

7. Impacts of Free Radicals on the Dynamics of Marine Microbial Populations

Ji-Dong Gu

Laboratory of Environmental Toxicology, Department of Ecology & Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, P.R. China, and The Swire Institute of Marine Science, The University of Hong Kong, Shek O, Cape d'Aguilar, Hong Kong SAR, P.R. China

Introduction

Microorganisms are exposed to a wide range of stress in the environment. These stresses can be physical, chemical and biological in nature. In particular, a general concept has been gradually established about the starvation condition in the natural environment. This is especially true since majority of the natural population of microorganisms are living in environments including aquatic lakes, oceans and aquifer that are oligotrophic (Kjelleberg, 1993). Ocean is one of the most productive ecosystems, but the biological productivity is affected by many factors including ultra-violet (UV) radiation (Herndl et al., 1993). Among the UV light, UV A (320-400 nm) affects viability of cells and is responsible for inhibition of up to 50% of the total photosynthesis. UV B (290-320 nm) is capable of reducing recruitment and affects the trophic transfer. In addition, it is also possible that free radicals and related chemical species in the environment play an important role in controlling the number and activity of natural population of microorganisms. Information on this subject is very limited currently. Because it is well known that biological factors such as viruses limit the microbial population in the oceans, this review will attempt to discuss the implication of chemical species of radicals and their potential role in understanding the fundamental biology of the natural ecosystem.

Oxidative stress is defined as a disturbance in the prooxidant-antioxidant balance in favor of prooxidants (Storz and Zheng, 2000). Both aerobic and facultatively anaerobic microorganisms have evolved abilities in detoxifying peroxide and super oxide through catalases and super oxide dismutases, respectively. In contrast, strictly anaerobic bacteria lacking both enzymes are very sensitive to molecular oxygen (O_2). Oxygen molecule is the most important electron acceptor and oxidative species of oxygen are produced constantly in the biosphere. Information on the relationship between these destructive chemical species and their effect on natural microorganisms will also allow the quantitative assessment of the natural productivity. Such

information has not been documented for the natural environments. Limited reports relevant to this subject are, however, largely based on medically important species of bacteria and assays under laboratory conditions. On the molecular level, high osmolarity can induce the multi-system response through regulatory genes *envZ*, *ompR* and *kdpD* and similarly oxidative stress induces *oxyR* gene and SOS response *recA* and *lexA* genes (Neidardt, 1987). As the technological advancement being made in the past several years, it is feasible to examine this interesting and important issue to obtain crucial insights to the effects of radicals on natural population of organisms.

Free radicals in the aquatic environments

Free radicals can be formed by a number of processes, e.g., thermolysis, photolysis, ionizing radiolysis, redox reactions, the Fenton reaction, chain reactions and mechanical generation. In aqueous systems, photochemical processes produce free radicals. Highly reactive species including 1O_2 , OH^\bullet , H_2O_2 and organic peroxides in oxygenated waters react with a range of organic compounds and the surface molecules of microorganisms. In a typical summer, surface waters receive about $1 \text{ kW}\cdot\text{m}^{-2}$ of sunlight, which is equivalent to about $2 \text{ mol photo m}^{-2}$ in the region of 300-500 nm (Hoigné, 1990). Dissolved organic compounds in the surface water absorb a large portion of the photons and a portion is believed to react with nitrate or nitrite and Fe species (Tables 1 & 2). However, it is not known that to what extent microorganisms are impacted by the redox reaction and, in particular the generated free radical species under natural conditions. Electrochemically generated $^\bullet OH$ radicals are highly effective in killing test strain of *Escherichia coli* and cellular morphology was also observed to be deformed and lysed (Figures 1 and 2). Similarly, reactive species of the Fenton reaction also have similar effects on bacterial viability.

Table 1. Reactants produced by photochemical processes in natural waters

Products	Chemical Formula	Possible generation processes
Singlet oxygen	$^1\text{O}_2$	Light absorbing dissolved organic matter (humic acids)
Superoxide anion	O_2^-	Photolysis of Fe^{3+} complexes; deprotonation of HO_2
Hydroperoxyl	HO_2	Protonation of O_2^-
Hydrogen peroxide	H_2O_2	Photolysis of Fe^{3+} complexes, disprotonation of superoxide anion
Ozone	O_3	Uptake from atmosphere
Hydroxyl radical	OH	Photolysis of hydroxo or other Fe^{3+} complexes, of NO_3^- , NO_2^- , photolysis of H_2O_2
Organic peroxy radicals	ROO	Photolysis of dissolved organic material
Polar oxidation		

Modified from Stumm and Morgan, 1996

Such reactions have only been investigated on their role in the processes of ageing and carcinogenesis to a large extent (Dizdaroglu, 1991); their effects on marine organisms have not been fully realized. Because of the short time and difficulties in chemical analyses, relationship between free radical concentrations and effects on natural microorganisms has not been fully established.

Effects of oxidative stress on bacteria

Microorganisms in natural waters experience, apart from UV radiation, a wide range of stress including, availability of nutrients, predators and competing organisms. Beside the mostly investigated parameters such as temperature, pH and salinity, others including UV radiation, H_2O_2 and ferric ion concentrations do exert significant effects on bacterial growth and survival (Gauthier, 2000; Koga and Takumi, 1995; Wang et al., 2004). Both visible and UV radiation are the most drastic bactericidal agents of nature in aquatic environment, specifically in the

surface layer, and they can kill, inhibit or induce the marine bacteria to enter viable-but-non-culturable (VBNC) state (Gauthier, 2000). Under condition of starvation when growth nutrients are limited, which is a frequently encountered situation in both fresh water and marine environments, microorganisms may use a set of mechanisms to cope with the environmental stress. For example, marine *Vibrio*

spp evolved a long-term starvation resistance and multiple adaptations to heat, UV, near-UV stress (Nystrom et al., 1992). In an experiment studying diverse responses to UV-B radiation by several marine bacterial isolates including *Vibrio* spp., the DNA damage and sensitivity after the second UV-B exposure were considerably less than those of the first exposure, suggesting the possible presence of adaptation mechanism (Joux et al., 1999). UV light is known for cyclobutane pyrimidine dimers, pyrimidine-pyrimidinone photoproducts and protein-damages and the formation of cellular lesions (Jacobs and Sundin, 2001). Microorganisms also have adaptive system to repair the DNA damage to some extent implying that the interactions between the physical

Table 2. Formation of water with reactive oxygen intermediates through addition of four electrons

O_2	dioxygen
$\text{O}_2 + e + \text{H}^+ \longrightarrow \text{HO}_2^*$	perhydroxyl radical
$\text{HO}_2^* (\text{pH } 7.4) \longrightarrow \text{H}^+ + \text{O}_2^{\bullet -}$	superoxide anion radical
$\text{O}_2^{\bullet -} + e + 2\text{H}^+ \longrightarrow \text{H}_2\text{O}_2$	hydrogen peroxide
$\text{H}_2\text{O}_2 + e + \text{H}^+ \longrightarrow \text{H}_2\text{O}$	
$^*\text{OH} + e + \text{H}^+ \longrightarrow \text{H}_2\text{O}$	hydroxyl radical

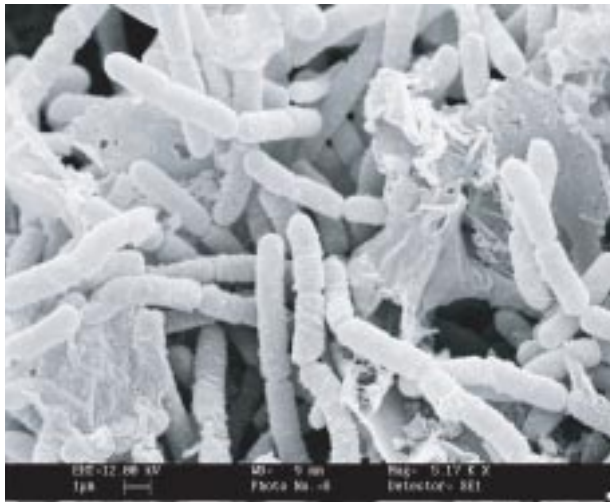


Figure 1. A scanning electron micrograph showing a population of marine bacteria on membrane filter



Figure 2. A scanning electron micrograph showing a population of marine bacteria after electrolytically disinfected on membrane filter

environment and microorganisms have long been evolved though this realization is yet to be appreciated widely by of microbial ecologists.

Toxic and reactive oxygen species are produced naturally in the atmospheric and aquatic environments. All aqueous environments containing dissolved oxygen, e.g., surface water, may also contain toxic forms of oxygen such as super oxide and hydroxyl radicals called reactive oxygen species (ROS). These ROS are capable of damaging protein, lipid and DNA through non-specific oxidative reaction. Because of this, hydroxyl radical is also important in degradation of dissolved organic carbon in the aquatic environment and furthermore in detoxification of a range of priority pollutants, e.g., methyl *tert*-butyl ether (Xu et al., 2004a, b) which are difficult to be degraded by specific microorganisms. Bacterial cells need to remove them before ROS can cause significant harm or damage to the individuals. Over the time of evolution and adaptation, different bacteria have

developed strategies to enzymatically decompose the oxidants through enzyme reactions, and the enzymes include peroxidase, catalase and super oxide dismutase. The oxidative stress defense adopted by bacteria includes: super oxide dismutases (SOD), catalase, alkyl hydroperoxide reductase, thiol peroxidase and cytochrome *c* peroxidase (van Vilet et al., 2002).

Cross protection against H₂O₂ challenge induced by nutrient starvation was observed in *V. parahaemolyticus*, which was believed to be based on the synthesis of a set of proteins referred to as starvation and stress proteins (Koga and Takumi, 1995). Another mechanism of resistance to hydrogen peroxide in *Vibrio* spp. was proposed that, under oxidative stress, some of the cells can immediately be disintegrated, releasing catalase for decomposition of hydrogen peroxide, providing an opportunity for the survival of other cells in the population (Ichise et al., 1999). In selective *Vibrio* spp., a pre-exposure of a bacterial population to mild-dosage of H₂O₂ may stimulate the onset of resistance to further exposure, a detoxification strategy by *Vibrio* spp. (Landre et al., 2000) and *Streptococcus pneumoniae* (Pericone et al., 2003). In natural ecosystems, this may have an important implication because newly formed bacterial cells may be exposed to various levels of radical stress from the very beginning of their life and such exposure, if not fatal, will enhance their resistance to repeated exposures. Such populations possibly tolerate higher concentrations of H₂O₂ during their lifetime. This kind of resistance may have fundamental genetic basis and such information will be very useful in the understanding of the resistant mechanisms.

UV effects

UV is probably the most important and effective physical factor controlling the aquatic productivity. UV light is lethal to most microorganisms and can damage other organisms to varying extent. Recently, results on several *Vibrio* species isolated from sediment and water of the intertidal mangrove system showed that effect of a combination of UV treatments showed distinguishable difference in the bacterial growth kinetics, e.g., lag phase time (λ) between bacterial isolates, but not so apparent in maximum biomass (A) or specific growth rate (μ_m) for *Vibrio vulnificus* MP-3 and *Vibrio cholerae* MP-1 (Wang et al., 2004). These investigators also observed that *Vibrio aestuariensis* MP-2 was the most sensitive to these treatments among the three isolates, as reflected in parameters of maximum biomass and lag time. More specifically, UV treatment followed by immediate incubation in darkness (UV+D) or under bright light

(UV+L) significantly affected the growth of *V. aestuarianus* MP-2 as illustrated by the calculated values of A , μ_m and λ . An increase of μ_m from 0.052 h⁻¹ of the non-UV to 0.0968 h⁻¹ of the UV treatment plus a dark period (UV+D) or 0.0870 h⁻¹ of the UV treatment plus a visible light period (UV+L) was observed and, at the same time, a decrease of λ from 7.3 of the non-UV to 3.8 hrs of the UV+D. In addition, UV+D tended to decrease λ of *V. vulnificus* MP-3, *V. cholerae* MP-1.

None of *V. vulnificus* MP-3 or *V. cholerae* MP-1 resulted in statistically significant differences in the A or μ_m under different UV treatments ($p > 0.05$). A significant increase of A from 0.7858 of non-UV to 1.0097 of the UV+L was detected with *V. aestuarianus* MP-2. The λ of *V. aestuarianus* MP-2 under UV+D treatment was only 3.8 hrs, approximately one third of that after the UV+L treatment (λ , 12.1 hrs). And the μ_m of *V. aestuarianus* MP-2s was significantly improved by approximately 2 fold under both UV treatments compared with the non-UV control. For all the three isolates, the λ with UV+D was the shortest among the three treatments and the differences in λ were highly significant ($p < 0.01$).

In the case of *V. vulnificus* MP-3 and *V. cholerae* MP-1, no significant differences were observed in the maximum biomass under the three treatments ($p > 0.05$). However, different bacterial isolates displayed variable responses to the same treatments. Under the same UV+L treatment, the lag time was 3.5 hrs for *V. vulnificus* MP-3, almost half that of *V. cholerae* MP-1 (6.6 hrs) and one-fourth that of *V. aestuarianus* MP-2 (12.1 hrs). Under UV+D treatment, the comparison of μ_m among the three bacterial strains was: *V. aestuarianus* MP-2 < *V. cholerae* MP-1 < *V. vulnificus* MP-3. This trend can also be found in maximum biomass though the difference in A between *V. cholerae* MP-1 and *V. aestuarianus* MP-2 was not statistically significant.

The wavelength of UV lamp used in this study was 254 nm, the most effective germicidal region of UV spectrum (Claus, 1989). Unexpectedly, the UV exposure did not seem to inhibit the bacterial growth of most of the isolates tested. These observed results might be due to the following reasons: firstly, the dose applied in this study was 238.5 J/m², lower than the daily UV doses recorded in the field at the surface of water (up to 3000 J/m²) (Demers et al., 1998). Therefore, the dosage tested in this experiment may not be bactericidal. Secondly, it has been proposed that photoadaptation could occur in marine bacteria, which leads to stronger resistance to the repetitive UV exposure (Joux et al., 1999; Nystrom et al., 1992). Since a second exposure was not attempted, the UV exposed plates were kept in the dark box for 1 hour (UV+D) before inoculation into nutrient broth and the

information from the experiments has some limitations. During this period of time, nucleotide excision repair, also called dark repair, and recombinational repair (post-replication repair) might have occurred (Joux et al., 1999). Other proteins such as DNA-binding protein or new proteins might have been produced accelerated entry into exponential growth phase in a shorter time. This mechanism is important for the bacterial populations to survive under the natural solar UV light exposure. Both *V. vulnificus* MP-3 and *V. cholerae* MP-1 exhibited very similar growth under UV+L and Non-UV treatment, suggesting the possible presence of photo enzymatic repair in these bacteria.

In this study, we also found that the bacteria on plates treated with UV+L and then kept under bright light after the UV exposure for 1 hr before the inoculation were able to process photo-repair of DNA damage. Since the specific mechanisms involved were not investigated, more work needs to be carried out to elucidate why *V. aestuarianus* MP-2 showed better growth under UV+L treatment than Non-UV treatment. It is also worthwhile to speculate that protein production by stress-response reaction may be involved during the white light exposure. Elucidation of the exact molecular basis of such mechanism will provide further basic understanding of the molecular biology involved and the nature of proteins. Since all three isolates were isolated from either inter-tidal mudflat sediment or surface water of the Mai Po Nature Reserve where there are large quantities of organic substances discharged through the urban wastewaters, high population density of microorganisms may also be a key factor for the selective enrichment of such characteristics of the bacteria in response to the chemical stress in the environment. Though the natural solar UV light may exert negative effect on bacterial activity, the photoactivated organic matter and other UV-absorbing compounds could have a major role in protection and rapid recovery of bacteria from UV stress (Kaiser and Herndl, 1997; Lyons et al., 1998). In this case, different environments may also have different mechanisms for bacteria to deal with the free radicals, which has a great potential for research in the near future.

Hydrogen Peroxide

Current knowledge supports the view that H₂O₂ brings about two types of bactericidal effects on *E. coli*: In mode I low concentrations of 1.0 to 3.0 mM H₂O₂ while in mode II, concentrations as high as 50mM (Pericone et al., 2003) are reported to be deleterious. During mode I killing, it is thought that DNA damage is the primary mechanism originated from hydroxyl radicals

(OH) generated from the Fenton reaction as: $\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{OH}^\bullet + \text{OH}^-$. Since the hydroxyl radical is highly reactive reactant (Duca, 1997; Symons et al., 1998), it reacts with DNA in a close proximity and causing non-repairable damage. From the Fenton reaction, the availability of Fe^{2+} may be a reaction-limiting factor. In mode II killing, the reaction proceeds at much slower rate. Although the detailed mechanisms of action have not been fully elucidated, it is generally believed that this lethal action is not DNA related it more likely involves inactivation of housekeeping enzymes, e.g., oxidation of the active site thiols.

In a recent investigation using several *Vibrio* species isolated from intertidal mudflat of a mangrove in Hong Kong in our laboratory, none of the tested isolates grew in the presence of 10 mM or higher H_2O_2 (Wang, 2003; Wang et al., 2004). At 1 mM H_2O_2 , *V. aestuarianus* MP-2 had a specific growth rate of only 0.12 ($\text{OD}_{600}/\text{hr}$), which was about half of *V. vulnificus* MP-3 under the same treatment. Using a H_2O_2 resistant *Streptococcus pneumoniae*, Pericone et al. (2003) observed its survival at 20mM H_2O_2 . In another investigation involving a marine oligotrophic ultramicrobacterium *Sphingomonas alaskensis* RB2256, limiting of specific nutrient was correlated to the increased viability of the bacteria after exposed to 25mM H_2O_2 for 60 min. (Ostrowski et al., 2001). *Pseudomonas fluorescens* was found to be resistant to H_2O_2 stress after adapted to carbon-starved condition (van Overbeek et al., 1995). It is commonly believed that excessive H_2O_2 can oxidize enzyme thiols, inactivate the catalytic site, and react with ferrous to form HO^\bullet attacking DNA structure and resulting in the gene mutagenesis and even cell death (Pott, 1994; Storz, 1999). Therefore, the bacterial growth of the three isolates was negatively affected by a comparatively higher concentration of H_2O_2 .

Catalase and super oxide dismutase (SOD) are inevitably involved in the resistance and survival of *Vibrio* spp. upon exposure to H_2O_2 . When challenged with both iron-depletion and oxidative stress, MnSOD can also be induced in place of FeSOD (Barnes et al., 1999; Kimoto et al., 2001; Touati et al., 1995). When exposed to 1 mM H_2O_2 challenge, the *A* reached by *V. aestuarianus* MP-2 was only 26% of the control, about one fourth of the *A* reached by other two isolates under the same treatment indicating early entry into cell-death phase. With 1 mM of H_2O_2 in the medium, this isolate needed about 8 hrs for entering exponential phase, almost 1.6 fold that of 0.1 mM treatment, though no significant difference in maximum biomass was observed. *V. vulnificus* was more sensitive to H_2O_2 challenge than most of other Gram-negative bacteria, but it could be induced to generate adaptation to further exposure by pre-

exposure (Landre et al., 2000). Since a second H_2O_2 exposure was not carried out in our experiments, it can only be postulated that *V. vulnificus* MP-3 could produce nascent proteins for tolerating further H_2O_2 exposure. Interestingly, *V. cholerae* MP-1 grew better when challenged with 1.0 mM H_2O_2 , as a result, higher values of the *A* and μ_m were achieved compared with those subjected to 0.1 mM H_2O_2 . This is similar to what has been observed on *E. coli* in which low concentration of H_2O_2 resulted in DNA damage that killed *E. coli* cells while higher concentration reduced the amount of such a damage (Imlay et al., 1988).

Other factors such as population physiological status, age of bacterial culture and pre-exposure may also have a role to play on bacterial tolerance to H_2O_2 challenge. Bacterial cells in the early-stationary phase or aged for a long period of time or pre-exposed to sub-lethal dosage are found to be more resistant to H_2O_2 exposure (Barnes *et al.*, 1999; Ichise et al., 1999; Kim et al., 2002). Starvation has long been thought to induce long-term survival under the stresses such as nutrient depletion, oxidative stress, UV or near-UV exposure. However, bacterial cells facing the nutrient-shortage can use global-regulators to deal with starvation, UV exposure and oxidative stress (Jenkins et al., 1988; Koga and Takumi, 1995; McDougald et al., 2002). This might be the case for the three environmental isolates obtained from Mai Po and Inner Deep Bay area.

It is also commonly known that bacteria are capable of forming community called biofilms (Figure 1). When it is formed, bacteria in the biofilms have much higher resistance to both physical and chemical stress. In doing so, they are better protected than the non biofilmed bacteria. Such aggregation of bacteria is very common in oligotrophic environments because the nutrients on surfaces tend to be higher than in the bulk solution phase. Bacteria under such condition are capable of synthesizing ample quantities of polysaccharides, which are physical barriers that protect individual cells. Such information is also important in comprehensive understanding of the ecology of these microorganisms in the marine environments.

Ferric Iron

Iron is also important to the growth of *Vibrio* spp. Serving as the co-factor of many important enzymes such as SOD, it has close connections with oxidative stress in bacterial cells and may also provide the attachment site to *V. cholerae* O1 in dechlorinated tap water for survival (Patel and Isaacson, 1994; Touati, 2000). With the rapid urbanization and industrialization in the areas around the Mai Po Nature Reserve and Inner Deep Bay, heavy metal pollution

is increasingly detected. The concentration of iron was higher than other heavy metals monitored (Che, 1999) in the inter-tidal mudflat sediments because oxidation of ferrous and reduction of ferric ions are regulated by the tidal activities. These phenomena prompted our interest to investigate the effects of Fe^{3+} , H_2O_2 and UV on the bacterial growth of three environmental isolates of and *Vibrio* spp. in Mai Po Nature Reserve.

As iron and free radicals bear physiological effects on microorganisms, an understanding of the effects of both of these parameters is essential. Maximum biomass (A) in term of OD_{600} and the lag time (λ) duration of the three isolates were significantly different for the range of Fe^{3+} concentrations tested ($p < 0.01$). For example, when the exogenous Fe^{3+} concentration was $10 \mu\text{M}$ in the culture medium, λ of *V. cholerae* MP-1 was 4.8 hrs, about half that of *V. aestuarianus* MP-2 (λ , 9.2 hrs) under the identical conditions. Grown in the nutrient broth supplemented with $100 \mu\text{M}$ of Fe^{3+} , the order of A reached among the three isolates is as follows: *V. aestuarianus* MP-2 > *V. vulnificus* MP-3 > *V. cholerae* MP-1. No significant difference was observed in the μ_m between *V. vulnificus* MP-3 and *V. cholerae* MP-1 for the Fe^{3+} concentration between 0 and $100 \mu\text{M}$ ($p > 0.05$). These two bacterial isolates seemed to show similar growth responses to the same Fe^{3+} concentrations. Interestingly, the highest A was observed in *V. aestuarianus* MP-2 in the presence of $10 \mu\text{M}$ of Fe^{3+} .

Iron plays an important role in bacterial cells as co-factor of enzymes, component of iron-sulfur proteins and terminal electron acceptor under anaerobic condition (Ehrlich, 2002). It is also of crucial importance in infection processes by pathogens or potential pathogens (Naidu and Yadav, 1997). The addition of Fe^{3+} stimulated the bacterial growth of *V. cholerae* MP-1 and decreased the λ in this experiment as supplement of $10 \mu\text{M}$ of Fe^{3+} to the nutrient broth medium resulted in the shortest λ (4.8 hrs), the highest A compared with other Fe^{3+} concentrations. In addition, iron may also play an important role in the survival of *V. cholerae* O1 in natural environment as attachment site (Patel and Isaacson, 1994). $50 \mu\text{M}$ of Fe^{3+} seemed to be optimal for both *V. vulnificus* MP-3 suggesting that individual bacterial isolate has different Fe^{3+} requirement for its growth. Fe^{3+} concentration as high as $100 \mu\text{M}$ did not enhance the growth of most isolates significantly (Che, 1999; Wang, 2004).

Ferritin, bacterioferritin and iron-binding proteins are examples of iron storage systems which can make the iron available when there is iron scarcity (van Vilet et al., 2002). Iron and oxidative stress are closely related in the facultative aerobic bacteria *Vibrio* spp.

Presence of excessive iron and oxygen can mediate the generation of damaging reactive oxygen species through Haber-Weiss and Fenton reactions. To cope with this, bacteria have complex and strict regulation of iron metabolism for the iron homeostasis inside their cells (van Vilet et al., 2002; Touati, 2000). This may also explain why not all three isolates showed significant inhibition of growth when high concentration of exogenous iron was provided in the culture medium. Iron has also been reported to play a role in the adaptation and tolerance to cold temperature in *V. vulnificus* (Bryan et al., 1999), which was consistent with the observed growth of *V. vulnificus* MP-3 at 15°C in another experiment (Wang et al., 2004). Because iron is so abundant in our environment but also a biologically limiting nutrient, especially in the ocean, sequestration of iron from the environment and effect of iron on biology of bacteria are indispensable information in microbial ecology.

Future perspectives

Due to the technological advances in instrumentation for detection of short-life chemical species and reaction kinetics taking place in extremely short period of time, further fundamental understanding of the mechanisms involving free radicals on bacteria in both natural environment and mesocosms will be helpful in realizing their effects on survival, growth and reproduction. Electron spin resonance has been used in identification of the free radical intermediates. Since molecular biology has been moving at a speed for complete sequencing of a number of bacteria nowadays, further elucidation of the basic mechanisms and molecular biology can be made with the DNA-based techniques. However, natural bacteria are living in the native environment and laboratory-based understanding should always be related to the natural conditions. Only when we achieve an understanding at such a level, can we claim that major progress has been made in understanding microorganisms in the ocean environment.

Conclusions

Very little is known about the oxidative stress of bacteria living in the ocean. Using three environmental isolates of *Vibrio* spp. from the intertidal environment, effects of specific nutrient-depletion, solar UV exposure and oxidative stress were investigated. These microorganisms developed different strategies for their own survival under challenge conditions. These data suggest that specific molecular mechanisms may exist which are responsible for microbial resistance and survival. At

the same time, the evolution of such ability by native microflora should serve to be an important part of our knowledge in understanding them in their natural environment. On the other hand, these resistant bacteria may also pose a potential threat to public health and should call for increasing attentions from both researchers and public health administrators.

Acknowledgements

Research in this laboratory is support by ITS of Hong Kong Government, AFCD and The University of Hong Kong. I would like to thank the initial research carried out by Yanling Wang (now at Georgia Institute of Technology, USA), and the Electron Microscopy Unit of Queen Mary Hospital of The University of Hong Kong for the scanning electron micrographs.

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