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## Article

**Keywords:** Type II photosensitized oxidation, Singlet oxygen, Phytodetritus, Pigmented and unpigmented bacteria, Carotenoids, Vaccenic acid, DNA repair system

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# Singlet oxygen-induced alteration of bacteria associated with phytodetritus: effect of bacterial pigments.

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20     **Abstract.**

We sought to determine whether the presence of carotenoids actively protects bacteria associated with senescent phytoplankton cells from damage caused by singlet oxygen ( $^1\text{O}_2$ ) resulting from the cessation of photosynthetic reactions. We irradiated senescent cells of the diatom *Thalassiosira sp.* associated with the bacterial strains *Pseudomonas stutzeri* (a ubiquitous planktonic unpigmented heterotrophic bacterium) or *Dinoroseobacter shibae* (an aerobic anoxygenic bacterium often associated with phytoplankton rich in the spheroidenone, carotenoid). The analysis focused on the photooxidation of bacterial membrane lipids (vaccenic acid) and the expression of genes involved in DNA repair systems. In *P. stutzeri* cells,  $^1\text{O}_2$  transferred from senescent algae was not completely scavenged by bacterial membrane MUFAs and reached the cytoplasm, allowing both  $^1\text{O}_2$  and UV radiation to cause DNA damage. In *D. shibae*, scavenging by bacterial membrane MUFAs quenching by spheroidenone allowed only a small fraction of  $^1\text{O}_2$  to reach the cytoplasm, where the efficient detoxifying activity of mutY limited their impact on the DNA of this strain, thus preventing oxidative stress. The fact that Rhodobacterales is the dominant order in bacterioplankton communities associated with algal blooms could thus be due to the protective effect of its constituent carotenoids against  $^1\text{O}_2$ - and UV-induced damage.

**Keywords.** Type II photosensitized oxidation; Singlet oxygen; Phytodetritus; Pigmented and unpigmented bacteria; Carotenoids; Vaccenic acid; DNA repair system.

## Introduction

Photosynthetic microorganisms (phytoplankton) such as diatoms, dinoflagellates and coccolithophores are the main primary producers in marine ecosystems, capable of producing organic matter using light energy and inorganic nutrients. This organic matter is heavily processed by bacteria, which mineralize approximately 50% of oceanic primary production (PP) <sup>1,2</sup>. The other 50% of PP either enters the marine food web or gets buried into seafloor sediments through a process called the 'biological pump' <sup>3</sup>. Bacterial activity in the oceans (secondary production) is therefore highly dependent on the abundance of primary producers <sup>1</sup>, and varies with phytoplankton blooms at large or small scales <sup>4</sup>.

Phytoplankton–bacteria interactions are highly intertwined, and changes in composition of phytoplankton blooms (in terms of abundance and species assemblages) lead to changes in bacterial-community composition <sup>5–7</sup>. As this microbial secondary production is a key process to better understand the functioning of marine ecosystems, there has been intensive research into the phylogenetic composition of bacterioplankton communities (i.e. free-living planktonic bacteria in marine ecosystems).

Singlet oxygen (<sup>1</sup>O<sub>2</sub>), which is produced by type II photosensitized oxidation processes in senescent phototrophic organisms <sup>8</sup>, is strongly electrophilic and lacks spin restriction <sup>9</sup> and therefore readily reacts with phytoplanktonic unsaturated lipids <sup>10,11</sup>, proteins <sup>12</sup>, and nucleic acids <sup>13,14</sup>. <sup>1</sup>O<sub>2</sub> can also react with the octadec-11-enoic (vaccenic) acid of the bacteria attached to this algal material, affording isomeric *trans* allylic hydroperoxyacids <sup>15,16</sup>. Recently, it was demonstrated that high solar irradiances strongly favor this transfer of <sup>1</sup>O<sub>2</sub> to bacteria <sup>17</sup>. The effect of exogenous pure <sup>1</sup>O<sub>2</sub> on Gram-negative and Gram-positive bacteria has already been investigated <sup>18,19</sup>, but the transfer of <sup>1</sup>O<sub>2</sub> from senescent phytoplankton to their attached bacteria has been only demonstrated by Rontani et al. in 2003 <sup>15</sup>. Further studies demonstrated: (i) the good correlation between the photodegradation state of bacteria attached to phytodetritus and

the photooxidation state of phytoplanktonic chlorophyll <sup>16</sup>, and (ii) inhibition of <sup>1</sup>O<sub>2</sub> transfer by the silica matrix of diatom frustules <sup>20</sup>. This transfer of <sup>1</sup>O<sub>2</sub> could strongly decrease the mineralization potential of bacteria attached and thus favor the preservation of phytoplanktonic material. The bacterial resistance to its deleterious effect could be a determining factor in the  
75 selection of microorganisms capable of interacting with senescent phytoplankton at the end of the bloom.

It is still hard to generalize bacterioplankton-community structures, but some important bacterial groups, such as *Bacteroidetes* and *Proteobacteria*, are typically abundant during bloom events, regardless of phytoplankton species <sup>6,21,22</sup>. Amongst them, *Roseobacter*, a genus  
80 that falls within the Alphaproteobacteria class, are often dominant in marine phytoplankton-associated bacterial communities <sup>23-27</sup>. Many studies investigating this compositional balance have given explanations for why strains belonging to the *Roseobacter* genus are so efficient at colonizing phytoplankton, including their high colonization capabilities <sup>28</sup>, their production of quorum-sensing molecules <sup>29</sup>, and their antimicrobial compounds <sup>30</sup>. Another physiological  
85 characteristic of the *Roseobacter* genus that could explain their high abundance in bacterioplankton assemblages is the particular aerobic anoxygenic phototrophic (AAP) metabolism that occurs among this taxon <sup>31</sup>. AAP bacteria are capable of photoheterotrophy, a metabolic pathway that enables them to produce energy from light, using photosynthetic reaction centers composed of bacteriochlorophyll-*a* (BChl *a*); <sup>32</sup>. AAP bacteria are also known  
90 to contain high amounts of pigments such as carotenoids <sup>33</sup>, which may protect these organisms against oxidative damage such as type II photosensitized oxidation, a process that can happen during late bloom phases <sup>34</sup>.

It has already been hypothesized that bacteria with high amounts of pigments such as carotenoids may be able to tolerate exposure to <sup>1</sup>O<sub>2</sub> <sup>19,35</sup>. This resistance to oxidative stress

95 could explain why the *Roseobacter* genus is highly represented among bacterioplankton communities during late bloom phases when the phytoplankton cells are senescent.

Here, we compared the effect of  $^1\text{O}_2$  produced during the senescence of a diatom (*Thalassiosira* sp.) on the lipids and DNA of two Gram-negative bacteria widely found in marine environments, one pelagic ubiquitous unpigmented bacterium, *Pseudomonas stutzeri*<sup>36</sup> 100 and one pigmented bacterium, *Dinoroseobacter shibae*<sup>37</sup> frequently associated with phytodetritus. For the first time, the impact of  $^1\text{O}_2$  produced during algal senescence on the physiology of associated bacteria was studied at the membrane-lipid and DNA levels.

## Material and methods

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**Phytodetritus production :** The non-axenic diatom *Thalassiosira* sp. RCC1714 was obtained from the Roscoff Marine station culture collection. Diatoms were grown in F/2 medium at 17°C in an environment-controlled cabinet under an irradiance of 83  $\mu\text{mol photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Osram, Lumilux, 12 h:12 h light/dark cycles). After two months of growth, cell density was measured 110 by counting under microscope. *Thalassiosira* sp. Cell death was induced by sonication on ice using a Branson Sonifier 450 ultrasound generator equipped with a Branson S450A cell disruptor probe at 240 W for 10 minutes (50% duty cycle). The sonicated cells were then aliquoted in 50-mL Falcon tubes and stored at -20 °C for later photooxidation experiments.

115 **Bacterial cultures :** *P. stutzeri* ATCC 14405 and *D. shibae* DSM 16493 were grown aerobically overnight on a rotary shaker in the dark at 17°C on marine broth medium (US Biological™) diluted 1:10 with sterilized artificial sea water<sup>38</sup>. These pre-cultures were used to inoculate the same medium to obtain under the same conditions an initial  $\text{OD}_{620\text{nm}}$  of about 0.020. In the middle of the exponential growth phase, the cultures were stopped and cell

120 abundances were counted based on epifluorescence in the presence of fluorochrome (4P,6-diamidine-2P-phenylindole dihydrochloride; DAPI) as previously described in Otto <sup>39</sup>.

**Photooxidation experiments:** Based on the recent results of Burot et al. <sup>17</sup> showing enhanced <sup>1</sup>O<sub>2</sub> transfer to bacteria at high irradiances, experiments were carried out in 100-mL sterilized quartz tubes using an Altas Suntest CPS + solar simulator with a Xenon NXE 1500 lamp producing a solar irradiance (2285  $\mu\text{mol photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) in the 300–800 nm range close to the levels generally observed at noon at the surface of Mediterranean Sea <sup>40</sup>. To limit irradiation to visible range, we also simultaneously carried out experiments in 100-mL glass (opaque to UV) tubes with irradiance reduced to 2038  $\mu\text{mol photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  in this case.

130 Phytodetritus and bacteria were mixed (at a 1:5 algal:bacterial cells ratio) in K medium<sup>41</sup> (K 25  $\times$  diluted with sterilized artificial sea water) at a final volume of 100 mL, and immediately exposed to light in the solar simulator. Tubes were held at 17°C by immersion in a water bath connected to a cryothermostat. Four sampling times were defined: 0.5, 2, 3.5 and 5 h. At each sampling time, 15 mL and 10 mL samples were collected in triplicate for q-RT PCR and lipids analysis, respectively. The 15-mL samples were filtered on 0.8- $\mu\text{m}$  polycarbonate filters (to collect only attached bacteria on particles and not the free-living organisms) and stored at -80°C until further RNA extraction. The 10-mL samples were filtered on 0.8- $\mu\text{m}$  glass fiber filters (GF/F) and stored at -20°C until further lipid extraction. Each experiment was conducted in four separate tubes prepared simultaneously in the same conditions. A single tube was sacrificed at each sampling timepoint.

Control experiments were conducted without phytodetritus. Bacteria were mixed with sterilized artificial sea water added with 0.1 g. L<sup>-1</sup> of lactate/acetate (50:50) as carbon source, then placed in 100 mL sterilized quartz tubes and immediately exposed to light in the solar simulator. Three sampling times for the controls were defined: 0, 2 and 5 h. At each sampling

145 time, a 15 mL sample was filtered on 0.2- $\mu$ m polycarbonate filters (to recover bacteria that are all free-living in this case) and stored at -80°C until further RNA extraction. For the lipid analysis, the samples were centrifuged with a Beckman & Coulter Allegra X-22-series centrifuge at 20,000  $g$  for 30 min, then pelleted and stored at -20°C. Here again, the three samplings were conducted in separate control tubes prepared in the same conditions.

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### **Lipid analyses**

*Lipid extraction:* Samples (GF/F filters) were reduced with excess  $\text{NaBH}_4$  after adding MeOH (25 mL, 30 min) to reduce labile hydroperoxides (resulting from photo- or autoxidation) to alcohols, which are more amenable to analysis using gas chromatography–mass spectrometry (GC-MS) as described elsewhere <sup>42</sup>. Briefly, Water (25 mL) and KOH (2.8 g) were then added, 155 and the resulting mixture was saponified by refluxing (2 h), then cooled, acidified with HCl (2 N) to pH 1, and extracted with dichloromethane (DCM; 3  $\times$  20 mL).

*Derivatization:* Dry TLEs were silylated in a mixture of pyridine/*bis*-(trimethylsilyl) 160 trifluoroacetamide (BSTFA; Supelco; 2:1, v/v) as previously described <sup>42</sup> and analyzed by gas chromatography–tandem mass spectrometry (GC-MS/MS).

*Gas chromatography–tandem mass spectrometry (GC-MS/MS):* GC-MS and GC-MS/MS analyses were performed on an Agilent 7890A/7010A tandem-quadrupole gas chromatograph 165 system (Agilent Technologies, Les Ulis, France) using a cross-linked 5% phenyl-methylpolysiloxane (Agilent; HP-5MS ultra inert, 30 m  $\times$  0.25 mm, 0.25- $\mu$ m film thickness) capillary column. The operating conditions used have been described previously by Rontani et al. <sup>42</sup>. Vaccenic acid photooxidation products were quantified in multiple-reaction-monitoring (MRM) mode using the transitions  $m/z$  339  $\rightarrow$  131 for the parent vaccenic acid and  $m/z$  199  $\rightarrow$

170 129,  $m/z$  213  $\rightarrow$  129,  $m/z$  357  $\rightarrow$  129 and  $m/z$  371  $\rightarrow$  129 for its oxidation products <sup>11</sup>. Precursor  
ions were selected from the most intense ions (and specific fragmentations) observed in electron  
ionization (EI) mass spectra. Collision-induced dissociation (CID) was optimized by using  
collision energies at 5, 10, 15 and 20 eV. Quantification involved peak integration and  
determination of individual response factors using standards and Mass Hunter software (Agilent  
175 Technologies, Les Ulis, France).

*Standard compounds:* Vaccenic acid was obtained from Sigma-Aldrich and oxidized using  
Fe<sup>2+</sup>/ascorbate <sup>43</sup>. Subsequent reduction of the resulting hydroperoxides in methanol with  
excess NaBH<sub>4</sub> afforded the corresponding hydroxyacids.

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**Quantitative RT-PCR :** RNA extractions were performed with the High Pure RNA Isolation  
Kit (Roche Applied Science, United States) as per the supplier's instructions. After extraction,  
any potential DNA contaminations were removed using the TURBO DNA-free™ kit  
(Ambion® by Life Technologies™) as per the manufacturer's instructions. RNA quantification  
185 and 260/230 and 260/280 ratio measurement were performed by spectrophotometry (NanoDrop  
2000c, Thermo Scientific). Absence of DNA contamination was verified on RNA samples for  
each targeted gene (as described below) by PCR without the reverse transcription step. Reverse  
transcription (RT) reactions were carried out using random primers with the PrimeScript™ RT  
reagent Kit (TaKaRa) as per the supplier's instructions.

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To plot the standard curves, fragments of the targeted genes were amplified and cloned  
into pGEMT-easy vectors (Promega) following the supplier's instructions. The primers used to  
amplify and clone the targeted genes for *P. stutzeri* and *D. shibae* are reported in Table S1. The  
PCR reactions (20  $\mu$ L) contained 50 ng of genomic DNA from *P. stutzeri* or *D. shibae*, 0.5  $\mu$ M  
of each primer, and 10  $\mu$ L of GoTaq® G2 Hot Start Master Mix (Promega). The PCR program

195 consisted in an initial denaturation step of 5 min at 98°C to activate the polymerase, then cycling  
(30 repetitions) with a 30 s denaturation step at 98°C, a 30 s hybridization step at the appropriate  
temperature for the selected primer pair, and an elongation phase of 20 s at 72°C. A final  
elongation phase at 72°C for 2 min was added for easy cloning in a T vector. The cloned DNA  
fragments were checked by sequencing. After purification, the plasmid concentrations were  
200 determined by spectrophotometry and used to produce the standard curves for qPCR allowing  
quantification of the synthesized cDNAs.

The various target cDNAs were quantified by qPCR (qPCR Master Mix, Promega) as per  
the supplier's instructions. Each qPCR run performed used 1 µL of the obtained cDNA and 0.5  
µM of each primer in a final 20 µL reaction volume. The qPCR program consisted in an initial  
205 denaturation step of 2 min at 98°C, then cycling (40 repetitions) with a 20 s denaturation step  
at 98°C, a 20 s hybridization step at the appropriate temperature for the selected primer pair,  
and an elongation phase of 20 s at 72°C. Experiments were done using the CFX Opus Real-  
Time PCR system (Bio-Rad) and the associated software. The starting quantity was determined  
using Bio-Rad CFX manager 2.1 software and transformed into copies per µL of cDNA. The  
210 quantified expression level of the housekeeping gene (*rpoD*) encoding for a  $\sigma^{70}$  subunit of the  
RNA polymerase was used to normalize gene expression. Each extracted RNA was analyzed  
in 6 technical replicates (two independent reverse transcriptions and three qPCR analyses per  
cDNA).

215 **Data Availability :** The sequences generated during the current study are available in  
the Genbank repository, the accession numbers are : #2741504, #2741532, #2741540,  
#2741543, #2741547, #2741570, #2741574, #2741584

**Ethical approval.** This article does not contain any studies with human participants  
220 performed by any of the authors.

## Results and discussion

The aim of this work was to determine whether bacterial pigmentation plays a protective role against the  $^1\text{O}_2$  damage when the bacteria are attached to lightened phytodetritus. To do so, we irradiated senescent cells of the diatom *Thalassiosira* sp. associated with the bacterial strains *P. stutzeri* (a ubiquitous heterotrophic bacterium without carotenoids) and *D. shibae* (an AAP containing a high proportion of the carotenoid spheroidenone). Experiments were carried out under two radiative fluxes (1) at high solar irradiance (PAR, Photosynthetically active radiation, + UV) close to that generally observed at noon at the surface of the Mediterranean Sea<sup>40</sup> and (2) after limiting irradiation to visible range (PAR only) to determine the effect of  $^1\text{O}_2$  only. Analysis focused on the effects of  $^1\text{O}_2$  on photooxidation of the major MUFA of these bacteria (vaccenic acid) and on activation of their DNA repair systems.

**Type II photosensitized oxidation of bacterial vaccenic acid :** Oxidative damage in attached cells of *P. stutzeri* and *D. shibae* was monitored using the concentration of photooxidation products of vaccenic acid ( $\text{pmol}\cdot\text{L}^{-1}$ ). Indeed, vaccenic acid is found in high proportions in *P. stutzeri* and *D. shibae*<sup>37,44</sup> but is only present in trace amounts in the bacterial community associated with the non-axenic strain of *Thalassiosira* sp. Employed<sup>17</sup>. Type-II photosensitized oxidation of vaccenic acid involves the addition of  $^1\text{O}_2$  to the two carbon atoms of its  $\Delta^{11}$  double bond to afford 11-hydroperoxyoctadec-12(*trans*)-enoic and 12-hydroperoxyoctadec-10(*trans*)-enoic acids<sup>11</sup>. These hydroperoxides can be easily converted to 13-hydroperoxyoctadec-11(*trans*)-enoic and 10-hydroperoxyoctadec-11(*trans*)-enoic acids, respectively, via stereoselective radical allylic rearrangement<sup>45</sup>. We used stable allylic hydroxyacids arising from  $\text{NaBH}_4$  reduction of these hydroperoxyacids as tracers of photooxidation of bacterial membranes.

For both bacterial strains, only trace amounts of vaccenic acid photooxidation products were detected before irradiation and in the different algae-free controls (Fig. 1). As previously observed<sup>15-17</sup>, oxidation of vaccenic acid strongly increased when irradiation was carried out in the presence of senescent phytoplankton cells (Fig. 1). The production of vaccenic acid oxidation products appeared to be maximal at 803 KJ.m<sup>-2</sup>, which corresponds to 30 min of exposure only.

In the case of *P. stutzeri*, the concentration of vaccenic acid oxidation products was significantly higher in the presence of solar light (PAR+UV) radiation compared with non-UV levels (Figs. 1A and 1B, t-test, n=18, p=0.0005 < 0.05). The sensitizer (chlorophyll of senescent *Thalassiosira* sp. cells), which catalyzes type-II photosensitized oxidation of bacterial vaccenic acid, absorbs in both the PAR<sup>8</sup> and UV<sup>46</sup> ranges. Consequently, an additional amount of <sup>1</sup>O<sub>2</sub> is produced during PAR+UV irradiation, thus inducing higher oxidation of vaccenic acid in membranes of *P. stutzeri*. In contrast, there was no significant difference in concentrations of vaccenic acid photooxidation products in *D. shibae* cells after irradiation by solar (PAR+UV) or PAR-only irradiance (Figs. 1C and 1D, t-test, n=18, p=0.203 > 0.05). This observation clearly shows that the additional amount of <sup>1</sup>O<sub>2</sub> produced by UV radiations is quenched by the carotenoid spheroidenone in the membranes of *D. shibae*. Although this carotenoid cannot totally prevent type-II photosensitized oxidation of MUFAs in *D. shibae* membranes (Fig. 1C and 1D), it seems to limit the damage caused by overproduction of <sup>1</sup>O<sub>2</sub> in the presence of UV.

Carotenoids containing two polar hydroxy groups (e.g. zeaxanthin), which lie across the monolayer membrane<sup>47</sup>, can efficiently protect against <sup>1</sup>O<sub>2</sub>-mediated bacterial killing<sup>48</sup>. In contrast, carotenoids such as spheroidenone that lack hydroxy groups can become completely embedded in the lipid layer of the membrane<sup>49</sup>. This embedment makes them less likely to react with <sup>1</sup>O<sub>2</sub>, which can therefore go on to rapidly damage unsaturated molecules in its vicinity. This can explain why spheroidenone cannot totally prevent vaccenic acid oxidation in

membranes of *D. shibae*. Note that spheroidenone may play a key role in photoprotection of *D. shibae* against UV radiation-induced damage: as spheroidenone absorbs strongly between 300 and 400 nm<sup>50</sup>, it can absorb harmful UV radiation.

275           **Effect of <sup>1</sup>O<sub>2</sub> on the DNA repair system genes** : The other aim of this work was to monitor the response to <sup>1</sup>O<sub>2</sub> of the DNA repair system in pigmented and non-pigmented bacteria. <sup>1</sup>O<sub>2</sub> is known to react with a wide range of cellular compounds, such as lipids, proteins, and DNA<sup>12-14</sup>.

Bacteria respond to adverse conditions by activating regulation pathways that increase  
280 their chances of survival. One such pathway—the aptly named SOS response—is induced by genome damage or disruption of DNA replication, and results in the synthesis of DNA repair enzymes and the inhibition of bacterial cell division. Bacterial cells use different strategies to protect themselves against the toxicity of <sup>1</sup>O<sub>2</sub>. These strategies usually involve ‘preventive’ detoxification enzymes, such as superoxide dismutase (SOD) or catalase (CAT)<sup>51,52</sup>, an  
285 efficient “Go system” with three detoxification proteins, i.e. MutM, MutT and MutY<sup>53</sup> assigned to DNA repair, or the quenching of <sup>1</sup>O<sub>2</sub> with antioxidant compounds such as carotenoid pigments<sup>54</sup>.

Amongst the nucleotides, <sup>1</sup>O<sub>2</sub> preferentially reacts with guanine (Fig. 2) as it has the lowest oxidation potential<sup>55,56</sup>. This leads to the formation of d-8-oxoGTP<sup>57</sup>. This modified  
290 nucleotide has high mutagenic potential, as it can mispair with adenine, leading to G → T transversion<sup>58</sup>, which is the main mutation observed. To monitor activation of the DNA repair system in response to <sup>1</sup>O<sub>2</sub> exposure, we measured the relative expression of several genes related to DNA repair after <sup>1</sup>O<sub>2</sub>-related damage as well as several stress-response transcription factors. In the genomes of both strains, three genes code for the detoxifying proteins of the “Go  
295 system”: *mutM* (or *fpG*), *mutY* and *mutT*. As shown in Fig. 2, MutM and MutY proteins are

glycosylases. MutM removes cytosine-paired d-8-oxoG while MutY removes adenine mispaired with d-8-oxoG <sup>59-61</sup>. MutT is a nucleotide triphosphatase that detoxifies the nucleotide pool, hydrolyzing d-8-oxoGTP into d-8-oxoGMP <sup>62</sup>.

<sup>1</sup>O<sub>2</sub> is also involved in regulation of the expression of genes associated with the bacterial SOS response to oxidative stress. Knowledge of the <sup>1</sup>O<sub>2</sub>-specific regulatory mechanisms in microorganisms is based on only a few model systems, including the anoxygenic bacterium *Rhodobacter sphaeroides* and several heterotrophic species such as *Escherichia coli*. In *R. sphaeroides*, stress response involves gene regulation by various alternative sigma factors RpoE and RpoH <sup>52,63,64</sup>. The sigma factor RpoE is maintained in an inactive state by forming a complex with its cognate anti-sigma factor ChrR <sup>52</sup>. Recent studies have shown that under exposure to <sup>1</sup>O<sub>2</sub>, RpoH becomes activated due to the rapid proteolysis of the RpoE:ChrR complex. RpoH (σ32) is the primary sigma factor controlling the heat shock response, but it is also known to be induced by other conditions (for a review, see <sup>65</sup>).

Amongst all the genes involved in the potential response to <sup>1</sup>O<sub>2</sub> exposure, we chose to focus on one representative of each type of response: *mutY*, *rpoH* (involved in stress response), and *chrR* (involved in <sup>1</sup>O<sub>2</sub>-induced response and *rpoH* regulation). Fig. 3 shows the relative expression of these genes in *P. stutzeri* and *D. shibae* after exposure to artificial (PAR) and solar (PAR + UV) light in the presence of senescent *Thalassiosira* cells. Note, however, that 8-oxoDG is not specific to <sup>1</sup>O<sub>2</sub>-induced alteration of DNA, but is also produced during the oxidation of DNA by other reactive oxygen species (ROS) (e.g., peroxy or hydroxyl radicals arising from hydroperoxide homolysis or H<sub>2</sub>O<sub>2</sub>). Consequently, the alteration of DNA observed in bacterial cytoplasm may also result from the action of these ROS (notably by H<sub>2</sub>O<sub>2</sub>, which is known to readily cross cell membranes <sup>66,67</sup>).

In *P. stutzeri*, *mutY* and *rpoH* were expressed from the beginning of exposure and reached peak expression (803 KJ.m<sup>-2</sup>) from 30 minutes (the experimental conditions did not allow

samples to be taken earlier) (Fig. 3A). In addition to being very fast, the response did not seem to be influenced by algae nor by UV light (Table S2), and so the slightest trace of singlet oxygen in an unpigmented organism leads to the expression of *mutY* and *rpoH*. The lower expression at 5 h under PAR + UV exposure (8028 kJ.m<sup>-2</sup>) could be explained by cells entering the stationary phase of growth, which arrives earlier than during exposure to PAR. *chrR* showed no difference in expression level under UV exposure but showed a very strong decrease in expression under PAR conditions.

In *D. shibae*, the presence of UV induced a significant increase of *mutY* and *chrR* expressions which is only observed in the presence of senescent *Thalassiosira* cells (Fig. 3B, Table S2). In the same way, *rpoH* expression showed completely different patterns between solar and PAR conditions but was not influenced by the presence of the algae.

These observations suggest that <sup>1</sup>O<sub>2</sub> arising from the algae has more difficulty penetrating the pigmented cells (comparison between pigmented and unpigmented cells in response to PAR exposure; Fig. 3, dotted bars). When UV is absent, only <sup>1</sup>O<sub>2</sub>-related oxidation remains. In this case, carotenoids are efficient enough to fight against this oxidant, therefore not allowing the <sup>1</sup>O<sub>2</sub> to enter the cell, and all three genes are expressed at a lower level. When algae and UV are present, leading to the production of more <sup>1</sup>O<sub>2</sub>, there is not enough carotenoid in the pigmented cell to fight against the increased oxidative stress, and so *mutY* and *chrR* show higher expression. However, relative expression levels increased more with the unpigmented than the pigmented bacteria, showing some kind of protection due to the presence of spheroidenone. This protection is evidenced by a lower expression of *rpoH* observed in *D. shibae* (Fig. 3B) indicative of the lack of oxidative stress in this pigmented attached bacterium.

## Conclusion

345 In this work, we studied the impact of type II photooxidation processes on different  
bacteria attached to senescent phytoplanktonic cells, with analysis focused on the  
photooxidation of bacterial membrane lipids (vaccenic acid) and the expression of genes  
involved in DNA repair systems. The aim was to determine whether the presence of carotenoids  
can actively protect bacteria against the damaging effects of  $^1\text{O}_2$  under PAR and PAR+UV  
350 irradiations. Comparison of the results obtained with the bacteria *D. shibae* (pigmented) and *P.*  
*stutzeri* (not pigmented) showed that under high irradiance conditions (that strongly favor the  
transfer of  $^1\text{O}_2$  from senescent algae to bacterial cells; <sup>17</sup>), the presence of spheroidenone in *D.*  
*shibae* cannot totally prevent  $^1\text{O}_2$ -induced oxidative alterations of unsaturated membrane lipids.  
However, the carotenoid spheroidenone appears to limit  $^1\text{O}_2$  damage, and it can absorb  
355 damaging UV radiation.

We also investigated the activation and regulation of the DNA repair system in response  
to exposure to  $^1\text{O}_2$  and UV radiation. In *P. stutzeri* cells, the  $^1\text{O}_2$  produced in senescent algae is  
not entirely scavenged by bacterial membrane MUFAs and so some  $^1\text{O}_2$  is able to reach the  
cytoplasm (Fig. 4A). Due to the lack of photoprotection across the membranes, DNA is thus  
360 damaged by both  $^1\text{O}_2$  and UV radiations. (Fig. 4A). However, in *D. shibae*, scavenging by  
bacterial membrane MUFAs quenching by spheroidenone limit the effects of  $^1\text{O}_2$  on bacterial  
membranes. Only a small fraction of UV radiation reaches the cytoplasm, where the efficient  
detoxifying activity of *mutY* limits its impact on the DNA of this strain, thus preventing  
oxidative stress (Fig. 4B). This limited impact is supported by the apparent inactivity of the  
365 *rpoH* gene responsible for oxidative stress response in photoheterotrophic bacteria.

Based on these results, the dominance of the *Roseobacter* genus in bacterioplankton  
communities associated to algal blooms could be attributed in part to the protective action of  
their carotenoids against  $^1\text{O}_2$  and UV-driven damage.

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**Originality-Significance Statement.** For the first time, the impact of  $^1\text{O}_2$  produced  
during algal senescence on the physiology of associated bacteria was studied at the membrane-  
lipid and DNA levels which places this current work in the top 10% of current research in  
environmental microbiology.

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**Table S1.** Sequences and hybridization temperatures (°C) of primers used for the cloning and amplification of targeted genes in *P. stutzeri* (*Ps*) and *D. shibae* (*Ds*).

| Target Gene           | Primer Name | Primer sequence 5' → 3'       | Fragment size (pb) | PCR hybridization temperature (°C) |
|-----------------------|-------------|-------------------------------|--------------------|------------------------------------|
| <b>16s <i>Ps</i></b>  | PS_16S-F    | AGC-TAG-TTG-GTG-AGG-TAA-AGG   | 291                | 60.0                               |
|                       | PS_16S-R    | GTA-TTA-CCG-CGG-CTG-CTG-GCA   |                    |                                    |
| <b><i>mutY Ps</i></b> | PS_mutY-F   | GGC-TAC-TTC-GAC-CGC-TTC-AT    | 74                 | 60.0                               |
|                       | PS_mutY-R   | AGC-ACT-TCA-TCT-TCC-TCG-GC    |                    |                                    |
| <b><i>rpoH Ps</i></b> | PS_rpoH-F   | GGT-CTG-ATG-AAG-GCG-GTC-AA    | 209                | 60.0                               |
|                       | PS_rpoH-R   | ACC-TCA-TCG-TTG-TTC-AGC-CA    |                    |                                    |
| <b><i>chrR Ps</i></b> | PS_chrR-F   | CGA-GTT-GGA-ATT-ACG-TCG-CG    | 109                | 60.0                               |
|                       | PS_chrR-R   | CGG-CTC-GAT-CAG-TTC-CTT-GT    |                    |                                    |
| <b><i>rpoD Ps</i></b> | PS_rpoD-F   | CAG-TCC-CGC-CTC-AAA-GAG-TT    | 148                | 60.0                               |
|                       | PS_rpoD-R   | CGT-TGA-TGC-CCA-TGT-CGT-TG    |                    |                                    |
| <b>16s <i>Ds</i></b>  | Rhodo 240F  | GGC-CCG-CGT-TGG-ATF-AGR-TA    | 276                | 62.0                               |
|                       | Univ 516R   | GTD-TTA-CCG-CGG-CKG-CTG-RCA   |                    |                                    |
| <b><i>mutY Ds</i></b> | DS_mutY-F   | GGC-TCT-CCG-AGG-TGA-TGC-TG    | 179                | 62.0                               |
|                       | DS_mutY-R   | CGC-GCA-TTT-CAG-CAG-GTT-G     |                    |                                    |
| <b><i>rpoH Ds</i></b> | DS_rpoH-F   | ATA-TAC-CAA-TCT-TCC-GGC-CCC-C | 106                | 60.0                               |
|                       | DS_rpoH-R   | GGC-CAG-CAT-GTA-TTC-CTC-TTC-G |                    |                                    |
| <b><i>chrR Ds</i></b> | DS_chrR-F   | AGC-TGC-CCA-AAT-CCA-TGA-CA    | 97                 | 62.0                               |
|                       | DS_chrR-R   | TTC-ACA-TCC-TCC-ACA-TCG-CC    |                    |                                    |
| <b><i>rpoD Ds</i></b> | DS_rpoD-F   | GCA-AGA-CCG-ATG-ACC-AGG-AC    | 150                | 60.0                               |
|                       | DS_rpoD-R   | TCT-GTT-CCG-AGC-TGA-CCT-GA    |                    |                                    |

All the primers have been designated from the complete genome of *P. stutzeri* (ACCESSION NUMBER CP002881) and *D. shibae* (ACCESSION NUMBER CP000830).

Table S2 : ANOVA analyses of data comparing the effects of *Thalassiosira* sp. input with solar irradiance, UV, solar light and PAR light on the vaccenic-acid oxidation product and the level of expression of *mutY*, *chrR* and *rpoH* (threshold,  $p < 0.005$ ).

|                                 | Source   | DDL | Somme des carrés | Moyenne des carrés | F       | Pr > F         |
|---------------------------------|--|-----|------------------|--------------------|---------|----------------|
| Vaccenique Ac oxidation product | Thalassiosira sp. effect with solar Irradiance | 1   | 9615978,78       | 9615978,78         | 82,91   | <b>0,00000</b> |
|                                 | specific effect of UV                          | 1   | 19299253,72      | 19299253,72        | 23,47   | <b>0,00008</b> |
|                                 | solar Light dose                               | 1   | 5143869,38       | 5143869,38         | 9,13    | <b>0,01287</b> |
|                                 | PAR Light dose                                 | 1   | 2366453,91       | 2366453,91         | 4,78    | 0,05358        |
| P Stutzeri                      | Thalassiosira sp. effect with solar Irradiance | 2   | 12,30            | 6,15               | 2,81    | <b>0,08675</b> |
|                                 | specific effect of UV                          | 3   | 0,76             | 0,25               | 0,17    | 0,91785        |
|                                 | solar Light dose                               | 1   | 14,41            | 14,41              | 12,99   | <b>0,00482</b> |
|                                 | PAR Light dose                                 | 1   | 0,94             | 0,94               | 1,99    | 0,18852        |
|                                 | Thalassiosira sp. effect with solar Irradiance | 2   | 1,30             | 0,65               | 0,33    | 0,72188        |
|                                 | specific effect of UV                          | 3   | 34,81            | 11,60              | 6,37    | <b>0,00331</b> |
|                                 | solar Light dose                               | 1   | 4,46             | 4,46               | 1,27    | 0,28665        |
|                                 | PAR Light dose                                 | 1   | 0,94             | 0,94               | 1,99    | 0,18852        |
| rpoH                            | Thalassiosira sp. effect with solar Irradiance | 2   | 23,80            | 11,90              | 4,46    | <b>0,02664</b> |
|                                 | specific effect of UV                          | 3   | 0,99             | 0,33               | 0,19    | 0,90177        |
|                                 | solar Light dose                               | 1   | 10,79            | 10,79              | 5,39    | <b>0,04269</b> |
|                                 | PAR Light dose                                 | 1   | 0,78             | 0,78               | 1,96    | 0,19213        |
| Vaccenique Ac oxidation product | Thalassiosira sp. effect with solar Irradiance | 1   | 1142347,63       | 1142347,63         | 16,31   | <b>0,00237</b> |
|                                 | specific effect of UV                          | 1   | 833878,21        | 833878,21          | 0,73    | 0,40090        |
|                                 | solar Light dose                               | 1   | 1557444,57       | 1557444,57         | 2,00    | 0,18718        |
|                                 | PAR Light dose                                 | 1   | 5287382,51       | 5287382,51         | 5,09    | <b>0,04769</b> |
| D. Shibaе                       | Thalassiosira sp. effect with solar Irradiance | 2   | 16,22            | 8,11               | 24,56   | <b>0,00001</b> |
|                                 | specific effect of UV                          | 3   | 129,74           | 43,25              | 151,30  | <b>0,00000</b> |
|                                 | solar Light dose                               | 1   | 2,99             | 2,99               | 12,68   | <b>0,00517</b> |
|                                 | PAR Light dose                                 | 1   | 0,00             | 0,00               | 0,00    | 0,98159        |
|                                 | Thalassiosira sp. effect with solar Irradiance | 2   | 9,61             | 4,81               | 23,14   | <b>0,00001</b> |
|                                 | specific effect of UV                          | 3   | 828,10           | 276,03             | 4090,43 | <b>0,00000</b> |
|                                 | solar Light dose                               | 1   | 0,60             | 0,60               | 6,99    | <b>0,02458</b> |
|                                 | PAR Light dose                                 | 1   | 0,01             | 0,01               | 0,22    | 0,65006        |
| rpoH                            | Thalassiosira sp. effect with solar Irradiance | 2   | 1,10             | 0,55               | 1,96    | 0,17018        |
|                                 | specific effect of UV                          | 3   | 117,53           | 39,18              | 236,09  | <b>0,00000</b> |
|                                 | solar Light dose                               | 1   | 0,06             | 0,06               | 0,24    | 0,63195        |
|                                 | PAR Light dose                                 | 1   | 0,35             | 0,35               | 4,73    | 0,05478        |

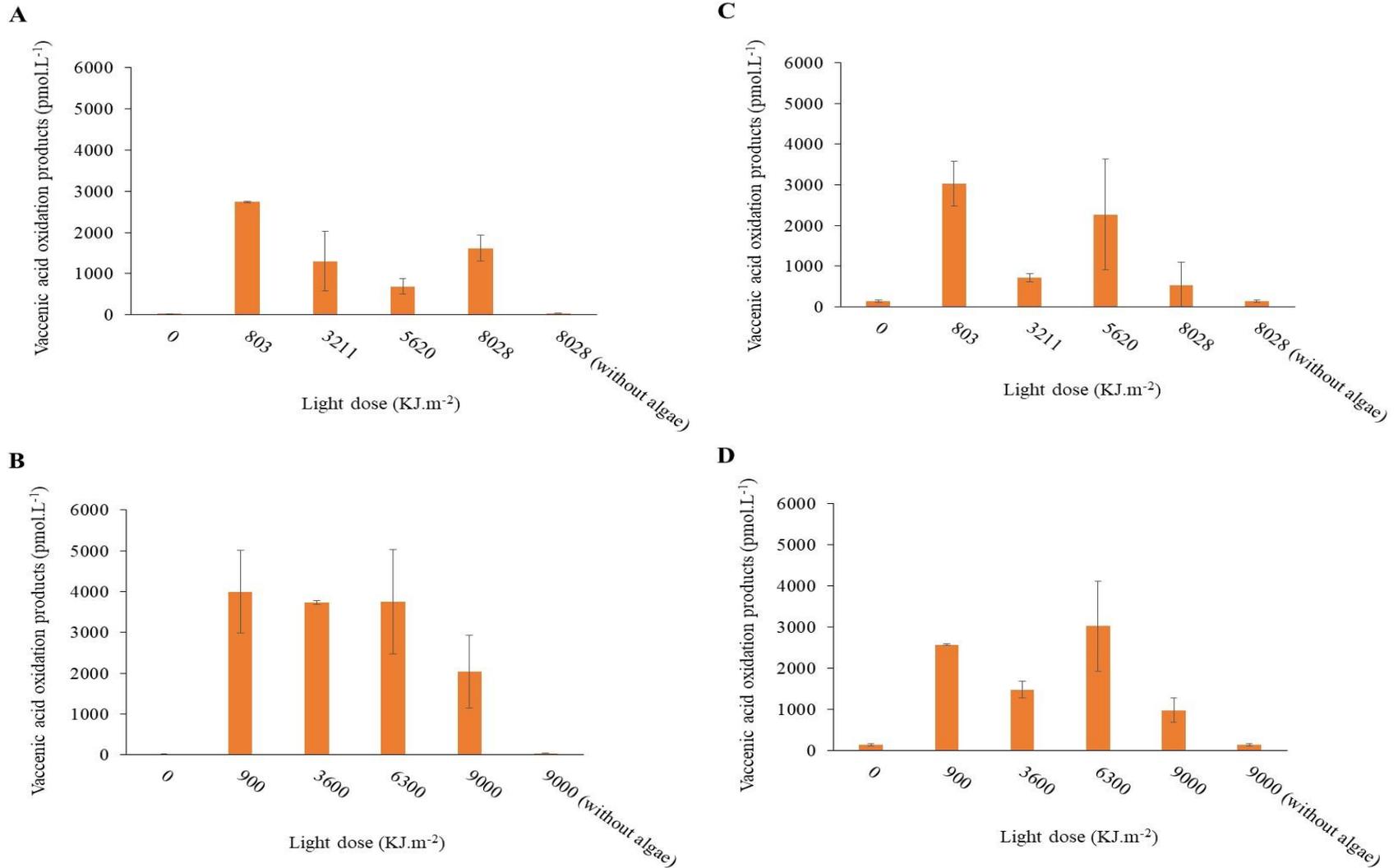


Fig. 1: Production of vaccenic acid oxidation products (pmol. L<sup>-1</sup>) during irradiation of *Thalassiosira* sp. cells associated with bacterial strains *Pseudomonas stutzeri* (A & B) or *Dinoroseobacter shibae* (C & D) with PAR and PAR + UV radiation, respectively.

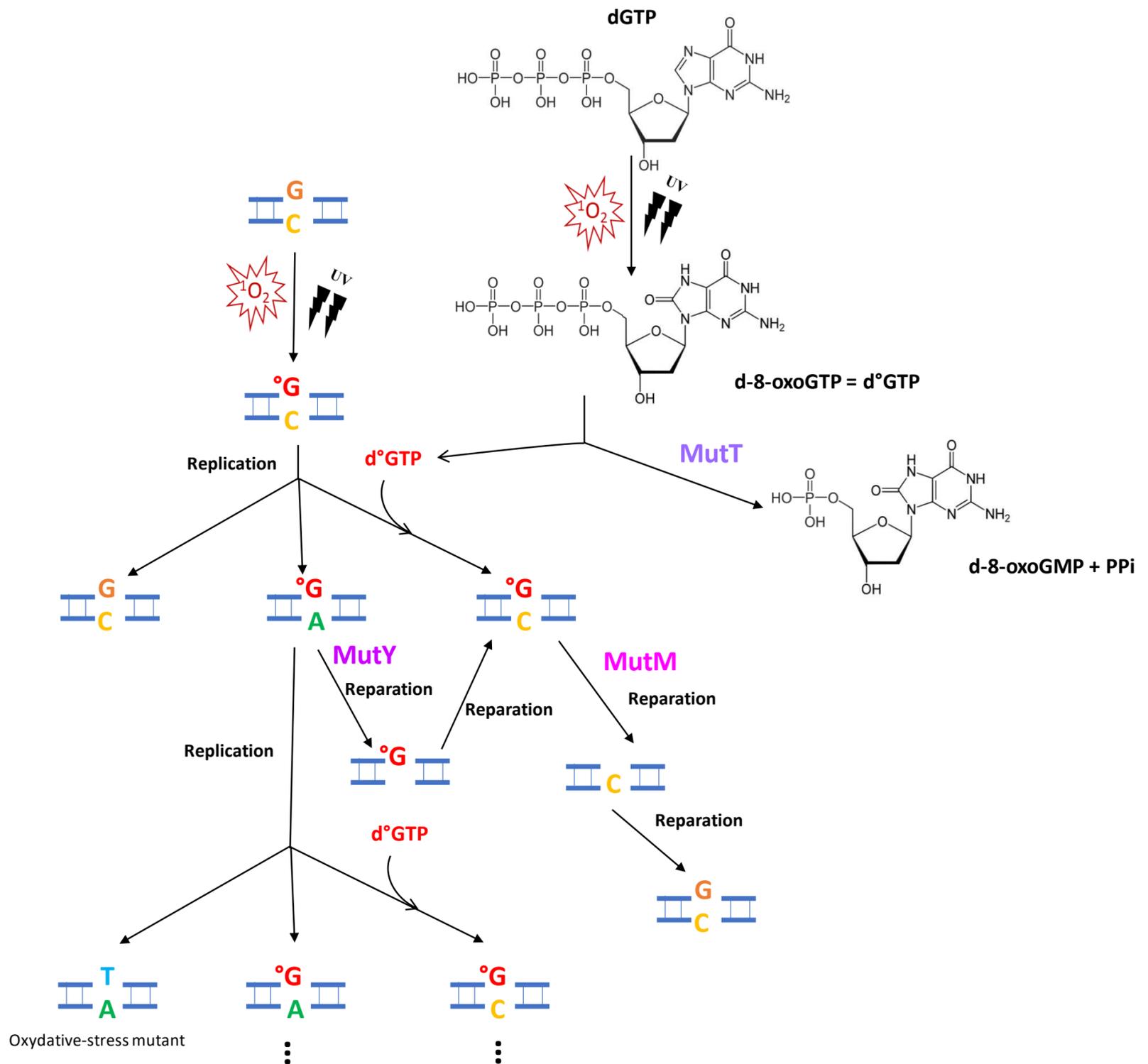


Fig. 2: The different scenarios related to 102 DNA damage and the role of the “Go system” proteins in repair. (°G = 8-oxo-7,8-dihydro-2'-deoxyguanosine = d-8-oxoG).



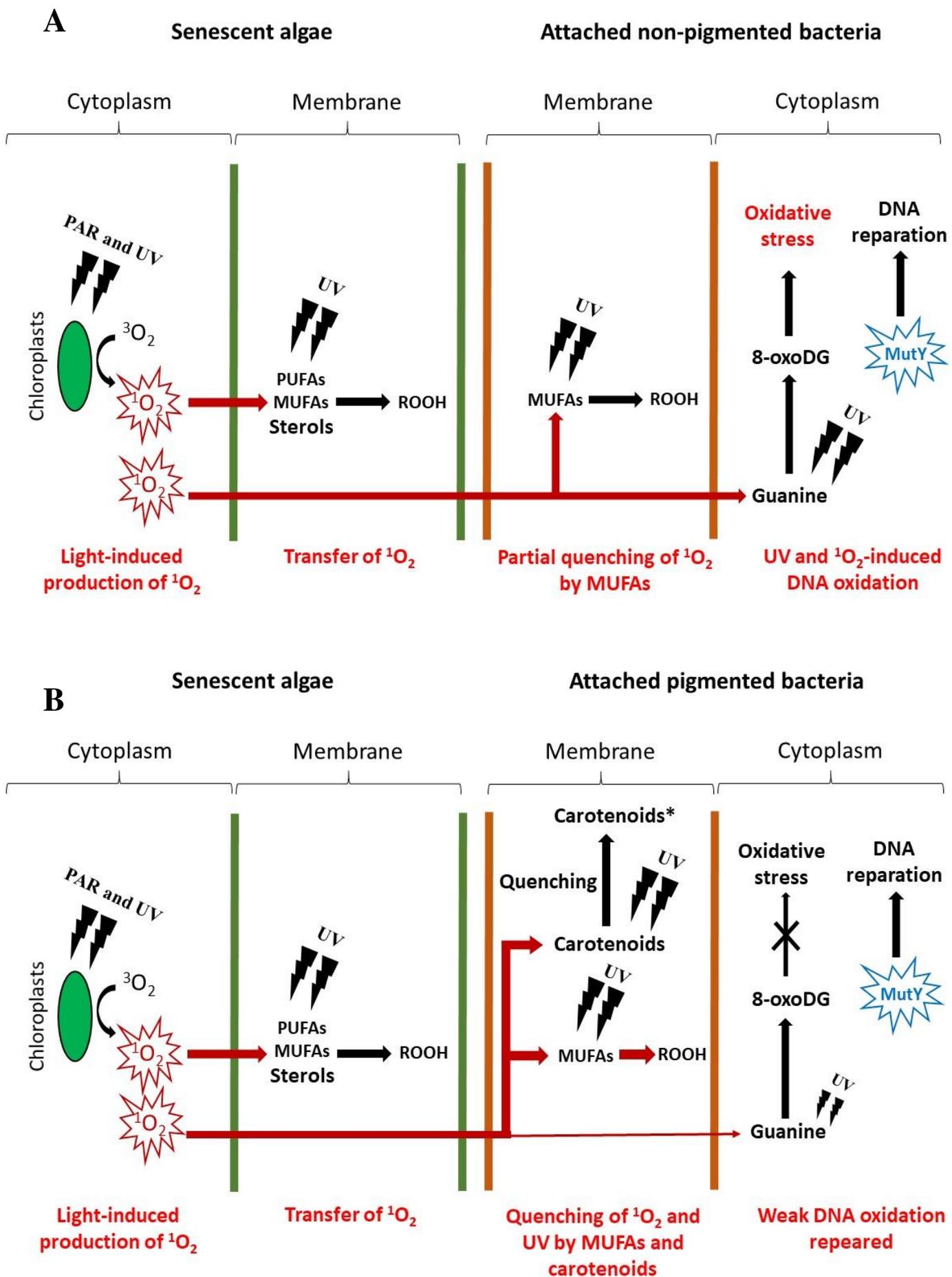


Fig. 4: Conceptual scheme showing the effect of singlet oxygen photochemically-produced in phytodetritus on membrane components and DNA of unpigmented (A) and pigmented (B) associated bacteria.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [allTableS.pdf](#)