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Review

Sources of marine superoxide dismutases: Characteristics and applications

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ABSTRACT

The ability of marine organism to cope with oxidative stress is one of the main factors that influence its survival in the marine environment, when senescence conditions prevail. The antioxidative defense system includes enzymatic and non-enzymatic components. Among the enzymatic system, superoxide dismutases are the first and most important of the antioxidant metalloenzymes. Four different types of metal centers have been detected in SODs, dividing this family into Cu/Zn, Ni, Mn and Fe-SODs. Its use was limited to non-drug applications in humans (include: cosmetic, food, agriculture, and chemical industries) and drug applications in animals. This paper is a review of the recent literatures on sources of marine SODs, the need for SOD and different applications in industry, covering the last decades. The most recent paper, patents and reviews on characterization and application are reviewed.

Contents

1. Introduction

Reactive oxygen species (ROS) are highly reactive oxygen-containing molecules produced during normal aerobic respiration process. The level of ROS control by the antioxidant system of the body [1,2]. Excessive amounts of ROS can damage macromolecules (e.g. proteins, lipids, and DNA) and cell membranes [3]. The antioxidative defense system includes enzymatic and non-enzymatic components [4]. Among the enzymatic system, superoxide dismutases (SOD; EC1.15.1.1) are the first and most important of the antioxidant metalloenzymes was reported by McCord and Fridovich (1969) when they observed dismutation of O_2^- into H_2O_2 and O_2 [5–7]:

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

H_2O_2 is converted by other enzymes like catalase (CAT) and peroxidases (GPx) into harmless product water (Fig. 1) [2,4]. The combined actions of these enzymes keep the levels of the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) low [8]. SOD is ubiquitous to all forms of life. SODs are classified based on the type of metal bound at the active site: Cu/Zn-SOD, Mn-SOD, Fe-SOD, and Ni-SOD [7,9].

Cu/Zn-SOD and Mn-SOD are found in both prokaryotes and eukaryotes, Cu/Zn-SOD is generally homodimeric and is present in diverse locations in different organisms [1,5]. It is found in the periplasm of gram-negative bacteria (sodC), cytoplasm and

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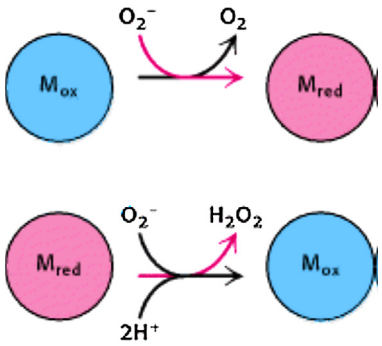

Fig. 1. Superoxide dismutase mechanism. The oxidized form of superoxide dismutase (M_{ox}) reacts with one superoxide ion to form O_2 and generate the reduced form of the enzyme (M_{red}). The reduced form then reacts with a second superoxide and two protons to form hydrogen peroxide and regenerate the oxidized form of the enzyme [2].

chloroplast of plants, intermembrane space of mitochondria, and several compartments such as nucleus, lysosome, peroxisome, cytosol (sod1), and extracellular milieu (sod3, EC SOD) in animals [7,9]. Unlike other organisms, plants have been two main subgroups of Cu/Zn-SODs: chloroplastic and cytosolic [10,11]. Cu/Zn-SOD plays important role in stationary phase survival and aerobic growth in bacteria and fungi [12]. Additionally, the periplasmic Cu/Zn-SOD in gram-negative pathogenic bacteria has been proposed to confer protection against the host defense responses [13].

Fe-SOD is found in prokaryotes (sodB), protozoans, and chloroplasts of algae and in plants two families of Fe-SODs have been found: the chloroplastic localized and the plastidial localized [3,5,14]. Fe and Mn-SODs are typically homodimers or homotetramers that probably evolved from a common ancestor. Due to their structural and sequence conservation, they can frequently bind both Fe and Mn, but attain significant activity with only the permitted metal cofactor [15]. Mn-SOD occurs in prokaryotes (sodA) and mitochondria of the eukaryotes (sod2), has the ability to catalyze the toxic superoxide anion into molecular oxygen and hydrogen peroxide [6,15]. Additionally Ni-SOD has recently been purified from several aerobic soil bacteria of Streptomyces [5,16]. Three common isoforms could be distinguished by their differential sensitivities to different chemicals. Cu/Zn-SOD is very sensitive to H_2O_2 and cyanide while Mn-SOD is insensitive to H_2O_2 and cyanide, and Fe-SOD cannot be inhibited by cyanide, but it is very sensitive to H_2O_2 [14].

In the 1990s, an antioxidant enzyme SOD was introduced into the market. Although the enzyme initially showed great promise in therapeutic applications, it did not perform up to expectations. Consequently, its use was limited to non-drug applications in humans (include: cosmetic, food, agriculture, and chemical industries) and drug applications in animals [1,17,18].

A marine enzyme may be a unique protein molecule not found in any terrestrial organism or it may be a known enzyme from a terrestrial source but with novel properties. This review is intended to start from a thorough analysis of habitat-related properties presenting marine SODs with novel chemical biodiversity. Additionally this paper describes the different bioprocess engineering approaches adopted for the production of marine SODs derived mainly from all of kingdom (e.g. Bacteria, Chromista, Plantas, fungi and Animalia). We insert each organism in kingdoms by information of site *World Register of Marine Species* [19].

2. Superoxide dismutases in marine bacteria

Marine bacteria are abundant and play critical roles in the ocean environment. As the technology that allows us to study these microscopic organisms evolves, so does our understanding of who they are and what they do [20]. SODs have been identified in a number of marine bacteria (Table 1). Except for one case, all of marine bioluminescent bacteria contain a ferri protein enzyme. The Fe-SODs from *Photobacterium leiognathi* (symbiont) and from *Photobacterium sepia* (free living) have been purified. Although the two enzymes are closely similar, various differences exist. The isoelectric point found for SODs from *P. sepia* and SODs from *P. leiognathi* were 4.1 and 4.4, respectively. Both SODs showed a high thermal stability and contains 1.6 g atoms of iron/molecule [21]. The SODs enzyme from *P. leiognathi* assumed that is a dimer containing one iron atom/subunit. In the Fe-SOD from *P. leiognathi* histidine and tryptophan residues are probably involved in the catalytic activity and that one tyrosine and one tryptophan residue/subunit may be metal ligands. Neither carboxyl groups nor tyrosine residues seem to be involved in the catalytic site [22].

Table 1
Superoxide dismutases (SODs) from marine bacteria sources.

Source	Important properties	Reference
Photobacterium leiognathi	p*I* 4.4 A high thermostable enzyme Histidine and tryptophan residues involved in the catalytic activity Tyrosine and one tryptophan residue/subunit may be metal ligands	[21,22]
Photobacterium sepia	p*I* 4.1 A high thermostable enzyme	[22]
Cyanobacterium Synechococcus	N-terminal similarity to both the Fe-SODs and the Mn-SODs of *Escherichia coli*	[23]
Nodularia Aphanizomenon Anabaena	May have a role in the photoadaptation of diazotrophic cyanobacteria and help to protect them from light injury	[24]
Geobacillus sp.	Molecular mass 50.23 kDa p*I* 4.65 The recombinant enzyme had high thermostability at 50 °C The enzyme also showed striking stability over a wide range of pH 5.0–11.0 Good tolerance to some inhibitors, detergents, and denaturants	[17]

Three constitutive forms of SODs activity have been demonstrated in the cyanobacterial marine picoplankter *Synechococcus* sp. WH 7803. Three distinct SODs activities were observed, an Fe-SODs, a Cu/Zn-SODs and a third form which has not been identified. All three types appear to be located in both the soluble cytoplasmic and membrane fractions. Growth of *Synechococcus* cells in artificial sea water (ASW) medium containing no added iron resulted in no alteration in the activity of the Fe-SODs. Growth of cultures in the absence of copper or zinc resulted in differential changes in the activities of the Cu/Zn-SODs and the unidentified SODs [23].

The abundance and cellular location of Fe-SOD in trichomes of *Nodularia*, *Aphanizomenon* and *Anabaena*, and in trichomes of a cultured *Nodularia* strain. For trichomes collected from natural populations the areal concentration of Fe-SOD labeling decreased with depth. An increase in the Fe-SOD content, particularly evident in scum samples that are continuously exposed to high irradiances, may have a role in the photo adaptation of diazotrophic cyanobacteria and help to protect them from light injury in the Baltic Sea [24]. A new gene encoding a SODs was identified from a thermophile *Geobacillus* sp. EPT3 isolated from a deep-sea hydrothermal field in east Pacific. The open reading frame of this gene encoded 437 amino acid residues. The recombinant SODs were determined to be a homodimer with monomeric molecular mass of 59.0 kDa. In comparison with other Mn–SODs, the manganese-binding sites are conserved in the sequence (His260, His308, Asp392, His396). The recombinant enzyme had high thermostability at 50 °C. The enzyme also showed striking stability

over a wide range of pH 5.0–11.0. At tested conditions, the recombinant SODs from *Geobacillus* sp. EPT3 showed a relatively good tolerance to some inhibitors, detergents, and denaturants, such as β-mercaptoethanol, dithiothreitol, phenylmethylsulfonyl fluoride, Chaps, Triton X-100, urea, and guanidine hydrochloride [17].

3. Superoxide dismutases in marine Chromista

Cavalier-Smith in 1981 established Chromista as a kingdom distinct from Plantae and Protozoa because of the evidence that chromist chloroplasts were acquired secondarily by enslavement of a red alga, itself a member of kingdom Plantae, and their unique membrane topology [25]. SODs have been identified in a number of marine Chromista and describe their properties (Table 2).

Lingulodinium polyedrum (formerly *Gonyaulax polyedra*) cells kept in the presence of Hg^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+}, are under oxidative stress. Thus, the phytotoxicity of metal ions in marine environment could be due to either direct or indirect generation of ROS produced during oxidative stress. Induction of SOD activity, in particular the Mn-SOD isoform, by exposure to these metals suggests that this may be an important adaptive response against the phytotoxic effects of pollutant metals in this species of dinoflagellate [26]. Regulation of *L. polyedrum* Fe-SOD can take place at different steps of gene expression. Translation of Fe-SOD varies over the course of a 24-h light-dark cycle, whereas an increase in the Fe-SOD transcript pool occurs after exposure to toxic metal ions, a novel finding. In both cases, a prompt induction of Fe-SOD expression is critical for controlling the steady-state levels of O_2 and thus preventing oxidative damage within subcellular sites highly prone to oxidative stress such as chloroplasts [27]. A hyperoxidative status and increased oxidative damage suggest a correlation between acute metal treatment and oxidative stress in chloroplasts of *L. polyedrum*. Despite concomitant β-carotene induction, acute exposure to metals is damaging and appears to exceed the antioxidant defense. Nevertheless, elevated SODs activities seem to be important in the attenuation of oxidative damage to chloroplastic molecules under chronic conditions of metal stress. Furthermore, reduced light-harvesting capacity due to low peridinin levels is probably a relevant effect of metals in *L. polyedrum*, particularly under chronic Hg^{2+}, Cd^{2+}, and Pb^{2+} treatments. Such antioxidant response at the subcellular site where oxidative [28].

The ability of phytoplankton to cope with oxidative stress is one of the main factors that influence its survival in the marine environment, when senescence conditions prevail. In a first attempt to investigate the antioxidant strategies of different phytoplanktonic groups face to oxidative stress, the SOD activity and photosynthetic pigment content along the growth curves of the dinoflagellate *L. polyedrum* (Stein) Dodge, the diatom *Minutocellus polymorphus* (Hargraves and Guillard) Hasle, von Stosch and Syvertsen were evaluated in batch-cultures. The antioxidant response during senescence in batch-cultures differs according to the species.

Table 2
Superoxide dismutases (SODs) from marine Chromista sources.

Source	Important properties	Reference
Lingulodinium polyedrum (formerly *Gonyaulax polyedra*)	Increase in the Fe-SOD transcript pool occurs after exposure to toxic metal ions	[26–29]
Minutocellus polymorphus	Important role to prevent oxidative stress triggered by a number of factors that affects growth, such as nutrient and light availability	[29]
Thallassiosira weissflogii	A more stable SOD in a broad pH range from 4 to 12, higher temperature, and in the presence of proteases	[30,31]
Nitzschia closterium	UV-B radiation decreased SOD activity	[32]

Induction of SOD activity may occur either in the early exponential or stationary growth phases, which is important to prevent oxidative stress triggered by a number of factors that affects growth, such as nutrient and light availability [29]. A cDNA clone of 1114 bp encoding a putative Mn-SOD from diatom *Thallassiosira weissflogii* was cloned by the PCR technique. Nucleotide sequence analysis of this cDNA clone revealed that it was translated into 201 amino acid residues. When the sequence was compared with Mn-SODs from *Vibrio mimicus* and *Escherichia coli*, as well as two Fe-SODs from *E. coli* and *P. leiognathi*, this SOD showed higher homology to Mn-SOD. The recombinant enzyme was heated at 55 °C with a time-dependent assay; the time interval for 50% inactivation was 23 min, and its thermal inactivation rate constant K_d was 3.03×10^{-2} min^{-1}. The enzyme was inactivated either in acidic pH (below 4.0) or in the presence of imidazole (above 1.6 M) and had only a moderate effect under SDS (above 4%), whereas it was not affected under an alkaline pH (above 9.0). The atomic absorption spectrometric assay showed that 0.6 atom of iron/manganese (3:1) was present in each subunit of SOD. Reconstitution study was suggested that diatom SOD was cambialistic (Fe/Mn)-SOD [30]. Their results clearly demonstrated that diatoms have at least two SOD isozymes to regulate oxidative stress [7]. Zhang et al., studied the effect of UV-B radiation on the growth and antioxidant systems of *Nitzschia closterium* (Ehrenb.) W. Sm. The relative growth rate of *N. closterium* declined with increasing dose of UV-B radiation, which showed that UV-B radiation can inhibit its growth. When the UV radiation dose was 0.54 kJ/m^2, the relative growth rate of *N. closterium* decreased by 25.6% ($P < 0.05$, *t*-test). It was a negative correlation between the relative growth rate and UV-B radiation dose. The relationship between the UV-B radiation dose and the content of both Chl *a* and carotenoid was negative. Superoxide anion radical (O_2^-) production and the concentration of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) also increased with the increasing of UV-B radiation. Antioxidant systems, non-enzymic components (carotenoid and glutathione content) and enzymic components (superoxide dismutase (SOD) and catalase (CAT) activity), decreased as a result of enhanced UV-B radiation. When the exogenous glutathione (GSH) was added, the effects of UVB radiation on the growth of the *N. closterium* was alleviated. These results suggest that enhanced UV-B radiation suppressed the antioxidant systems and caused some active oxygen species to accumulate, which in turns retarded the development of the marine microalgae [31].

4. Superoxide dismutases in marine Plantas

The Kingdom Plantae contains organisms that can produce their own food using sunlight, a process known as photosynthesis. Most of them live on land or in fresh water. Only a few plants such as eel grass and mangrove trees are found in shallow salt water [32]. SODs have been recognized in a number of marine Plantas and describe their properties (Table 3).

The major SOD of the unicellular red alga, *Porphyridium cruentum*, has been purified to homogeneity. This enzyme has a Molecular mass of 40,000 and is composed of two subunits of equal size, which are joined by non-covalent interactions. Manganese constituted 0.13% of this SOD. This is equivalent to one manganese atom/molecule of enzyme. Cyanide at 5 mM and H_2O_2 at 3 mM had no effect on the activity of this SOD but 20 mM azide caused 50% inhibition. The isoelectric point, assessed by isoelectric focusing, is 4.2. The optical spectrum of this enzyme exhibited a maximum at 280 nm (E_m = 49,000 M^{-1} cm^{-1}) and a broad band centered at 450 nm (E_m = 170 M^{-1} cm^{-1}). Exposure to a pH of 3.8 in the presence of 8.0 M urea labilized the manganese and allowed the preparation of a colorless and inactive apoenzyme which could

Table 3
Superoxide dismutases (SODs) from marine plantae sources.

Source	Important properties	Reference
Porphyridium cruentum	Molecular mass 40 kDa Composed of two subunits of equal size p*I* 4.2 Maximum optical spectrum at 280 nm (E_m = 49,000 M^{-1} cm^{-1}) Broad band centered at 450 nm (E_m = 170 M^{-1} cm^{-1})	[33]
Tetraselmis gracilis	Cadmium promoted the induction of SOD activity Important to prevent oxidative stress such as nutrient and light availability	[29,34]
Bruguiera gymnorrhiza	Full length cDNA encoding a 153-amino-acid sequence of cytosolic Cu/Zn-SOD NaCl treatment the transcript level of cytosolic. Cu/Zn-SOD increased in young and mature leaves rather than in old leaves Expression of the cytosolic Cu/Zn-SOD gene was induced by exogenous abscisic acid	[10]
Platymonas subcordiformis	UV-B radiation decreased the SOD activity in both *P. subcordiformis*	[31]
Avicennia marina	A decrease in mRNA levels was observed for *Sod1* with osmotic stress treatment The transgenic plants were more tolerant to methyl viologen mediated oxidative stress in comparison to the untransformed control plants and also withstood salinity stress	[11,35]
Enteromorpha linza	Molecular mass is near 46 kDa A homodimeric protein ElFe-SOD was inhibited by hydrogen peroxide, insensitive to potassium cyanide The optimal temperature for its maximal enzyme activity was 35 °C	[14]
Sonneratia alba	Molecular mass 25 kDa All iron-binding sites (His 27, His 80, Asp 164 and His 168) of SaFe-SOD were conserved PH stability in the pH range of 3.5–9.5 at 25 °C A thermostable enzyme	[3]

be reconstituted by subsequent treatment with $MnCl_2$. The reconstituted enzyme was found to have regained both manganese and activity [33].

Okamoto et al. studied the effects of cadmium on protein production and the growth of the marine prasinophyte *Tetraselmis gracilis* (Kylin) Butcher. When chronically exposed to sublethal concentrations of cadmium, *T. gracilis* contained high levels of SOD activity, one of the main enzymes of the cells antioxidant defense mechanism. Under these growth conditions, total SOD activity in crude extracts was increased by 41% (at 1.5 ppm) and 107% (at 3.0 ppm). Assays of SOD activity in non-denaturing polyacrylamide gels also showed a similar induction by cadmium. The accumulation of heavy metals along the marine food chain, similar effects to those observed in *T. gracilis* may occur in organisms at higher trophic levels, causing even more severe cellular damage because of the elevated cadmium concentrations [34]. The SOD activity and photosynthetic pigment content along the growth curves of the prasinophycean *T. gracilis* were evaluated in batch-cultures. Total SOD activity was determined by an indirect method involving the inhibition of cytochrome *c* reduction. All three species of microalgae had reduced SOD activity at the end of their growth. *T. gracilis* exhibited a remarkable increase approximately 85% in β-carotene concentration after 10–14 days of growth. Therefore, the antioxidant response during senescence in batch-cultures differs according to the species. Induction of SOD activity may occur either in the early exponential or stationary growth phases, which is important to prevent oxidative stress triggered by a number of factors that affects growth, such as nutrient and light availability. The steady-state balance between the prooxidant conditions and the antioxidant strategies selected by each species in the course of evolution will determine the nature and the magnitude of the antioxidant responses presented during growth [29].

Cytosolic Cu/Zn-SOD is expressed to cope with both osmotic and ion stresses in the cytosol of *Bruguiera gymnorrhiza* leaves. The importance of chloroplastic SODs and other chloroplastic antioxidant enzymes under various environmental stress conditions has been pointed out, as the chloroplast is easily damaged by oxidative stress due to excess electrons in photosystems. One possible mechanism is a leak of active oxygen species from chloroplasts and/or mitochondria [10].

Sod1 cDNA from *Avicennia marina* encoded full-length proteins with 152 amino acids. Sod1t transcript was found to be unaltered in response to NaCl stress. A decrease in mRNA levels was observed for Sod1 with osmotic stress treatment. Sod1 mRNA levels were induced by iron, light stress and by direct H_2O_2 stress treatment, thus confirming their role in oxidative stress response [35]. Southern hybridization of *A. marina* genomic DNA using Sod1, revealed that this gene in *A. marina* genome is present as a single copy. The cDNA was cloned into a binary vector (pCAMBIA1300) and Basmati-1. Southern hybridization analysis of transgenic transformed into indica rice var Pusa rice plants revealed stable integration of the Sod1 transgene in the rice genome. The mRNA transcript of Sod1 was detected by Northern hybridization in the transgenic rice plants. SOD isozyme assay of the transgenic rice plants revealed the stable expression of the transgenic Sod1 protein. The transgenic plants were more tolerant to methyl viologen mediated oxidative stress in comparison to the untransformed control plants. The transgenic plants also withstood salinity stress of 150 mM of NaCl for a period of eight days while the untransformed control plants wilted at the end of the stress treatment in hydroponics. Pot grown transgenic plants could also tolerate salinity stress better than the untransformed control plants, when irrigated with saline water. The transgenic plants also revealed better tolerance to drought stress in comparison to untransformed control plants [11].

The *Enteromorpha linza* SOD (ElSOD) was purified by a four-step protocol consisting of phosphate buffer extraction, ammonium sulphate precipitation, ion exchange chromatography and gel filtration chromatograph. The SDS-PAGE exhibited ElSOD a single band near 23 kDa and the gel filtration study showed ElSODs molecular mass is near 46 kDa in non-denatured condition, indicated it's a homodimeric protein. ElSOD is an iron-cofactored SOD (Fe-SOD) because it was inhibited by hydrogen peroxide, insensitive to potassium cyanide. The optimal temperature for its maximal enzyme activity was 35 °C, and it still had 29.8% relative activity at 0 °C, then they classified ElSOD as a cold-adapted enzyme. ElSOD was stable when temperature was below 40 °C or the pH was within the range of 5–10. The first 11 N-terminal amino acids of ElSOD were ALELKAPPYEL, comparison of its N-terminal sequence with other Fe-SOD N-terminal sequences at the same position suggested it is possibly a chloroplastic Fe-SOD. Other characterizations indicated that ElSOD is a cold-adapted SOD, which showed its potential value in antioxidant utilization [14].

A novel iron SOD (Fe-SOD) gene from *Sonneratia alba* was cloned and then expressed in *E. coli* Rosetta-gami, designated as SaFe-SOD. The DNA sequence of SaFe-SOD contained a 786-bp open reading frame which encodes a 261 aminoacid protein of 30.0 kDa. The 651-bp fragment coding for putative mature SaFe-SOD was amplified and inserted into pET15b for expression. This recombinant SaFe-SOD was subsequently isolated by Ni-trap column protein purification system. The apparent molecular mass of the purified enzyme was 25 kDa on SDS-PAGE. In comparison with Fe-SODs from other plant species, all iron-binding sites, His 27, His 80, Asp

164 and His 168, of SaFe-SOD were conserved. SaFe-SOD was found to have good pH stability in the pH range of 3.5–9.5 at 25 °C after 1 h incubation and was relatively stable and showed 78% activity when incubated in 50 °C for 1 h. Quantitative real-time PCR experiments demonstrated that SaFe-SOD was expressed in leaf, stem, flower, fruit and root tissues with the highest expression in leaf tissues. The SaFe-SOD gene was cloned from *S. alba* and characterized the recombinant SaFe-SOD expressed in *E. coli* cells. This enzyme was of high yield and enzymatically active [3].

5. Superoxide dismutases in marine fungi

Marine fungi are distinct from terrestrial and freshwater fungi in their taxonomy and morphology, as well as in their adaptations to an aquatic habitat. They are an ecological, not a taxonomic group and cannot be defined by nutritional or physiological requirements. Obligate marine fungi are those that grow and sporulate exclusively in a marine or estuarine habitat and are permanently or intermittently submerged. Facultative marine fungi are those that normally occupy freshwater habitats or terrestrial milieus but are able to grow (and possibly to sporulate) in the marine environment [36]. SODs have been recognized in a number of marine fungi and describe their properties (Table 4).

A gene (*dhsod-1*) encoding a Cu-Zn-SOD of the marine yeast *Debaryomyces hansenii* was cloned using mRNA by the RT-PCR technique. The deduced amino-acid sequence shows 27% homology with that of cytosolic SOD from *Saccharomyces cerevisiae* and *Neurospora crassa*, as well as lower homologies (between 55% and 65%) with the corresponding enzyme of other eukaryotic organisms, including human. The gene sequence encodes a protein of 153 amino acids with a calculated molecular mass of 15.92 kDa, in agreement with the observed characteristics of the purified protein from *D. hansenii*. The *dhsod-1* sequence has been deposited in the public data library of the NCBI under Accession Number AFO 16383 [37]. Additionally, the cytosolic form of Cu/Zn-SOD was isolated from the marine yeast *D. hansenii*. This enzyme has a subunit mass of 18 kDa. The preparation was found to be heterogeneous by IF electrophoresis with two p*I* ranges: 5.14–4.0 and 1.6–1.8. The enzyme preparation had a remarkably strong stability at pH 6.0–7.0, surviving boiling for 10 min without losing more than 60% of activity. In sequencing analysis, a peptide obtained by trypsin digestion was found to have 85% identity to the *S. cerevisiae* Cu/Zn-SOD [38]. Cu/Zn-SOD (SODC) is a cytosolic enzyme which catalyses the dismutation of the superoxide radical. The encoding region of this gene (sod1) has been cloned from several strains of marine yeast belonging to the genus Debaryomyces (dvv sod1, dvy sod1 and dh sod1-61) through genomic DNA–PCR amplification and deposited in the NCBI databank under accession nos. AF301019, AF327449 and AF327448, respectively. Fragments of 480–486 nucleotides were obtained, which contain information for products of 153–156 amino acids with calculated molecular masses of 15.8–16.6 kDa. The deduced amino acid sequence shows that *Debaryomyces vanrijiae* enzymes present three additional amino acids not closely related to the active site conformation. In addition, in *D. vanrijiae* var. vanrijiae (strain 020), one histidine residue is apparently replaced by a proline [39]. The activity and expression of SOD was analyzed in a copper-tolerant yeast, *Cryptococcus* sp. N6. Using cell extracts, total proteins in the cell extracts were separated by non-denaturing PAGE and subsequently staining to detect SOD activity. Two distinct bands exhibiting SOD activity appeared on native PAGE: one band, with higher mobility, appeared when the cells were grown without $CuSO_4$, and the other band appeared when the cells were grown with 10 mM $CuSO_4$. Cells grown with 3 mM $CuSO_4$ produced both SOD isoforms. Western blot analysis, using a monoclonal antibody against human SOD-1, showed that SOD protein was expressed in the absence of $CuSO_4$ and that the expression level increased when the cells were grown with 3 or 10 mM $CuSO_4$. The molecular mass of SOD from strain N6 was approx 18 kDa. Treatment of the cells with the protein synthesis inhibitor, cycloheximide at 0.5 μg ml^{-1}, did not affect cell growth in the absence of $CuSO_4$ but significantly inhibited growth in the presence of 10 mM $CuSO_4$ and inhibited expression of SOD protein. This suggests that SOD may play a role in cell growth in the presence of high concentrations of $CuSO_4$. In the presence of the other metal ions tested, only band A appeared. The SOD protein level increased when cells were cultured with Cu^{2+}, Cr^{2+}, Fe^{3+} and Ni^{2+}, and decreased with the addition of Mn^{2+} to the medium [40]. In general the presence of Cu/Zn-SOD in cytosol, as well as Mn-SOD in the mitochondrial matrix of yeast, has been accepted. The absence of Cu/Zn-SOD in a pigmented yeast has been accepted as a general rule. Some authors suggest that the absence of Cu/Zn-SOD in pigmented yeast is complemented by the presence of caroteno proteins that act as an extra mitochondrial antioxidant [41]

Table 4
superoxide dismutases (SODs) from marine fungi sources.

Source	Important properties	Reference
Debaryomyces hansenii	Molecular mass 15.92 kDa. This enzyme has a subunit mass of 18 kDa. The preparation was found to be heterogeneous by IF electrophoresis with two p*I* ranges: 5.14–4.0 and 1.6–1.8 The enzyme preparation had a remarkably strong stability at pH 6.0–7.0, surviving boiling for 10 min without losing more than 60% of activity	[37,38]
Several marine strains of the genus Debaryomyces	Molecular masses of 15.8–16.6 kDa. In *D. vanrijiae* var. vanrijiae (strain 020), one histidine residue is apparently replaced by a proline	[39]
Cryptococcus sp. N6	Molecular mass 18 kDa Treatment $CuSO_4$ inhibited expression of SOD protein Increased when cells were cultured with Cu^{2+}, Cr^{2+}, Fe^{3+} and Ni^{2+} Decreased with the addition of Mn^{2+} to the medium	[40]
Rhodotorula spp. and Udeniomyces spp.	The absence of SODC is not a rule for pigmented yeast Fragments of 485–487 nucleotides were obtained, which contain information for theoretical products of 153–154 amino acids	[41]

6. Superoxide dismutases in marine Animalia

A large amount of all life on earth live in marine or brackish water. The exact fraction is not known, but keep in mind that the oceans constitute 99.7% of the habitable volume on earth. The World Register of Marine Species estimates that there exist 230,000 marine species. More than 200,000 of them are accounted for. Marine invertebrates make up a major portion, including commonly known phyla such as Porifera (sponges), Cnidarians (e.g. jellyfish, anemones and corals), Arthropoda (e.g. crabs, shrimps, lobsters and sea spiders), Mollusca (e.g. sea snails, sea slugs, mussels, scallops and octopuses) and Echinodermata (e.g. sea stars, urchins and sea cucumbers). Marine animals with a chord string, chordata, include sea squirts and vertebrates like agnathans, fish and mammals [42]. SODs have been recognized in a number of marine Animalia and describe their properties (Table 5).

Cu/Zn-SOD from the bathophile teleost *Lampanyctus crocodilus* (LSOD) showed a high degree of homology with the sequence of the enzymes from other teleostean fish species. The apparent molecular mass of LSOD was determined by SDS polyacrylamide gel

Table 5
Superoxide dismutases (SODs) from Animalia marine sources.

Source	Important properties	Reference
Lampanyctus crocodilus	Molecular mass 37.6 kDa p*I* 6.35 A higher thermo stable enzyme	[43]
Xiphias gladius	Higher content of arginine and tyrosine High homology with the other eukaryotic enzymes Low homology with the *Photobacterium leiognuthi* enzyme	[44,45]
Gadus morhua	High sensitivity to DDC Relative thermostability	[46,47]
Prionace glauca	Low isoelectric point The enzyme activity is unusually independent of ionic strength The isolated enzyme has 30% of its copper in the reduced state	[50,51]
Ilisha elongate, Dorosoma nasus, Platycephalus indicus, Tylosurerst rongylurus, Lutjanus fulviflamma, Lutjanus johni, Lethrinus coccineus, Lethrinus kallopterus, Johnius aneus, Johnius caruta, Otolithus ruber, Liza macrolpis, Pampus argenteus, Therapon theraps, Therapon puta, Upeneus tragula, Mulloidicthys auriflamma, Siganus oramin, Siganus javeus, Chorinemus lysan, Caranx kalla, Leiagnathu fsasciatus, Scolopsis phaeops, Gerres flamentosus, Plectorhynchu schotaf, Spilotichthys pictus, Argyrops filamentosus, Acanthopagrus bifasciatus, Acanthopagrus latus, Scatophagus argus, Synaptura orientalis, Cynoglossus arel	The active enzyme is a dimer In the heart electropherogram the number of major SOD bands varies between one and three The SOD zone of activity of all species show an anodic migration The fastest band was observed in *Siganus oramin* and the slowest one in the *Lutjanus johni*, *Caranxkalla*, and *Acanthopagrus bifasciatus*	[52]
Paralichthys olivaceus	The Cu/Zn-SOD has four same suhunits of 16 kDa The molecular mass of the native SOD is 65 kDa The dominant amino acids of the SOD were Ser, Thr, Pro and Glu A thermostable enzyme p*I* about 6.3	[4,53,54]
Scrobicularia plana, Cerastodemza edule, Mya arenaria	In hemoglobin containing species, elevated SOD activity in body fluids and in well-perfused tissues is triggered by the hemoglobin content	[43]
Uca longisignalis, Clibanarius vittatus, Penaeus setiferus, Ocypode quadrata, Menippe mercenaria, Homarus americanus, Crassostrea Virginica, Urosalpinx cinera, Fasciolaria tulipa, Loliguncula brevis, Octopus vulgaris	Cu/Zn-SOD is sensitive toward cyanide (CN) and hydrogen peroxide (H_2O_2) The cytosolic SOD is a homodimeric protein, which exists in a monomer–dimer equilibrium (24–48 kDa) Contains approximately 1 Mn per subunit	[18]
Callinectes sapidus	Insensitive to CN/H_2O_2 The latter activity was not inhibited by CN or H_2O_2, indicating the absence of the Cu/Zn-SOD enzyme cytMn-SOD activity in the hepatopancreas changes during the molting cycle of the crab	[18,56,57]
Sparus aurata	Insensitive to malondialdehyde (MDA) or HNE Not significantly modified in light mitochondrial (LMF) fractions by any treatment Significant increases were observed in the cytosolic fraction (CF) Mn-SOD insensitive to cyanide	[58]
Mytilus edulis	Two main bands of Cu/Zn-SOD were obtained at p*I* 4.7 and 4.6	[59]
Crassostrea gigas	The purified protein appears as a single band with an apparent molecular mass of 20 kDa. The *Cg*-EcSOD is a monomer with intramolecular disulfide bonds The *Cg*-EcSOD consists of 174 amino acids	[2]
Litopenaeus vannamei	The coding sequence predicts a 287 residues protein A unique 61 amino acids extension at the N-terminus and lacking a mitochondrial-targeting sequence Molecular mass of cMn-SOD is 24.8 kDa p*I* 6.04	[60]
Haliotis discus discus	The open reading frame sequences of Cu/Zn- and Mn-SODs encoded 154 and 226 amino acids, respectively The mRNA levels of both SODs were increased in general during the metal or thermal treatments	[61]
Cliona celata	The value of SOD activity 163.4 U/g total protein in wet tissues	[62]
Pinctada fucata	The pfSOD mRNA was abundantly expressed in haemocytes and gill After challenge with lipopolysaccharide (LPS), expression of pfSOD mRNA in haemocytes was increased	[5]
Alvinella pompejana	ApMn-SOD1 a high thermostable enzyme. Their half-lives were similarly long at 65 °C (<110 min) but exhibited a twofold difference at 80 °C (20.8 vs 9.8 min)	[63]
Apostichopus japonicus	Increase levels of *Sod* mRNA by thermal and osmotic stresses	[64]

electrophoresis in reducing conditions; the subunit molecular mass was 18.8 kDa value that is remarkably higher than the one reported for other Cu/Zn-SODs. The isoelectric point, assessed by isoelectric focusing, is 6.35. The catalytic properties of LSOD are very similar to those of the bovine enzyme, albeit with higher sensitivity to thermal denaturation. The apparent molecular mass of LSOD (37.6 kDa) is higher than the other Cu/Zn-SOD variants studied. The amino acid sequence of LSOD reveals interesting substitutions compared to the bovine enzyme [43]. Cu/Zn-SODs was also isolated from swordfish (*Xiphias gladius* L.) liver. The SOD has a higher content of arginine and tyrosine than the corresponding bovine enzyme and appears to dissociate more readily into subunits [44]. Comparison of the amino acid sequence of swordfish liver Cu/Zn-SOD with the bovine, human, horse, yeast and *P. leiognathi* indicates that the swordfish enzyme has a high homology with the other eukaryotic enzymes. Low homology is, however, observed with the *P. leiognathi* enzyme [45].

The activities of SOD in different species of fish are measured: (saithe (*Pollachius virens*), mackerel (*Scomber scombrus*), cod (*Gadus morhua*), capelin (*Mallotus villosus*), rainbow trout (*Salmo irideus*),

sprat (*Sprattus sprattus*), Norway pout (*Boreogadus esmarkii*), blue whiting (*Micromesistius poutassou*), great silver smelt (*Argentina silus*) and roe and milt from mackerel). SOD when measured in whole fish, a fivefold variation between the lowest (blue whiting) and the highest value (rainbow trout). Except for the high values determined in mackerel and rainbow trout, the activities were fairly constant. The content of Mn-SOD varied between 15% and 50% of the total, except for rainbow trout in which about 94% of total enzyme activity was found when measured in the presence of sodium cyanide. The reason for this very high mitochondrial activity is unclear. The enzyme activities in different tissues in saithe and mackerel are measured. The localization of SOD in saithe differ from that reported in carp. While carp had a very high activity in the liver compared with other tissues, saithe had the highest activities in heart, spleen and roe. These results give more evidence to the postulation that the red muscle in fish fulfills a metabolic role which differs from that performed by this tissue in higher vertebrates [46,47].

The SODs were measured in the rete mirabile and gas gland epithelium area of the swim bladder of the toadfish *Opsanus tau*. When the concentration of enzyme in the swim bladder was compared with the concentration in other organs (kidney, heart, gills) of the same fish, the swim bladder was found to have the highest concentration of SOD. Therefore, SOD may play an important role in the protection of swim bladder tissues against high concentrations of oxygen [48].

Rete mirabile and gas gland epithelium from the swim bladders of six species of marine fishes (*Tautoga onitis*, *Stenotomus chrysops*, *Anguilla rostrata*, *Prionotus carolinus, Centropristes striatus*) were assayed for SOD activity. Correlation of the results of these assays with measurements of the concentration of oxygen in the lumen of the normal steady state swim bladders revealed that swim bladders in species containing higher levels of oxygen also exhibited higher levels of SOD activity in the rete mirabile/gas gland epithelium region [49].

A Cu/Zn-SOD was purified for the first time from an elasmobranch species (*Prionace glauca*) and showed the following differences with respect to other animal SOD. Three bands were obtained when the purified enzyme was subjected to polyacrylamide gel electrophoresis, both on protein and activity staining. The enzyme displays a low isoelectric point. Isoelectric point values of 4.8, 4.6, 4.5 respectively, were obtained for the three components. The amino acid composition was determined on the mixture of the three forms. It is apparent that the absence of Trp is in accordance with the UV spectrum of the guanidine-treated enzyme. The enzyme activity is unusually independent of ionic strength. The isolated enzyme has 30% of its copper in the reduced state [50]. Calabrese et al. determined the complete amino acid sequence for the Cu/Zn-SOD from the shark *P. glauca*. The active site region showed the substitution of an Arg for Lys at position 134, which is important for electrostatic facilitation of the diffusion of 4 to the catalytically active copper. This change may be related to observed alterations of electrostatic parameters of the enzyme (p*K* of the pH dependence of the enzyme activity, rate of inactivation by H_2O_2), although it preserves a high efficiency of dismutation at neutral pH [51].

Al Hassan studied the tissue extracts of heart, kidney, gills and eye lens were electrophoretically examined for SOD activity in 32 species of teleostean fish (*Ilisha elongate, Dorosoma nasus, Platycephalus indicus, Tylosurerst rongylurus, Lutjanus fulviflamma, Lutjanus johni, Lethrinus coccineus, Lethrinus kallopterus, Johnius aneus, Johnius caruta, Otolithus ruber, Liza macrolpis, Pampus argenteus, Therapon theraps, Therapon puta, Upeneus tragula, Mulloidicthys auriflamma*, *Siganus oramin, Siganus javeus*, *Chorinemus lysan*, *Caranx kalla*, *Leiagnathu fsasciatus, Scolopsis phaeops, Gerres flamentosus, Plectorhynchu schotaf, Spilotichthys pictus, Argyrops filamentosus, Acanthopagrus bifasciatus, Acanthopagrus latus, Scatophagus argus, Synaptura orientalis, Cynoglossus arel*). SOD activity was found in all tissues investigated. The heart, kidney, gill and eye lens extracts from all fish species studied revealed three types of SOD. The SOD phenotypes could be explained by a two-allele hypothesis; individuals with single-band phenotypes are presumably homozygous for allelic genes and the three-banded phenotypes reflect heterozygous fish. The three-banded heterozygous phenotypes suggest that the active enzyme is a dimer. In the heart electropherogram the number of major SOD bands varies between one and three. Some variations in the mobility of SOD isozymes between the species were observed. Except for *L. kallopterus*, the SOD zone of activity of all species shows an anodic migration. The fastest band was observed in *S. oramin* and the slowest one in the *L. johni*, *C. kalla*, and *A. bifasciatus* [52].

A unique Cu/Zn-SOD was found and isolated from plaice *Paralichthys olivaceus* skin. Surprisingly, the properties of purified fish skin SOD were very different from those of SOD from other sources reported so far. The purified SOD was composed of four same subunits of 16 kDa and the molecular mass of the native SOD was found to be around 65 kDa. The dominant amino acids of the SOD were Ser, Thr, Pro and Glu. Above 70 °C, thermostability of the SOD was much lower than that of bovine erythrocyte Cu/Zn-SOD. It also was observed that SOD-Fs had p*I* about 6.3 on isoelectrofocusing gel [53]. Cu/Zn-SOD has been purified to homogeneity from Japanese flounder *P. olivaceus* hepato-pancreas. The purified enzyme gave a single protein band with molecular mass of 17.8 kDa under reducing conditions, and showed approximately equal proportions of 17.8 and 36 kDa molecular mass under non-reducing conditions. Three bands were obtained when the purified enzyme was subjected to native-PAGE, both on protein and activity staining, but the electrophoretic mobility of the purified enzyme differed from that of bovine erythrocyte Cu/Zn-SOD. Isoelectric point values of 5.9, 6.0 and 6.2, respectively, were obtained for the three components. The N-terminal amino acid sequence of the purified enzyme was determined for 25 amino acid residues, and the sequence was compared with other Cu/Zn-SODs. The N-terminal alanine residue was unacetylated, as in the case of swordfish SOD. Above 60 °C, the thermostability of the enzyme was much lower than that of bovine Cu/Zn-SOD. The activity of Japanese flounder SOD remained at a constant level above pH 3, but was reduced dramatically to approximately 10% of the standard reaction at pH 2. When the enzymes were pre-incubated for 1 h at different temperatures from 4 to 70 °C, the activities of Japanese flounder Cu/Zn-SOD remained constant at temperature below 50 °C. On the other hand, a sudden decline in activity of the Japanese flounder SOD occurred above 60 °C. Heated at 70 °C, Japanese flounder SOD was completely inactivated [54]. Additionally, Mn-SOD from *P. olivaceus* hepatopancreas has been purified with high purification and recovery. The molecular mass of the purified enzyme was estimated to be 26 kDa by SDS-PAGE under reducing conditions. In addition, the electrophoretic mobility of this enzyme was observed to be faster than that of Japanese flounder Cu/Zn-SOD. On the other hand, the N-terminal amino acid sequence of this Mn-SOD was determined to be 16 amino acid residues, and the sequence showed high homology to other Mn-SODs but not Japanese flounder Cu/Zn-SOD. Analysis of nucleotide and deduced amino acid sequences revealed that the Mn-SOD cDNA consisted of a 64 bp 5′-non-coding region, a 675 bp open reading frame encoding 225 amino acids, and a 465 bp 3′-non-coding region. The first 27 amino acids containing a mitochondria-targeting signal were highly conserved among other Mn-SODs [4].

Comparing SOD levels and types in marine benthic invertebrates it appears, that high SOD activities are often connected to the presence of intra- and extracellular respiratory pigments. The reason for this could be the tendency of these pigments to form reactive oxygen species via autoxidation. These radicals need to be removed

quickly, even at the expense of H_2O_2 formation, and SOD activity in the body fluids would be one possible way of achieving this. This means, that in hemoglobin containing species, elevated SOD activity in body fluids and in well-perfused tissues is triggered by the hemoglobin content. Sulphide exposure does not affect total SOD activity in tissues, but could lead to an increase of sulphide-insensitive Mn-SOD activity [55].

Brouwer et al. studied the *Decapod Crustacea* include Blue crabs (*Callinectes sapidus*), gulf mud fiddler crabs (*Ucalongisignalis*), striped-legged hermit crabs (*Clibanarius Vittatus*), white shrimp (*Penaeus setiferus*), ghost crabs (*Ocypode quadrata*), stone crabs (*Menippe mercenaria*) and American lobsters (*Homarus americanus*). To avoid potential effects of the molt cycle, all animals collected were in the intermolt stage. The Mollusks include the common oyster (*Crassostrea Virginica*) [class Bivalvia], the oyster drill (*Urosalpinx cinera*), and the tulip shell (*Fasciolaria tulipa)* [class Gastropoda], the brief squid (*Loliguncula brevis*), and the common octopus (*Octopus vulgaris*) [class Cephalopoda]. These studies showed that the paradigm that all oxygen-respiring eukaryotes have a cytosolic Cu/Zn-SOD and that Mn-SOD occurs exclusively in the mitochondria does not apply to a large group of marine arthropods. These organisms use two distinct forms of Mn-SOD, one in the cytosol and the second in the mitochondria, in defense against potentially toxic superoxide. The functional and regulatory properties of this novel system, and its evolutionary origin, are under investigation [18].

Blue crab) *C. sapidus*) have a novel cytosolic form of Mn-SOD, which is different from the mitochondrial enzyme in that it has an abnormal mitochondrial-targeting sequence. The lack of Cu/Zn-SOD is common to all marine decapod crustaceans (crabs, shrimp, hermit crabs, and lobsters). The paradigm that all oxygen- respiring eukaryotes have cytosolic Cu/Zn-SOD and that Mn-SOD is localized to the mitochondria appears not to apply to a large group of marine arthropods [20]. Assays for malondialdehyde, a common product of lipid peroxidation, indicated that the defense mechanisms induced by copper exposure were not entirely sufficient to prevent oxidative damage to cellular membranes. It seems therefore that copper chelation and increased antioxidant defenses are effective, in view of the large amounts of copper accumulated in the cells, in limiting oxidative tissue damage. The molecular mechanisms that play a role in protection against copper toxicity and copper-induced oxidative stress appear to be similar among organisms as diverse as yeast, crustacea, and mammals [56]. Demonstrated that crabs and other decapod crustaceans that are dependent on copper (haemocyanin) for oxygen transport have developed unique features of copper metabolism. Whereas most animals, and aerobic eukaryotes in general, have a cytosolic copper-dependent form of SOD, crabs have a manganese-dependent enzyme. Molecular phylogeny analysis suggested the Mn-SOD gene duplication is as old as the origin of the arthropod phylum. cytMn-SOD activity in the hepatopancreas changes during the molting cycle of the crab [57].

New active SOD isoforms in fish (*Sparus aurata*) were detected by isoelectrofocusing in the light mitochondrial (LMF) and cytosolic (CF) fractions. extracts with malondialdehyde (MDA) to clarify the effects of aldehydes, Cu/Zn-SOD and Mn-SOD isoforms were purified and amino acid analysis was carried out. The new bands found in LMF and CF fractions were reproduced in vitro after incubation of pure SODs with malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE), the new SOD bands formed being coincident with the loss of Lys or His residues. Lysine residues were preferentially derivatized after treatment of Cu/Zn-SOD with MDA, but in Mn-SOD the lysine residues were modified only after treatment with MDA, while the histidine residues were modified only by HNE. No change of SOD activity was detected after MDA or HNE exposure, although at the higher aldehyde concentrations used protein aggregates were formed. Therefore, the appearance of new active SOD bands, after isoelectrofocusing separation, can be proposed as a biomarker of oxidative stress. Most of the new SOD bands could be reproduced in vitro by incubation of fish liver cell-free [58].

Manduzio et al. reported the characterization of three isoforms of Cu/Zn-SOD in the blue mussel *Mytilus edulis* and they showed that one of these isoforms is strongly inducible. Cytosolic extracts of digestive gland and gills from adult blue mussels were analyzed by polyacrylamide gel electrophoresis or isoelectric focusing followed by in situ staining for SOD activity. Two main bands of Cu/Zn-SOD were obtained at p*I* 4.7 and 4.6 corresponded to native apparent molecular mass values of 205 and 155 kDa. Blue mussels from chemically contaminated area in Le Havre harbor exhibited a third Cu/Zn-SOD isoform characterized by a more acidic isoelectric point (p*I* 4.55) and a native apparent molecular mass of 130 kDa. When maintained in clean marine water, mussels from this area showed a transitory decrease in total SOD activity accompanied by the disappearance of the SOD-3 band. Conversely, the exposure (4 and 8 h, and 3 and 7 days) of control blue mussels to copper ($25\ mg\ l^{-1}$) markedly increased SOD-3 band while the total SOD activity did not systematically change. Taken together their results suggested that the variations of SOD expression pattern in *M. edulis* could be used as a tool for the marine environment monitoring [59].

Gonzalez et al. have characterized in the oyster *Crassostrea gigas* an extracellular SOD (*Cg*-EcSOD) which appears to bind lipopolysaccharides (LPS). This was the first evidence of a SOD which appear to display LPS-binding property. They purified the protein from the oyster plasma and identified as a Cu/Zn-SOD according to its N-terminal sequencing and biological activity. LPS recognition mechanisms can be provided by several actors which can interplay such as plasma LBP-binding protein (LBP), membrane bound or soluble forms of CD14 and integrins as they observed in their experiments the formation of aggregation with the purified *Cg*-EcSOD, they can assume. *Cg*-EcSOD expression and synthesis are restricted to hemocytes as revealed by in situ hybridization and immunocytochemistry. *Cg*-EcSOD-expressing hemocytes were seen in blood circulation, in connective tissues, and closely associated to endothelium blood vessels. *Cg*-EcSOD presents in its amino acid sequence a LPS-binding motif found in the endotoxin receptor CD14 and they showed that the protein displays an affinity to *E. coli* bacteria and with LPS and Lipid A. Oyster SOD consists of 174 amino acids and its sequence appears to be significantly similar to the proteins from the extracellular SOD family while it shows lower identity with the oyster cytosolic SOD (14% identity). The oyster SOD they have isolated shares 20% amino acid sequence identity with the extracellular human and nematode SODs and it was consequently named *Cg*-EcSOD. *Cg*-EcSOD is also very similar (94% identity) to previously described oyster sequences named cavortin isolated from *C. gigas* oyster from a New Zealand farm (AY256853) but which would be an SOD [2].

Manganese containing SOD is normally a nuclear-encoded mitochondrial enzyme in eukaryotic organisms; however, a cytoplasmic manganese SOD (cMn-SOD) was found in crustaceans that use hemocyanin as oxygen carrier. The complete cDNA and deduced amino acid sequence of a cMn-SOD from *Litopenaeus vannamei* were determined. The coding sequence predicts a 287 residues protein with a unique 61 amino acids extension at the N-terminus and lacking a mitochondrial-targeting sequence. Phylogenetic analysis clusters cMn-SODs and mitochondrial Mn-SODs in two separate groups. cMn-SOD transcripts were detected in hemocytes, heart, hepatopancreas, intestine, nervous system, muscle, pleopods and gills. Since hemocytes are key defense cells and their reactions produce superoxide radicals, the infection by white spot syndrome virus on the cMn-SOD transcript levels were investigated and found to increase transiently 1 h post-infection and then decrease as the viral infection progressed to levels significantly lower than uninfected controls by 12 h post-infection [60].

Complementary DNAs encoding Cu/Zn-SOD (SOD1) and Mn-SOD (SOD2) were isolated from disk abalone, *Haliotis discus discus*. The open reading frame sequences of Cu/Zn- and Mn-SODs encoded 154 and 226 amino acids, respectively. Multiple sequence alignments using the deduced amino acid sequences revealed that both abalone SODs showed considerable sequence similarities with their orthologues from diverse aerobic organisms, in which the amino acid residues forming metal ligands were highly conserved. All phylogenetic trees for both SOD genes inferred from maximum likelihood and Bayesian inference analyses presented the monophyletic status of Teleostei and Aves/Tetrapoda clades, and recovered relatively close genetic affiliation of *H. discus discus* with some molluscan species. Expression of both SODs at mRNA levels was highly modulated in various tissues (gill, muscle and hepatopancreas from juveniles, and haemocytes from adults) by experimental exposures to copper, zinc and cadmium and also by thermal treatments. The mRNA levels of both SODs were increased in general during the metal or thermal treatments; however, the transcriptional responses of SOD genes were quite variable depending upon isoforms and tissues based on semi-quantitative and/or real-time RT-PCR assays. Moreover, the availability of sequences for both isoforms will make it possible to visualize the specific response of each SOD isoform to a given stress factor at a particular time in Haliotidae species [61].

The SOD in the cosmopolitan sponge *Cliona celata* has been described as a useful biomarker for marine pollution in other marine invertebrates. The quantification of the catalytic activity for SOD is quite complex because its substrate is an unstable free radical. Statistical treatment of the data indicates that the reference value for the specific SOD activity in *C. celata* should be in the interval [0–535.5] U/mg of total protein in wet tissues, for normal populations [62].

Anju et al. reported the cloning of a Cu/Zn-SOD (designated as pfSOD) from the pearl oyster (*Pinctada fucata*) using rapid amplification of cDNA ends (RACE) PCR. The full-length cDNA of this Cu/Zn-SOD contains an open reading frame (ORF) of 471 bp coding for 156 amino acids. No signal peptide was identified at the N-terminal amino acid sequence of Cu/Zn-SOD indicating that this pfSOD encodes a cytoplasmic Cu/Zn-SOD. This is supported by the presence of conserved amino acids required for binding copper and zinc. Semi-quantitative analysis in adult tissues showed that the pfSOD mRNA was abundantly expressed in haemocytes and gill and scarcely expressed in other tissues tested. After challenge with lipopolysaccharide (LPS), expression of pfSOD mRNA in haemocytes was increased, reaching the highest level at 8 h, then dropping to basal levels at 36 h. These results suggest that Cu/Zn-SOD might be used as a bioindicator of the aquatic environmental pollution and cellular stress in pearl oyster [5].

Alvinella pompejana (Polychaeta, Alvinellidae) is one of the most thermotolerant marine eukaryotes known to date. It inhabits chimney walls of deep-sea hydrothermal vents along the East Pacific Rise (EPR) and is exposed to various challenging conditions (e.g. high temperature, hypoxia and the presence of sulphides, heavy metals and radiations), which increase the production of dangerous reactive oxygen species (ROS). Two different allelic forms of a Mn-SOD involved in ROS detoxification, ApMn-SOD1 and ApMn-SOD2, and differing only by two substitutions (M110L and A138G) were identified in an *A. pompejana* cDNA library. RFLP screening of 60 individuals from different localities along the EPR showed that ApMn-SOD2 was rare (2%) and only found in the heterozygous state. Dynamic light scattering measurements and residual enzymatic activity experiments showed that the most frequent form (ApMn-SOD1) was the most resistant to temperature. Their half-lives were similarly long at 65 °C (<110 min) but exhibited a twofold difference at 80 °C (20.8 vs 9.8 min). Those properties are likely to be explained by the occurrence of an additional sulphur-containing hydrogen bond involving the M110 residue and the effect of the A138 residue on the backbone entropy [63].

The combined effects of acute temperature and salinity on expressions of SOD mRNA were investigated in the sea cucumber *Apostichopus japonicas* Selenka. There were 12 treatments (combinations of temperature at 16, 20, 24 and 28 °C and salinity at 22, 27 and 32 ppt). In low salinity environments, the cellular level stress was indicated by SOD mRNA, and the maximal expression of all genes occurred at 6 h after stresses. The up-regulation of SOD mRNA indicated the emergence of protein denaturation and oxidative damage and also suggested an increase in energy consumption at high temperature and low salinity [64].

7. Importance and applications

Antioxidant enzymes are emerging as a new addition to the pool of industrial enzymes and are surpassing all other enzymes in terms of the volume of research and production [1]. The antioxidative defense system includes enzymatic (such as SOD) and non-enzymatic components (such as glutathione, flavonoids, urate, ascorbate, tocopherols, carotenoids, ubiquinol, and minerals) can also be used for this purpose. The non-enzymatic antioxidants are generally administered as dietary sources, as it is the cheapest route. The dietary antioxidant bioavailability is dependent on a number of factors like food processing, food deprivation, stability of the antioxidant, stabilizing effect of food matrix to restrain the release of lipophilic antioxidants, the isomeric form present in it especially in case of carotenoids and the conjugated form in which it is present apart from the physicochemical and biopharmaceutical properties of the active agent. [65]. SOD is among the most potent antioxidants known in the nature. Therefore, administration of SOD can afford more effective protection against acute massive oxidative insults. SOD supplementation has been shown to prevent or reverse the adverse effect in several of the above conditions in a number of therapeutic clinical trials carried out in human subjects [66]. Since SOD catalyzes production of H_2O_2, which is also a powerful oxidant, some of the studies used SOD in combination with H_2O_2 metabolizing antioxidants such as catalase. Mutations in *sod* gene, its deficiency, and certain SOD genotypes are proposed to directly correlate with several disorders such as amyotrophic lateral sclerosis [67]. Thaler et al. proposed this to be the reason why vegetarians show lower rates of several types of cancer and chronic cardiovascular diseases as compared with omnivores [68].

Petersen et al. discovered that SOD binds directly to collagen, which it protects from oxidation. They noted that SOD significantly protects type I collagen from oxidative breakdown. Furthermore, they noted this interaction may play an essential physiological role in preventing fragmentation of collagen during oxidative stress [69]. Although SOD's benefits go beyond the mere neutralization of superoxide anions, the threat of exposure to superoxide should not be underestimated. Superoxide anions are strongly implicated in the development of numerous degenerative diseases, including atherosclerosis, stroke, heart attack, chronic and acute inflammatory conditions, and various other age-related disorders [70]. SOD may be an effective antioxidant therapy for managing the detrimental consequences of inflammatory diseases, as well as for mitigating other conditions associated with uncontrolled overproduction of superoxide [71]. The SOD prompted immune cells (macrophages) to release the anti-inflammatory cytokine interleukin-10 rather than inflammatory tumor necrosis factor, which the cells may release under conditions of oxidative stress. Subsequent studies of live animals showed that SOD levels increased when SOD/gliadin was administered orally [72]. The Japanese researchers noted that orally active SOD prevented tumor progression promoted by inflammation, and that it may have

Table 6
Applications of SOD.

Applications	Reference
Treatment of inflammation in animals	[86,93]
Clinical purposes and specific applications in the food industry systems	[3,58,66,67,70,85,87,93,94]
Preservation of perishables like vegetables	[88]
Preservation of biologicals, like organs for transplantation and sperms	[89,90]
Reduction in tobacco and alcohol-induced damage	[91]
Biosensor for O_2^-	[92]
Cosmetics for the protection of the skin	[3,30]
Prevent alcohol-induced hangover	[95]
Removal of Amadori and Maillard products	[96]
Mutagenicity testing	[97,98]

elicited these effects by scavenging the inflammatory superoxide anion [73]. Elevated SOD activity can be therapeutically useful by protecting against oxidative stress-induced neurotoxicity. Acutely increased extracellular-SOD (EC-SOD) activity protects against neurobehavioral impairment caused by acute ischemia. Chronically increased EC-SOD activity may also be therapeutically useful by protecting against chronic oxidative stress-induced neurobehavioral damage that accumulates during the aging process. Novel EC-SOD mimetics may be useful in attenuating aging-induced cognitive impairments and other aspects of physiological decline with aging [74]. And evidence suggests that boosting falling SOD levels may help guard against disease and extend life span [75]. Low SOD levels in humans have also been associated with a host of degenerative diseases, including fibromyalgia [76], diabetes [77], cancer [78], multiple sclerosis [79], Alzheimer's and Parkinson's disease [80]. Increased level of SOD in plants, can protect the host against physical and chemical stress, and improve the biomass production with larger shoot, crown, and root systems [81–83]. The importance of oxidative stress in microorganisms is reflected in fermentation industries where strong approaches are followed to increase the O_2 flux into fermentation medium, resulting in oxidative cell damage and poor productivity. Similarly, ROS-generating chemicals are used as food preservatives to prevent microbial growth [84]. In pathogens, SOD performs a very important role in the dismutation of extracellular ROS derived from oxidative burst of phagocytes (Table 6) [85].

8. Conclusions

The enormous biodiversity in the marine ecosystems provides a huge natural reservoir for novel and useful biocatalysts [99,100]. This article has provided many examples of SOD from marine sources which are being used as successful biocatalysts. Their use highlights a thriving and exciting area of industrial biotechnology.

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