) at different pCO_2 increased linearly with the incubation temperature (28-35°C). The variations in bacterial growth profile corresponding to incubation period (h) of bacterial community structure isolated from the Eastern Harbor, Alexandria, Egypt showed that the highest number of dominant bacterial species was 3 species isolated at 32°C, while two dominant bacterial species were detected at 25 and only one dominant species was recorded at 28 and 35°C. On the other hand, the highest number of dominant bacterial species was 3 at $pCO_2$385, followed by 2 species at $pCO_2$280 and only one species at 750 and 1050, respectively (Figure 4).

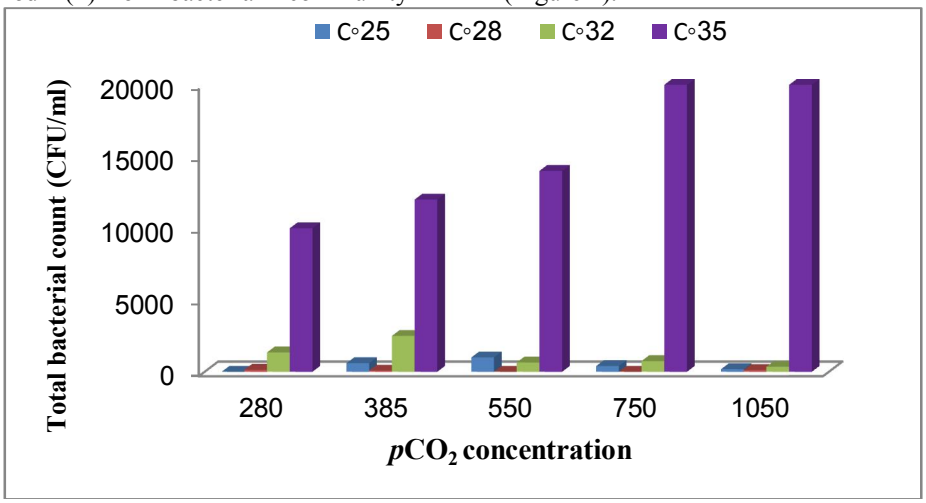

Figure 3: Total bacterial count (CFU/ml) at different pCO2 (280, 385, 550, 750 and 1050) and the incubation temperature (25, 28, 32 and 35°C)

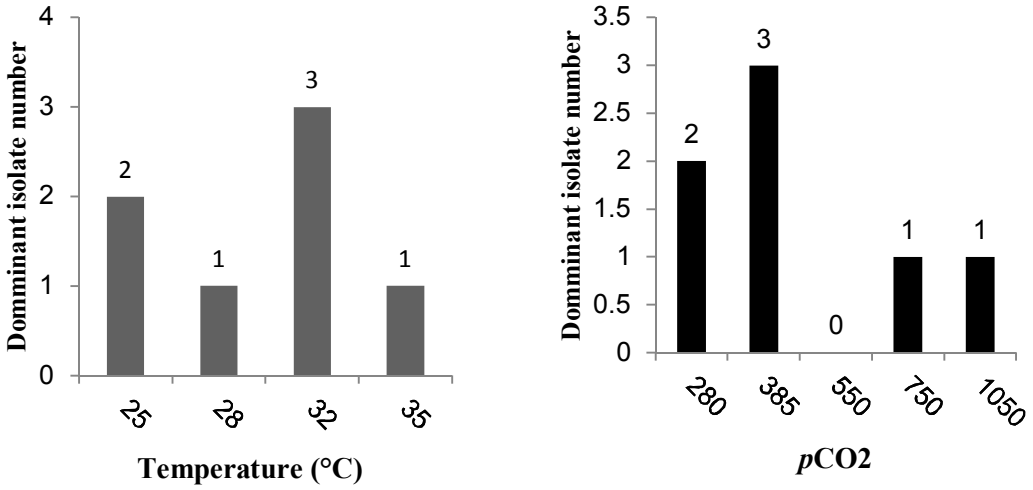

Figure 4: Number of dominant bacterial isolates corresponding to variation in the incubation temperature and variation in pCO2 concentration

These identified bacterial species are tabulated according to dominancy in each pCO_2 concentration and temperature as illustrated in Table 1.

A multiple sequence alignment between the obtained sequences is obtained. Sequencing data were aligned against the 16S rRNA sequences of the Ribosomal Database Project (http://rdp.cme.msu.edu/). It has been found that the bacterial isolate coded HW1 had a 97% identical counterpart with respect to its 16S rRNA sequence. Both HW5 and HW6 had 96% identical counterpart with respect to its 16S rRNA sequence. Both HW2 and HW3 had 95% identity, while the isolate HW4 94% identity. However, the isolate coded HW7 showed 81% identify with its most closely related

sequence. The most closely related species and the percentages of identity, as well as, the 16S rRNA sequences of the isolates were submitted to GenBank and their accession numbers are presented in Table 1.

The seven bacterial isolates HW1-HW7 were affiliated according to their 16S rDNA to *Bacillus cereus* HW1, *Psychrobacter maritimus* HW2, *Shima marina* HW3, *Pseudoalteromonas atlantica* HW4, *Bacillus horikoshii* HW5, *Oceanicola marinus* HW6 and *Oceanicola nanhaiensis* HW7, respectively.

Table 1: Accession number of the experimental 16S rDNA sequence and similarity percentage to the closest known species

Strain code	Accession no.	Most related species	Similarity (%)	Dominancy conditions
HW1	NR 074540.1	*Bacillus cereus* ATCC 14579	97	$PCO_2$385, 25°C
HW2	NR 027225.1	*Psychrobacter maritimus* strain Pi2-20	95	$PCO_2$385, 25°C
HW3	NR 043969.1	*Shima marina* strain CL-TAO3	95	$PCO_2$280, 28°C
HW4	NR 026218.1	*Pseudoalteromonas atlantica* strain IAM 12927	94	$PCO_2$280, 32°C
HW5	NR 040852.1	*Bacillus horikoshii* strain DSM8719	96	$PCO_2$750, 32°C
HW6	NR 043969.1	*Oceanicola marinus* strain AZO-C	96	$PCO_2$1050, 32°C
HW7	NR 043797.1	*Oceanicola nanhaiensis* strain SS011B1-20	81	$PCO_2$385, 35°C

The marine isolate *O. nanhaiensis* HW7, as the most dominant bacterial species was more examined for the biodegradation of organic matter (such as; protein, lipids and carbohydrates); that was driven by the hydrolytic activity of extracellular enzymes, which are predominately produced by bacteria. *Oceanicola nanhaiensis* HW7 exhibited a fluctuation in hydrolytic activities against several carbon sources. The highest enzymatic activity was for lipase followed by agarase, while the lowest activity was for cellulase. Moreover, the highest lipase activity was recorded at $pCO_2$385µatm (AU= 25) followed by (AU= 24) at $pCO_2$280 µatm and $pCO_2$550 µatm, while it was (AU= 22.5) and (AU= 22.3) at $pCO_2$750 µatm and $pCO_2$1050 µatm, respectively.

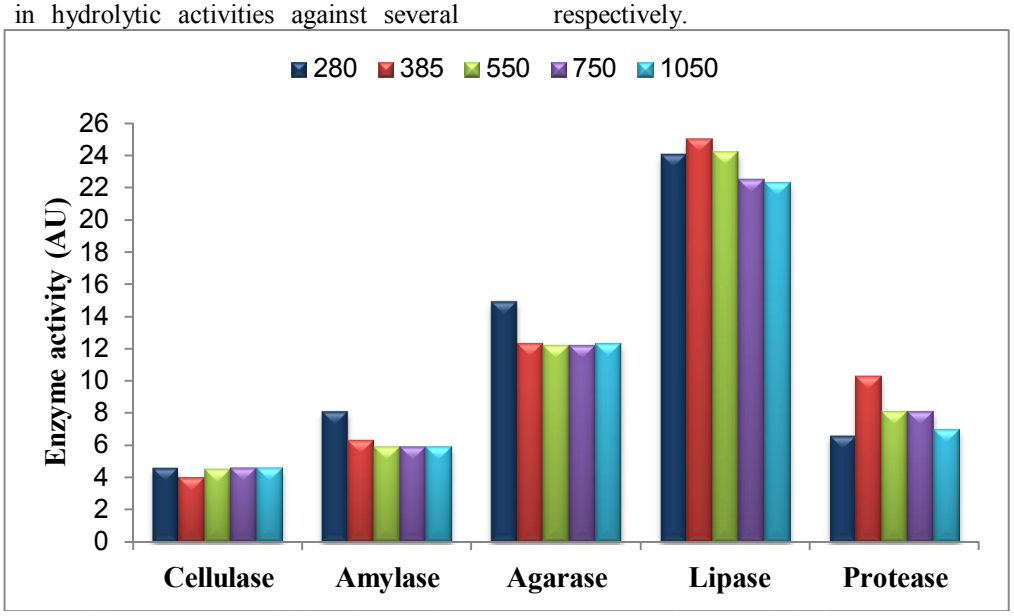

Figure 7: Different enzyme activities of *O. nanhaiensis* HW7 corresponding to variation in pCO2 concentration (µatm)

4. Discussion

As the atmospheric CO_2 concentration rises, more CO_2 will dissolve in the oceans, leading to a reduction in pH [35].

The bacterial community structure was slightly affected by the change in pH resulting in the increase of pCO_2 in seawater. On the long run, however, this change may have a significant effect in changing the whole picture of the marine biological community. This may because it controls both appearance and disappearance of specific species that play roles in the ocean such as: the food chain cycle, negative or positive interactions with other marine organisms and degrading organic matter to simple components. Although, these conditions became better for the dominancy of harmful bacteria species that may be flourished; the opposite may also happen and the most beneficial species thrive instead. On the other hand, the marine environment is not appropriate for the fungi (molds and yeasts) growth because of its pH

ranged in the alkaline level. Therefore, marine fungi will be absolutely affected in realistic and experimental range of pCO_2. This is because fungi prefer acidic pH to flourish in (4-6). However, the marine fungi will not effectively play roles according to this fact.

Yam et al, 2009 [36] support such hypothesis. They stated that the ocean sequestration of carbon dioxide and direct injection of CO_2 into bathypelagic layers is one of the climate change mitigation options. It is essential to assess the potential environmental impacts on the marine ecosystem. In bathypelagic layers, bacteria are dominant organisms and play significant roles in oceanic carbon cycling through the utilization and transformation of organic matter concerning other microbial processes, especially the heterotrophic bacteria; however, results have often been inconsistent.

The data of running study showed that, the highest consumption of glucose and oxygen was recorded in consistence with the dominancy of glucose degrading bacteria. It was reported by Khandeparker et al, 2011 [37] which belonged to the *Bacillus* genera followed by *Vibrio*, *Marinobacter*, *Exiquinobacterium*, *Alteromonas*, *Enterobacter* and *Aeromona*s have abilities to produce an array of complex carbohydrate degrading enzymes. Based on these findings and based on the identified bacteria from pCO_2 perturbation experiment the high glucose and oxygen uptake at pCO_2= 385µatm and at temperature 25 and 35°C could be attributed to the presence of glucose degrading bacteria *Bacillus cereus* HW1 and *Psychrobacter maritimus* HW2 at temperature 25°C and *Oceanicola nanhaiensis* HW7 at temperature 35°C. Moreover, the high oxygen uptake at of pCO_2= 750 and 1050 µatm at 32°C could be explained on the bases of presence of the dominant species *Bacillus horikoshii* HW5 and *Oceanicola marinus* HW6, respectively.

The fluctuation in the bacterial count within the same temperature (28-32°C) indicates that the dominancy of some species upper others because they have the ability in the consumption of carbon sources and/or secretion of agents against other marine organisms; while, at 35°C only one species was dominant. This may explain the increase in the bacterial counts as a factor with pH within the same temperature.

Yam et al, 2009 [36] performed laboratory experiments by acidifying bathypelagic seawater with CO_2 gas or buffer solutions to examine the impact on bacterial activities (abundance, production rate, and proportion of viable cells). Yam et al, 2009 [36] observed, from the laboratory experiments, some potential effects by artificial changes in CO_2 concentration, pH, or both, on bacterial activities. It was suggested that trophic conditions of bacterial assemblage strongly influence the magnitude of the impacts on bacterial activities and metabolisms by CO_2 sequestration.

On the other side, the ocean acidification effects on bacterial communities predominantly stems from complex systems such as symbiotic microbial communities of corals or large-scale mesocosm experiments. At a reduced pH, coral microbial communities were found to shift to bacteria associated with stressed or diseased hosts [38, 39], which could, however, not be confirmed at natural CO_2 vent sites Yam et al, 2009 [40]. Furthermore, a decrease in the relative abundance of *Alphaproteobacteria* and an increase in the relative abundance of *Flavobacteriales* were observed in natural biofilms from the Great Barrier Reef [41].

Polysaccharides are a major component of marine organic matter and comprise up to 15% of sinking and suspended particulate organic carbon [42-44] and up to 32% of dissolved organic carbon [45]. They can account for more than 50% of total phytoplankton primary production [46] and provide a labile energy and carbon source to heterotrophic bacterioplankton in form of structural cell components, storage glucan, and phytoplankton exudates. Intense production of polysaccharides included in phytoplankton biomass and exudates occurs during bloom events [46-48].

Heterotrophic bacteria are the main producers of CO_2 in the ocean and drive organic matter turnover and sustain food webs [48-51]. Marine microbial communities are engines of globally important process, such as the carbon, nitrogen and sulphur cycles [52]. Concerning other microbial processes and especially heterotrophic bacteria however, results have often been inconsistent and Liu et al, 2010 [10] concluded that “more research is needed at multi-species and community scales”.

Krause et al, 2012 [35] stated that bacteria susceptible to changes in pH were different members of *Gammaproteobacteria*, *Flavobacteriaceae*, *Rhodobacteraceae*, *Campylobacteraceae* and further less abundant groups. Their specific response to reduced pH was often context-dependent. Bacterial abundance was not influenced by pH. They suggested that already moderate changes in pH have the potential to cause compositional shifts, depending on the community assembly and environmental factors.

As mentioned, seven isolates obtained by the current study were identified as: *Bacillus cereus* HW1, *Psychrobacter maritimus* HW2, *Shima marina* HW3, *Pseudoalteromonas atlantica* HW4, *Bacillus horikoshii* HW5, *Oceanicola marinus* HW6 and

Oceanicola nanhaiensis HW7, respectively. Clearly they were of marine origin or adapted bacteria.

Species of *B. marinus, B. badius B. subtilis, B. cereus, B. licheniformis, B. firmus, and B. lentus* were often isolated from marine habitats [53, 54]. Recent studies on marine bacilli [55] showed that strains of *B. marinus, B. subtilis, B. pumilus, B. licheniformis, B. cereus*, and *B. mycoides* are common inhabitants of the Pacific Ocean. Romanenko et al, 2004 [56] isolated four strains of Gram-negative, aerobic, psychrotolerant, non-motile, non-pigmented bacteria; they were isolated from coastal sea-ice and sediment samples. *Psychrobacter maritimus* sp. nov. and *Psychrobacter arenosus* sp. nov., were isolated from coastal sea-ice and sediments of the Sea of Japan. *Pseudoalteromonas atlantica* is a marine bacterium, which has been shown to act as a primary producer of biofilms and exhibit virulence against edible crab species, through the secretion of extracellular products. *Bacillus horikoshii* is a facultative anaerobe bacterium. It is a Gram positive, alkaliphilic and alkalitolerant, aerobic Endospore-forming bacteria.

The genus *Oceanicola* is a member of the *Alphaproteobacteria* that was first described by Cho and Giovannoni, 2004 [57]. Most *Oceanicola* spp. are isolated from seawater in different marine environments [58-61]. Indeed, Gu et al, 2006 [58] isolated Gram-negative, non-motile, rod-shaped bacterium; *Oceanicola nanhaiensis* strain SS011B1-20(T) from sediments of the South China Sea. Its growth occurred at NaCl concentrations between 0 and 10% and at temperatures between 10 and 37°C.

Allgaier et al, 2008 [14] did find changes in the bacterial taxonomic composition in response to high CO_2 concentrations, which suggest that the effects of elevated pCO_2 are likely to vary among species. On the contrary, Alderkamp et al, 2006 [62] found that small changes in pH have direct effects on the bacterial community composition. Their species belonged to *Gammaproteobacteria*, *Flavobacteriaceae*, *Rhodobacteraceae* and *Campylobacteraceae* as phylogenetic groups responding most notably to the differences in pH. Moreover, Alderkamp et al, 2006 [62] confirmed that isolate MED 165 belongs to *Rhodobacteraceae* and is 99.8% similar to *Roseobacter* sp. AY 576690; whereas MED 217 belongs to *Flavobacteriaceae* and is 99.6% similar to *Cytophaga* sp. AY 745817. Both MED 165 and MED 217 were isolated from 1:20 seawater dilution cultures enriched with inorganic phosphorous.

Oceanicola nanhaiensis HW7 isolated from the Eastern Harbor, Alexandria, Egypt had proved its hydrolysis ability towards different common carbon sources studied (cellulose, starch, agar, lipids and proteins). This explains its potentiality to be continued under the study conditions of pH, temperature, salinity and nutrient availability.

The pH is known as an important regulating factor for bacterial extracellular enzyme activity in aquatic environments [63]. Nevertheless, potential impacts of ocean acidification on bacterial growth and degradation activity are only poorly investigated [63-65]. Hence, results from these studies are not sufficient to answer questions concerning the effects of current seawater acidification, inducing moderate pH changes on large scales due to rising atmospheric CO_2 [13].

The main source of CO_2 in the ocean is the decomposition and subsequent respiration of organic molecules by heterotrophic bacteria. However, very little is known about potential effects of ocean acidification on bacterial degradation activity [66]. The bacterial degradation of polysaccharides is initiated by the activity of extracellular enzymes [63, 67]. Thereby, macromolecules are enzymatically hydrolyzed outside of bacterial cells into units of low molecular weight that are small enough to be transported across the cytoplasmic membrane.

Our results conducted that the degradation of polysaccharides and proteins by *O. nanhaiensis* HW7 was not accelerated by increasing pCO_2 during experimental simulation of ocean acidification. The opposite direction was supported by our data.

The study done by Pointek et al, 2010 [66] revealed that the degradation of polysaccharides, a major component of marine organic matter, by bacterial extracellular enzymes was significantly accelerated during experimental simulation of ocean acidification. These results strongly suggest that ocean acidification will impact bacterial organic matter degradation by changing reaction velocities of extracellular enzymes. Also, Pointek et al, 2010 [66] concluded that the activity of extracellular enzymes in aquatic environments was shown to respond sensitively to changing pH.

Conclusion

The current results demonstrated that, contrary to some expectations, high pCO_2 did not negatively affect bacterial growth but increased growth efficiency in the case of *Oceanicola nanhaiensis* strain SS011B1-20 at high temperature. They conducted that the degradation of polysaccharides and proteins was accelerated by decreasing pCO_2 during the experimental simulation of ocean acidification. We agree with Hallegraeff, 2010 [68] who concluded that changes in phytoplankton communities provide a sensitive early warning for climate-driven perturbations to marine ecosystems.

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