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Oxidative damage and antioxidative system in algae

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ABSTRACT

Reactive oxygen species (ROS) typically produce in algae and act as secondary messengers in numerous cellular processes. Under abiotic stresses, the balance between production and suppression of ROS disappears and causes increase of ROS. Increasing excessive ROS can cause damage to various cellular components comprising cell membranes, proteins and lipids. Algae have an antioxidant defense system to overcome on oxidative damage. Antioxidant defense mechanisms are of two types, namely enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants include superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase. The non-enzymatic antioxidants include carotenoids, tocopherol, ascorbic acid, glutathione, flavonoids and phenolic compounds. In this review, we describe the various types of ROS and their production, and antioxidant defense mechanisms for ROS suppression.

1. Introduction

Algae are autotroph organisms that use light and inorganic nutrients. These organisms have valuable compounds include polyunsaturated fatty acids (PUFA), carotenoids, phycobiliproteins, polysaccharides and phycotoxins [1]. Algae exist in dry and aquatic environments, and some have nutritional value and are used as food, and other some have biodiesel application [2]. Aerobic organisms use oxygen to breathe their energy. Electron leak in the electron chains in the plasma membrane, mitochondria, and chloroplasts produce reactive oxygen species (ROS) [3,4].

ROS is very harmful at high concentrations and lead to oxidative damage. Abiotic stresses cause oxidative stress through excessive production of ROS. ROS can react with biomolecules and inactivate or change them and cause to organelle dysfunction, alteration in cell structure and mutagenesis. Algae have antioxidant defense systems for cope on the harmful effects of ROS and maintain them under oxidative stress [5–7].

ROS act as a secondary messenger in biological processes such as response to environmental stresses. The balance between production and suppression of ROS determines its role. Control the level of ROS is vital due to the multifunctional role of ROS for avoid any oxidative damage and not to remove them completely. Antioxidative system including the non-enzymatic and enzymatic antioxidants causes ROS detoxification [8,9].

The present review focused on kinds of ROS, their site of creation, and their role as messenger and inducer of oxidative stress. Further, role of Antioxidative defense system in combating damage posed by overproduced ROS under stresses has been illuminated in detail.

2. Generation of reactive oxygen species

ROS generate in chloroplasts, peroxisomes, cytosol, and mitochondria and are as a by-product of respiration. ROS are produced in thylakoid membranes when the absorption of light is more than the requirement of photosynthetic apparatus. Photosystem I (Psi) is the most main source of ROS production due to the completion of the electron transfer in the thylakoid membrane on its stromal side. PSII caused ROS production when the excitation energy delivery to the PSII reaction center and the electron transport chain between the photosystems limited. ROS activity depends on its type and reaction conditions. For example HO* has a very high chemical activity and is able to oxidize the most organic molecules and H_2O_2 has moderate activity. The ROS activity decreases in the order $HO^{\cdot} > {}^1O_2 > H_2O_2 > O_2^{\cdot-}$ in biological environments [10,11].

2.1. Singlet oxygen

Singlet oxygen ($^1O_2$) counters with chief biological compounds such as unsaturated fatty acids of membrane lipids and is electrophilic. O_2 reacts with singlet or triplet excited state of a pigment molecule and produces $^1O_2$ in photosynthetic tissues. Carotenoids and tocopherols are

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scavengers of $^1O_2$ [12,13].

2.2. Hydrogen peroxide

Hydrogen Peroxide (H_2O_2) produces by lessening of oxygen by two electrons. Superoxide dismutase causes the generation of H_2O_2 by dismutation of $O_2^{\cdot -}$. H_2O_2 suppressed by peroxidases and catalases [14].

2.3. Superoxide anion radical

PSI by electron reduction of oxygen causes generation of superoxide anion radical ($O_2^{\cdot -}$), but PSII produces low amount of $O_2^{\cdot -}$. Superoxide dismutase detoxifies $O_2^{\cdot -}$ via the dismutation reaction [15].

2.4. Hydroxyl radical

Hydroxyl radical ($HO^{\cdot}$) creates of homolytic cleavage of water (H_2O $HO^{\cdot}$ + $H^{\cdot}$). $HO^{\bullet}$ radicals rapidly attack to proteins and lipids and lead to oxidative injury [16].

3. Oxidative damage

Imbalance between pro-oxidant and antioxidant homeostasis augmented ROS and oxidative damage. ROS disrupt cell function by lipid peroxidation, oxidizing proteins and damaging nucleic acids (Fig. 1).

3.1. Lipid peroxidation

Lipids, as the key components of the membrane, are the primary goal of the ROS and lead to lipid peroxidation by removing hydrogen from the unsaturated chain of fatty acids. Therefore, lipid peroxidation determinates cellular harmful in living organism under oxidative stress. Lipid peroxidation produced wide types of cytotoxic products from polyunsaturated acids such as malondialdehyde (MDA) and aldehydes. MDA is an identifier for oxidative hurt. Chloroplasts have a composite system of membranes rich of polyunsaturated fatty acids in algae, which are main targets for peroxidation [17,18].

3.2. Protein oxidation

ROS cause amino acids oxidation, change of electrical charge of proteins, breakup of peptide chain, cross-linking of proteins and increase susceptibility to proteolysis by proteases. Proteins have sulfur comprising amino acids or thiol groups are the target of ROS. Cysteine and methionine residues are mainly more susceptible to oxidation. Oxidation of methionine residues or sulfhydryl groups lead to conformational alternations, unfolding and degradation proteins. ROS generated disulfide bonds between sulfur-containing amino acids and destroyed the function and structure of proteins. Oxidized proteins lead cellular dysfunction making their deletion necessary. They are normally recognized and degraded by proteasomal complexes. If oxidized proteins are not removed, they collected and change the cell function [19,20].

3.3. DNA oxidation

Hydroxyl radicals attack to the base and saccharide in DNA and cause saccharide fragmentation and strand breaks. Strand break are more destructive and lethal than base damage in cells. ROS cause alteration in DNA bases for example thymine and guanine residues in

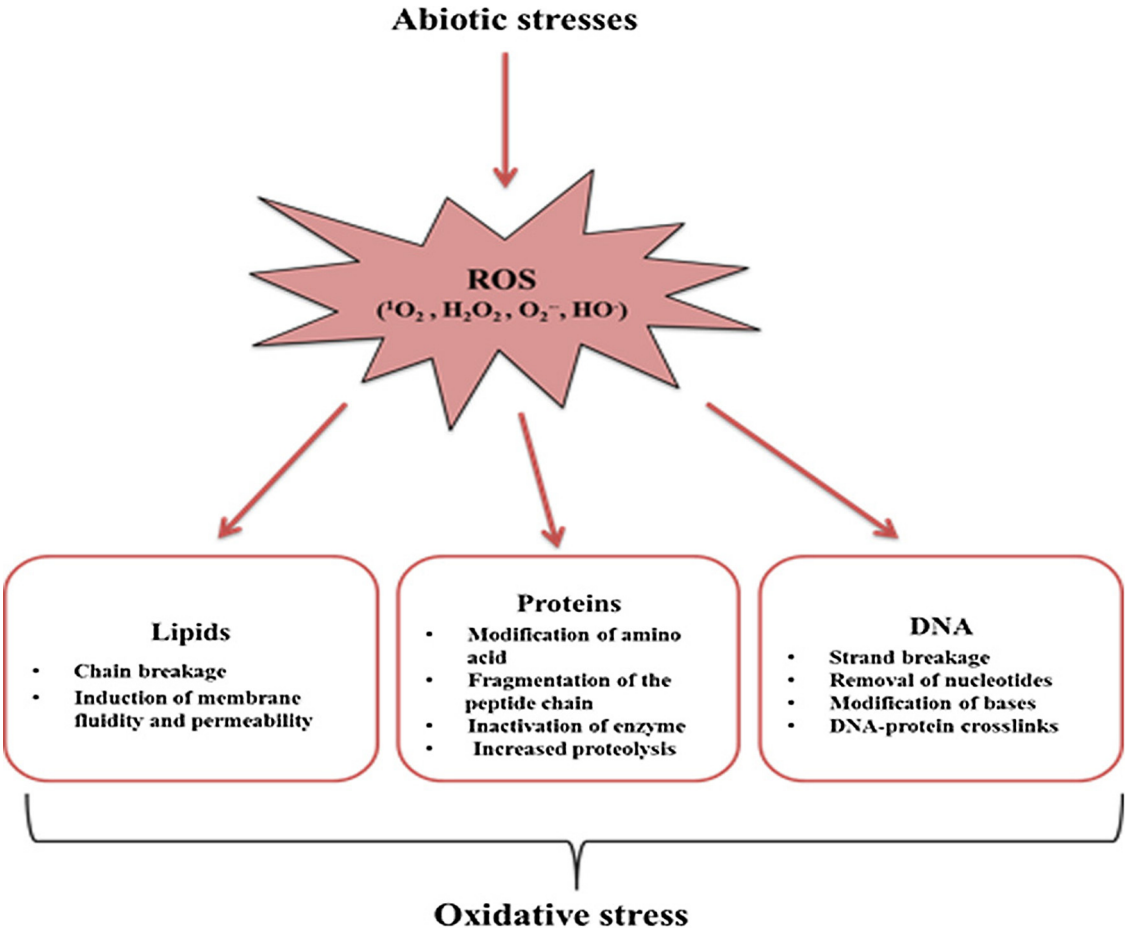

Fig. 1. Reactive oxygen species (ROS) induce oxidative damage to proteins, lipids and DNA [48].

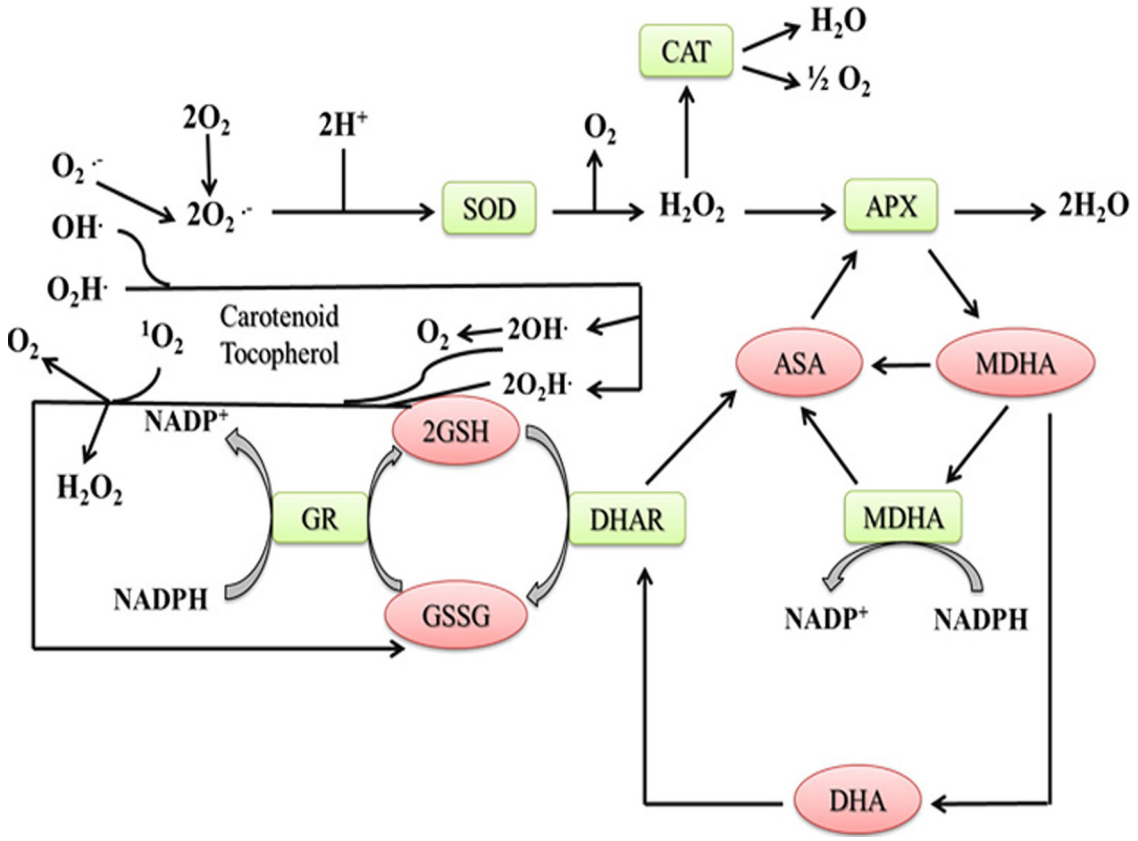

Fig. 2. Enzymatic and non-enzymatic antioxidants in algae. ASC, Ascorbate; APX, Ascorbate peroxidase; CAT, Catalase; DHA, Dehydroascorbate; GSH, Glutathione; GR, Glutathione reductase; GSSG, glutathione disulfide; MDHA, Monodehydroascorbate; SOD, Superoxide dismutase; DHA, Dehydroascorbate reductase [49].

DNA can be hydroxylated or degraded. Oxidation of thymine residues stop replication by DNA polymerase and transcription by RNA polymerase [21,22].

4. Antioxidative defense system in algae

Algae applied two defensive strategies for cope on ROS before they can damage to cellular difference components (Fig. 2). The first system comprise antioxidant enzymes (high molecular weight) such as superoxide dismutases, glutathione reductase, catalases, ascorbate peroxidase and non-enzymatic (low molecular weight) that comprise ascorbate, flavonoids, carotenoids, glutathione, tocopherols and phenols. The second mechanism is repair enzymes that repair and remove damaged macromolecules. The enzymatic antioxidants are important in the detoxification of the destructive effects of formed ROS by electron transport ($O_2^{\bullet -}$, H_2O_2, $HO^{\bullet}$), but the non-enzymatic antioxidants are more effective in the prevention of ROS prodction by the excitation energy transfer ($^1O_2$) [23–25].

4.1. Enzymatic components of antioxidative defense system

4.1.1. Superoxide dismutase

Superoxide dismutase (SOD) is a metalloprotein and first line of defense against oxidative stress that catalyzes the dismutation of superoxide radicals into oxygen and hydrogen peroxide. SOD include three isoforms that determined by their metal center cofactors: Cu/ZnSOD found in the thylakoid membranes and cytosol of higher plants, certain dinoflagellates and charophycean green algae, MnSOD located in mitochondria, and FeSOD found in the chloroplast stroma. The SOD activity enhanced by abiotic stresses and acts a defense tool [26–28].

4.1.2. Catalase

Catalase (CAT) is tetrameric enzyme contain heme that has a main role to convert H_2O_2 into H_2O and O_2. CAT has three groups. Manganese catalases exist in prokaryotes. Catalase peroxidases act as both catalases and peroxidases and have been found in prokaryotes and some eukaryotes. Classical catalases (cat) include heme groups and covert H_2O_2 to H_2O and O_2 in a two-step process. First, one molecule of H_2O_2 is reduced to water and the Fe^{3+} of the catalase is altered to cat (Fe[V]O). Second, the cat(Fe[V]O) is converted back to Fe^{3+} while another molecule of H_2O_2 is reduced to H_2O and O_2 [29].

4.1.3. Ascorbate peroxidase

Ascorbate peroxidase (APX) is a heme enzyme and converts the H_2O_2 into H_2O through ascorbate as electron donor. APX is existed in plants and algae. Two cytosolic APX (cAPX) isoenzymes have been showed in the red algae *Galdieria partita* and *G. sulphuraria* [30,31].

4.1.4. Glutathione reductase and glutathione peroxidase

Glutathione reductase (GR) is a flavoprotein oxidoreductase that exists in eukaryotes and prokaryotes. GR is a great enzyme of the ascorbate-glutathione cycle and have a necessary function in defense system against ROS by maintain the reduced status of GSH. Like Ascorbate peroxidase, Glutathione peroxidase decomposes H_2O_2 to H_2O by GSH [32,33].

4.2. Nonenzymatic components of antioxidative defense system

4.2.1. Glutathione

The Tripeptide glutathione is the major low molecular weight thiol and formed of glutamate, cysteine and glycine. Reduced glutathione by oxidizing itself into oxidized glutathione via ascorbate-glutathione cycle causes the regeneration of ascorbate. Glutathione involves in

adjust redox potential for amino acids and proteins, scavenging oxidative damage, non-specific reductant, substrate/cofactor for enzyme-catalyzed reactions, reconstruction of protein disulfide bonds and suppression of H_2O_2 and organic peroxides (ROOH) [34,35].

4.2.2. Ascorbate

Ascorbic acid (ascorbate, vitamin C) is a water-soluble antioxidant that acts as substrate for ascorbate peroxidase, electron donor for $^{\cdot}OH$ radical, good scavenger, reducing antioxidant and donating its electrons to ROS. Ascorbate suppresses H_2O_2 thought ascorbate-glutathione cycle. [36].

4.2.3. Carotenoids

Carotenoids are isoprenoid and lipophilic compounds and are colored yellow, orange or red that 750 kinds of carotenoid are exist in plants and microorganisms. Carotenoids include carotenes such as lycopene, α- carotene and β-carotene, and xanthophylls with oxygen as hydroxyl groups (e.g. lutein), as oxy-groups (e.g. canthaxanthin) or as a combination of both of them (e.g. astaxanthin). Some of xanthophylls (violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, lutein, loroxanthin, astaxanthin and canthaxanthin) produced by microalgae whereas others (diatoxanthin, diadinoxanthin and fucoxanthin) also synthesized by brown algae. Carotenoids have antioxidant characterizes and cause maintain of cells against free radicals, inhibition of lipid peroxidation, increase the stability of the photosynthetic apparatus and protection of integrity membranes [37–39].

4.2.4. Tocopherols

Tocopherols (vitamin E) have an aromatic ring and a long hydrocarbon chain. Tocopherols play in oxidationreduction reactions by the aromatic ring. Tocopherols destroy reactive types of oxygen and protect unsaturated fatty acids from oxidation. Among the four tocopherols (α-, β, ϒ and ℓ-tocopherols), α-tocopherol is a key antioxidant because it can suppress $^1O_2$, reduce $O_2^{\cdot\ -}$ and finish lipid peroxidation reaction [40].

4.2.5. Phenolic compounds

Phenolic compounds include more than 8000 compounds and divided into 10 various groups according to their chemical structure. Phenolic compounds are as main natural antioxidants through single electron transfer and hydrogen atom transfer. Many studies showed that the phenolic content in microalgae is lower than or equal to the minimum amounts founded in terrestrial plants. Also, types of flavonoids, such as isoflavones, flavanones, flavonols, and dihydrochalcones produced in microalgae. Microalgae synthesized complex phenolic compounds, so identify of phenolic compounds are required in microalgae. Marine algae have many polyphenols such as phlorotannins that have antioxidant activities. Phenolic are involved in defense mechanisms against environmental stresses [41–43].

5. ROS signaling

ROS in low concentration have significant roles in the defense against infection, the cell signaling and the apoptosis. $O_2^{\cdot\ -}$, $OH^{\cdot}$ and H_2O_2 have more signaling capacity because they can transfer of membranes via aquaporins [44]. The transduction of H_2O_2- based signals is centered on sulfur chemistry, with the main player being the reversible oxidative modification of cellular sulfur-containing groups (e.g., cysteine residues and thioredoxin), which in turn results in disturbances of metabolism and signaling pathways in microorganisms. H_2O_2 oxidized cysteine thiol groups of phosphatases in mitogen activated protein kinase (MAPK) pathways, which act in signaling response for various environmental stimuli. Late studies showed moreover change of gene expression, ROS have an essential role for resistance under oxidative stress by post-translational changes. Some of mitogen-activated protein kinase family includes extracellular signal-regulated kinases, JNK, and p38 play a role in cellular processes including proliferation, differentiation, and apoptosis regulated by ROS [45–47].

6. Conclusion

ROS have two divergent functions in algae; in low concentration they are as signaling molecules that regulate cellular processes, whereas they cause damage to cellular components in high concentration. The balance between production and suppression of ROS disappears under stress condition. In order to avoid the oxidative damage, algae have a complex antioxidative defense system include of enzymatic and non-enzymatic components.

Declaration of Competing Interest

The authors declare no conflict of interest.

References

[1] G. Markou, E. Nerantzis, Microalgae for high-value compounds and biofuels production: are view with focus on cultivation under stress conditions, Biotechnol. Adv. 8 (2013) 1532–1542.
[2] W.L. Chu, Y.S. Yuen, C.Y. Wong, M.L. Teoh, S.M. Phang, Isolation and culture of microalgae from the windmill Island region, Antarctica, Kuala Lumpur, Malaysia, Proceedings on Malaysian International Seminar on Antarctica: Opportunities for Research, 5–6 2001, pp. 53–59. August (2001).
[3] R.G. Alscher, J.L. Donahue, C.L. Cramer, Reactive oxygen species and antioxidants: relationships in green cells, Physiol. Plant. 100 (1997) 224–233.
[4] C.H. Foyer, Oxygen metabolism and electron transport in photosynthesis, in: J. Scandalios (Ed.), Molecular Biology of Free Radical Scavenging Systems, Cold Spring Harbor Laboratory Press, New York, NY, 1997, pp. 587–621.
[5] B. Halliwell, J. Gutteridge, Free Radicals in Biology and Medicine, Oxford Science Publications, New York, 1999 pp.;936.
[6] S.N. Lim, P.C.K. Cheung, V.E.C. Ooi, P.O. Ang, Evaluation of antioxidative activity of extracts from brown seaweed, *Sargassum siliquastrum*, J. Agric. Food Chem. 50 (2002) 3862–3866.
[7] E. Vranová, D. Inzé, F. Van Breusegem, Signal transduction during oxidative stress, J. Exp. Bot. 53 (2002) 1227–1236.
[8] R. Desikan, S.A.H. Mackerness, S.J.T. Hancock, J. Neill, Regulation of the *Arabidopsis* transcriptome by oxidative stress, Plant Physiol. 127 (2001) 159–172.
[9] G. Noctor, C.H. Foyer, Ascorbate and glutathione: keeping active oxygen under control, Annu. Rev. Plant Biol. 49 (1998) 249–279.
[10] K. Asada, Production and scavenging of reactive oxygen species in chloroplasts and their functions, Plant Physiol. 141 (2006) 391–396.
[11] K. Apel, H. Hirt, Reactive oxygen species: metabolism, oxidative stress, and signal transduction, Annu. Rev. Plant Biol. 55 (2004) 373–399.
[12] C. Schweitzer, R. Schmidt, Physical mechanisms of generation and deactivation of singlet oxygen, Chem. Rev. 103 (2003) 1685–1757.
[13] C. Triantaphylidès, M. Krischke, F.A. Hoeberichts, B. Ksas, G. Gresser, M. Havaux, Singlet oxygen is the major reactive oxygen species involved in photooxidative damage to plants, Plant Physiol. 148 (2008) 960–968.
[14] M. Ruzzi, E. Sartori, A. Moscatelli, I.V. Khudyakov, N.J. Turro, Time-resolved EPR study of singlet oxygen in the gas phase, J. Phys. Chem. A 117 (2013) 5232–5240.
[15] P. Pospíšil, Production of reactive oxygen species by Photosystem II, Biochim. Biophys. Acta 1787 (2009) 1151–1160.
[16] B. Halliwell, Plant Physiol. 141 (2006) 312–322.
[17] N. Garg, G. Manchanda, ROS generation in plants: boon or bane, Plant Biosyst. - Int. J. Deal. With All Asp. Plant Biol. 143 (2009) 8–96.
[18] B. Halliwell, S. Chirico, Lipid peroxidation: its mechanism, measurement, and significance, Am. J. Clin. Nutr. 57 (1993) 7155–7245.
[19] F.J. Kelly, I.S. Mudway, Protein oxidation at the air-lung interface, Amino Acids 25 (2003) 375–396.
[20] L. Lyras, N.J. Cairns, A. Jenner, P. Jenner, B. Halliwell, An assessment of oxidative damage to proteins, lipids, and DNA in brain from patients with Alzheimer's disease, J. Neurochem. 68 (1997) 2061–2069.
[21] L.H. Breimer, T. Lindahl, Excision of oxidized thymine from DNA, J. Biol. Chem. 259 (1984) 5543–5548.
[22] A. Martinez, R. Kolter, Protection of DNA during oxidative stress by the nonspecific DNA-binding protein Dps, J. Bacteriol. 179 (1997) 5188–5194.
[23] K.S. Pikula, A.M. Zakharenko, V. Aruoja, K.S. Golokhvast, A.M. Tsatsakis, Oxidative stress and its biomarkers in microalgal ecotoxicology–A minireview, Curr. Opin. Toxicol. 13 (2019) 8–15.
[24] S. Jahan, I.B. Yusoff, Y.B. Alias, A.F. Bakar, Reviews of the toxicity behavior of five potential engineered nanomaterials (ENMs) into the aquatic ecosystem, Toxicol. Rep. 4 (2017) 211–220.
[25] T. Xia, M. Kovochich, M. Liong, L. Madler, B. Gilbert, H.B. Shi, J.I. Yeh, J.I. Zink, A.E. Nel, Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties, ACS Nano 2 (2008) 2121–2134.
[26] K. Asada, The water-water cycle in chloroplasts: scavenging of active oxygens and

dissipation of excess photons, Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 (1999) 601–639.
[27] K.J. Davies, Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems, IUBMB Life 50 (2000) 279–289.
[28] N. Mallick, F.H. Mohn, Reactive oxygen species: response of algal cells, J. Plant Physiol. 157 (2000) 183–193.
[29] M.J. Mate, M. Zamocky, L.M. Nykyri, C. Herzog, P.M. Alzari, C. Betzel, F. Koller, I. Fita, Structure of catalase-A from *Saccharomyces cerevisiae*, J. Mol. Biol. 286 (1999) 135–149.
[30] C. Oesterhelt, S. Vogelbein, R.P. Shrestha, M. Stanke, A.P. Weber, The genome of the thermoacidophilic red microalga *Galdieria sulphuraria* encodes a small family of secreted class III peroxidases that might be involved in cell wall modification, Planta 227 (2008) 353–362.
[31] S. Sano, M. Ueda, S. Kitajima, T. Takeda, S. Shigeoka, N. Kurano, S. Miyachi, C. Miyake, A. Yokota, Characterization of ascorbate peroxidases from unicellular red alga *Galdieria partita*, Plant Cell Physiol. 42 (2001) 433–440.
[32] E.A. Edwards, S. Rawsthorne, P.M. Mullineaux, Subcellular distribution of multiple forms of glutathione reductase in leaves of pea (*Pisum sativum* L.), Planta 180 (1990) 278–284.
[33] M.C. Romero-Puertas, F.J. Corpas, L.M. Sandalio, M. Leterrier, M. Rodriguez Serrano, L.A.D. Rio, Glutathione reductase from pea leaves: response to abiotic stress and characterization of the peroxisomal isozyme, New Phytol. 170 (2006) 43–52.
[34] C.H. Foyer, B. Halliwell, The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism, Planta. 133 (1997) 21–25.
[35] B. Halliwell, J.M. Gutteridge, Free Radicals in Biology and Medicine, 3rd ed., Oxford University Press, Midsomer Norton, Avon, England, 1999.
[36] G.L. Wheeler, M.A. Jones, N. Smirnoff, The biosynthetic pathway of vitamin C in higher plants, Nature. 393 (1998) 365–369.
[37] N. Di Pietro, P. Di Tomo, A. Pandolfi, Carotenoids in cardiovascular disease prevention, JSM Atheroscler. 1 (2016) 1–13.
[38] T. Katsumata, T. Ishibashi, D. Kyle, A sub-chronic toxicity evaluation of a natural astaxanthin-rich carotenoid extract of *Paracoccus carotinifaciens* in rats, Toxicol. Rep. 1 (2014) 582–588.
[39] S. Sasso, G. Pohnert, M. Lohr, M. Mittag, C. Hertweck, Microalgae in the post-genomic era: a blooming reservoir for new natural products, FEMS Microbiol. Rev. 36 (2012) 761–785.
[40] Y. Takenaka, M. Miki, H. Yasuda, M. Mino, The effect of a-tocopherol as an anti-oxidant on the oxidation of membrane protein thiols induced by free radicals generated in different sites, Arch. Biochem. Biophys. 285 (1991) 344–351.
[41] M.L. Cornish, D.J. Garbary, Antioxidants from macroalgae: poten-tial applications in human health and nutrition, Algae. 25 (2010) 155–171.
[42] F. de la Coba, J. Aguilera, F.L. Figueroa, M.V. de Galvez, E. Herrera, Antioxidant activity of mycosporine-like amino acids isolated from three red macroalgae and one marine lichen, J. Appl. Phycol. 21 (2009) 161–169.
[43] L.S. Jahnke, Massive carotenoid accumulation in *Dunaliella bardawil* induced by ultraviolet-A radiation, J. Photochem. Photobiol. 48 (1999) 68–74.
[44] C.R. Reczek, N.S. Chandel, ROS-dependent signal transduction, Curr. Opin. Cell Biol. 33 (2015) 8–13.
[45] V.I. Lushchak, Adaptive response to oxidative stress: bacteria, fungi, plants and animals, Comp. Biochem. Physiol. C. 153 (2011) 175–190.
[46] K.A. Morano, C.M. Grant, W.S. Moye-Rowley, The response to heat shock and oxidative stress in *Saccharomyces cerevisiae*, Genetics. 190 (2012) 1157–1195.
[47] E.A. Veal, A.M. Day, B.A. Morgan, Hydrogen peroxide sensing and signaling, Mol. Cell 26 (2007) 1–14.
[48] P. Sharma, A.B. Jha, R.S. Dubey, M. Pessarakli, Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions, J. Bot. (2012), https://doi.org/10.1155/2012/217037.
[49] S.S. Gill, N. Tuteja, Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants, Plant Physiol. Biochem. 48 (2010) 909–930.