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Industrial Production of Superoxide Dismutase (SOD): A Mini Review

Rajesh Kanna Gopal[1] **and Sanniyasi Elumalai**[2*]

[1]*Department of Plant Biology and Plant Biotechnology, Presidency College (Autonomous), Chennai, India*

[2]*Department of Biotechnology, University of Madras, Chennai, India*

***Corresponding author:** Sanniyasi Elumalai, Department of Biotechnology, University of Madras, Chennai-600 025, TN, India, Tel: 9710116385; E-mail: dalbergia17@gmail.com

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Abstract

The use of chemical advances to mechanical research, improvement, and assembling has turned into a critical field. Since the creation of rough rennet in 1874, a few catalysts have been marketed, and utilized for restorative, supplementary, and different applications. Late headways in biotechnology now enable organizations to create more secure and more affordable chemicals with upgraded intensity and specificity. Cancer prevention agent catalysts are developing as another expansion to the pool of modern chemicals and are outperforming every single other compound as far as the volume of research and creation. In the 1990s, a cell reinforcement chemical-superoxide dismutase (SOD) was brought into the market. In spite of the fact that the catalyst at first demonstrated extraordinary guarantee in restorative applications, it didn't perform up to desires. Therefore, its utilization was restricted to non-tranquilize applications in people and medication applications in creatures. This survey compresses the ascent and fall of SOD at the mechanical level, the purposes behind this, and potential future push territories that should be tended to. The audit likewise concentrates on other modernly significant parts of SOD, for example, mechanical significance, catalyst designing, generation procedures, and process streamlining and scale-up.

Keywords: Antioxidant enzyme; Biotechnology; Superoxide dismutase; Photosynthesis

Introduction

Oxygen evolving photosynthetic organism especially blue-green algae (Cyanobacteria) has dramatically changed the reducing Earth's atmosphere [1] into more oxidant by photolysis of water between 3.2 and 2.4 billion years ago [2]. Which was followed by the build-up and development of aerobic organisms thereby started consuming oxygen (O_2) as a strong electron acceptor. As we know that O_2 is a strong electron acceptor, it may cause severe damaging effects to the own cells by destabilizing its own metabolism. This happens in live cells due to the generation of reactive oxygen species (ROS) during both the photosynthesis and respiration. The reactive oxygen species are uncontrollably synthesized as an intermediates during O_2 reduction ($1/2O_2$) or oxidation (O_2-). The most potent and highly ROS are singlet oxygen ($1/2O_2$), superoxide anion (O_2-), hydrogen peroxide (H_2O_2) and the hydroxyl free radical (OH-). The superoxide anion (O_2-) and hydroxyl free radical (OH-) are the most reactive ROS with the biomolecules including protein, lipid and nucleic acids due to the occurrence of unpaired electrons.

The hydroxyl free radical is one of the intermediate, generated due to the reaction of O_2- with the Fe-S clusters and releases an iron molecule during Fenton reaction [3]. The superoxide anion (O_2-) is negatively charged and thus cannot be diffused through membranes and oxidizes $[4Fe\text{-}4S]_2$+in to [3Fe-S] 1+ by releasing iron (Fe_2+). And thus, Fe_2+reacts with H_2O_2 and resulted in the generation of hydroxyl free radical (OH-).

$$Fe^{2+}+H_2O_2 \rightarrow OH^-+FeO_2^{+} + H+ \rightarrow Fe^{3+}+OH^-+OH$$

The hydroxyl free radical (OH-) can cause highly damaging effects on DNA neither superoxide anion (O_2-) nor hydrogen peroxide (H_2O_2) but they are the precursors for the proliferative generation of hydroxyl free radicals through Fenton reaction [3].

To overcome this problem, the living organisms had developed various defence mechanisms to shield themselves against the causing damages by ROS [3]. The Prokaryotic and Eukaryotic organisms have developed various defence mechanisms throughout the course of evolution and are enzymatic and non-enzymatic. Catalases, Superoxide dismutase (SOD) and Peroxidases are enzymatic whether glutathione, carotenoids, vitamins A, C and E etc. are non-enzymatic. The enzymatic mechanism is the primary antioxidant defence evolved within the organism but the non-enzymatic are secondary antioxidant defence consumed as intake from food.

Superoxide Dismutase (SOD)

As the name revels that the enzyme SOD (EC 1.15.1.1) performs the dismutation reaction on the superoxide anions (O_2-) generated during the metabolic activities of cells [4]. Generally, the SOD enzyme converts two molecules of superoxide anions (O_2-) into hydrogen peroxide (H_2O_2) and one molecule of oxygen (O_2). Followed by the conversion of H_2O_2 into water molecule by other enzymes like catalase and peroxidases.

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

Superoxide dismutase is a primary antioxidant enzyme and ubiquitous in nature present in all kinds of living organisms from Prokaryote to Eukaryote. Different forms of SODs are found and are distinguished based on the metal cofactors present at the active site of the enzyme. Copper and Zinc SOD (Cu/Zn-SOD), Nickel SOD (Ni-SOD), Manganese SOD (Mn-SOD) and Iron SOD (Fe-SOD). The

Cu/Zn-SOD reported to occur in the bacterial periplasm, cytoplasm and chloroplast of photosynthetic plants. Superoxide anions (O_2-) are impermeable through membrane and thus the Cu/Zn (sod1) SOD is dispersed in diverse locations including nucleus, lysosome, peroxisome and cytosol to detoxify O_2-. Extracellular form of Cu/Zn SOD (sod3) is also reported in animals. The SOD3 is different from SOD1 whereas the latter is a homodimer and the former is a homotetramer. The Fe SOD and Mn SOD are found in the Prokaryotes, but the former found in the chloroplast and the latter found in the mitochondria of higher organisms. For the last forty years, approximately 30,000 research articles have been published on the SOD and about 180 patents have been applied on the applications of SOD.

Pharmaceutical applications of SOD

The non-enzymatic antioxidants are the secondary and dietary antioxidants but the availability of such antioxidants is being reduced based on their properties like stability, solubility, isomers, processing with food, biotransformation in the gastrointestinal tract [5]. The induction of oxidative stress due to the generation of ROS leads to the cause of various diseases including cancer, asthma, diabetes, arthritis, atherosclerosis, aging, infertility, neurological disorders, ischemia-reperfusion injury, transplant rejection, autoimmune diseases, rheumatoid arthritis, septic shock-induced tissue injury [6]. Various clinical trials have been carried out in humans to hamper and control the ROS by supplementation of SOD and thereby to overcome such diseases. The proliferation of amyotrophic lateral sclerosis (ALS) have been reported due to the mutation in sod gene or its deficiency in human [7]. The expression fold of SOD can be optimized epigenetically in diet, which suppressed the CpG methylation in promoter and thus induced the expression fold of Mn SOD in the vegetarian group when compared with the omnivorous group. Minimal rates of chronic cardiovascular diseases and different types of cancer were reported in vegetarians than the omnivores [8]. Relatively, elevated levels of oxidative stress and suppressed levels of SOD were recorded in people smokes cigarette and drink alcohol [9]. The supplementation of SOD lowering the lactic acid content and hampering fatigue of diaphragm, protection against UV rays, prevent graying of hair, proliferates the growth of hair, heals wounds, reduce wrinkles on face, depigmentation in athletes [10].

Industrial applications of SOD

SOD formulation is also used along with the production of tobacco based products to minimize the free radical damage occurs in respiratory tract [11] and which can be reduce the hangover after consumption of alcohol [12]. In former days the SOD was a product from liver and serum of animals (pig, horse, bull and dog etc.) for biochemical purpose. Nowadays, it was also derived from plant sources (seeds, vegetables, cereals and fruits) but due to the minimal amount and high cost extraction methods it was not feasible for commercialization. Thus, the microbial sources were chosen which could be feasible for induction of SOD subjected to ROS stress and the large scale production of SOD. The proliferation of SOD was induced by increase in the air pressure from 1 to 6 bar in a batch cultivation of Yarrowia lipolytica [13].

Hence, the SOD plays an important role in pathogens to scavenge the extracellular ROS derived from the host defence mechanism. And due to this activity, many research works have been carried out as a target for drugs to protect the host against the pathogens like Plasmodium falciparum [14], Brucella abortus, Schistosoma mansoni [15] and antigenic agent in serodiagnosis [16]. It has shown to increase the efficiency of penetration through skin from topical creams, when fused with HIV-1 tat protein transduction domain or lysine-rich peptide [17]. The SOD in combination with chaperone proteins safe guards the proteins from its inhibition by H_2O_2 and withstand up to 45°C of temperature [18].

The applications of SOD extended to cosmetic and manufacturing of other supplementary products to protect from free radical damages. Nelson [19] investigated and reported that SOD can also prolong the survival period of organs for transplantation, sperms [20] food stuffs [21] laundry ingredients to remove Amadori and Maillard products [22] and as biosensors to detect superoxide anions (O_2-) [23]. In higher green photosynthetic plants, the SOD used as a shield against physio-chemical stresses including chilling, drought, salinity and high light exposure, O_3, metal ions, herbicides respectively thus enhances high yield biomass and its proliferation [24-26]. The food preservatives are subjected to generate ROS to avoid microbial contamination [27] and the high oxidative stress occurs during fermentation resulted in cell damage and poor productivity of fermented bioproducts. Several microbial sources of SOD have been optimized and characterized from Caulobacter, Brucella, Haemophilus, Pseudomonas and *Escherichia coli* [28]. The bacterium *Lactobacillus fermentum* ME-3 strain has been patented for the high SOD activity and also helps in the gastrointestinal and urogenital infections treatment [29]. Several animal and plant derived SODs were expressed using pET30a+vector in *E. coli* Rosetta DE3 pLysS with tRNAs for certain rare amino acids found in plant and animal SODs.

The famous PPL therapeutics (Netherland) who cloned the sheep Dolly has developed transgenic lambs for SOD production. The purified recombinant human Cu/Zn SOD expressed in *E. coli* yield up to 5% of Cu/Zn SOD [30]. The SOD3 expressed in pET-28a in *E. coli* yielded up to 26% of SOD of total cellular protein [31]. The overexpressing of SOD was successfully obtained by multicopy plasmid YG131 in *Kluyveromyces marxianus* L3 strain [32]. Supplemented with Cu^{2+} and Zn^{2+} yielded Cu/Zn SOD with the use of yeast chaperone [33] and the yield was 30-50% of total bacterial protein with 87-98% Cu saturation. The SOD can be overexpressed in milk through acidic protein (wap) promoter resulting in 0.7 mg/ml and 3 mg/ml in transgenic mice and rabbits respectively [34,35]. The yeast glyceraldehyde phosphate dehydrogenase promoter was used in the high level expression of human SOD in yeast [36]. The bacmid a baculovirus shuttle vector system used as a vector to overexpress Mn SOD in insects like *Bombyx mori* as a bioreactor [37]. The human liposarcoma (LSA) cells were used as a host for the synthesis of Mn SOD, but astoundingly it was secreted in the medium than being found in the mitochondria. The Chinese hamster ovary expression system yield very low amount of human SOD3 [38]. Recombinant SOD was attained from 0.5 l fed-batch bioreactor with 1.4×10^6 Units in one liter of the medium [39].

The SOD production from brewer's yeast was also patented by Suntory Ltd. in Japan [40]. The solid state and submerged fermentation methods obtained up to 2600 U/ml of SOD from *Bacillus subtilis* strain [41]. The SOD extracted from marine microbe *Photobacterium sepia* and *Photobacterium phosphoreum* learnt that it was auspicious to reduce UV-inducced erythema in sportsmen. The final concentration of the enzyme was 10 times high when compared with the yield from baker's yeast and stable for 2 years under 8°C without retarding its activity. The same drug have shown many pharmaceutical applications in human clinical trials [42].

Commercially available SOD as drug

Even though there are various sources and forms SOD, bovine-derived SOD commonly called as Orgotein available commercially to treat inflammation and radiation induced side-effects. It has been also approved by US-FDA to treat inflammation in cattle and pets and used in the treatment of familial amyotrophic lateral sclerosis (ALS) in 1995. Various patents were owned by Oxis International Inc. for the extraction of orgotein from animals and its therapeutic applications [43]. Superoxide dismutase from marine source is being used in cosmetics by L'oreal and different formulations and manufacturing processes were patented by the same company [44]. The recombinant SOD from yeast have enormous temperature stability up to 45°C and named as Biocell SOD developed by Brooks Industries (USA) in 1987 [45]. The Biocell SOD (0.1-0.5%) is being used in skin care formulations due to its temperature stability when heating and which are available in Arch (NJ, USA) a personal care product.

Nowadays the formulations of SOD are widely used commercially as moisturizes, sunscreens, skin-lightening creams, eye creams, nail polish and anti-hair fall sprays and available in prestigious brands such as Paula's Choice, Bioelements, Rachel Perry, The Herbarie, Dabao Cosmetics Co. Ltd., Supplement Spot LLC, Nature's Drugstore, Revitol Corporation, Avenue, Phytomer, Pevonia Botanica Skin Care and Estee Lauder [46]. SOD extract (extramel) from cantaloupe melon have been developed and marketed first by Bionov (France) and which awarded European anti-stress promising ingredient of the year in 2008. The encapsulated formulation of extramel is used as a cosmetic applications by Seppic (France) [47]. Isocell Pharma has developed an oral supplement SOD product Glisodin in combination with wheat Gliadin which not only prevent degradation of SOD in the gastrointestinal tract and also improved its uptake in intestine [48]. The same available as spray-dried powder with 1 U/mg of activity stable for 2 years at below 20°C and the same company has many patents on glisodin applications [49]. The glisodin is also marketed and manufactured by other companies also, the are PL Thomas Inc. (USA), Cell Logic Nutraceutical Solutions (Australia), PT Kalbe Farma Tbk (Indonesia), Syspharma Co. Ltd. (Korea), Millenium Biotechnologies Inc. (NH, USA), Nutrition Act Co. Ltd. (Japan), PURE-XP Ltd. (UK), and Novus Research Inc. (AZ, USA). It is also used in a pet health-promoting formulation by Nutramax Laboratories Inc. (MD, USA). In human the glisodin is effective in elevating antioxidant levels in serum and minimizes reducing pain in inflammatory conditions like arthritis. The glisodin formulations resurgex and resurgex plus from Millenium Biotechnologies (NH, USA) supplemented to people who are struggling with immunocompromised conditions such as AIDS or cancer [50] which was confirmed in 6 month clinical study on 25 HIV-1 patients [51].

Various sources of SOD from both native and recombinant are now commercially available in the form of biochemical reagents from different companies such as Roche, Sigma, Wako, Jena Bioscience, AMS Biotechnology Ltd, Worthington and Calzyme [52]. BTG and Chiron Corporation (CA, USA) received orphan drug designation using Cu/Zn SOD to hamper reperfusion injury in donor organs. Recombinant human SOD yielded more patents to Chiron Corporation (a part of Novartis). And they have performed various methods to improve thermostability, pharmacokinetics and catalytic efficiency of the enzyme [53].

SOD from blue-green algae

Algae are the large and diverse group of aquatic photosynthetic organisms exists in both the marine and freshwater forms which are ubiquitous in nature. Both microscopic and macroscopic forms of algae are found which are classified as Bacilloriophyceae (Diatoms), Chlorophyta, Euglenophyta, Dinoflagellates, Chrysophyta, Phaeophyta, Rhodophyta and Cyanobacteria. The cyanobacteria are the prokaryotic, photosynthetic microorganisms named due to the production of special blue-green pigment phycocyanin and reddish brown pigment phycoerythrin. The blue-green algae are one among the oxygen evolving microorganisms evolved approximately 3.5 billion years ago as a result of oxygenic photosynthesis. The cyanobacteria are placed in between the prokaryote (anaerobic bacteria) and the eukaryote (green algae) because, they do not have a well-defined nucleus and cell organelles at the same time they are photoautotrophic. The cyanobacteria are the well-known sources of neutraceutical compounds, for example; Spirulina sp. which has easily digestible protein and phycobiliproteins are the products from Synechococcus sp. have high commercial value. The sulfated polysaccharides are recently reported as anti-viral agents extracted from some of the filamentous cyanobacteria e.g., Nostoc sp.

Generally, the cultivation of cyanobacteria is not much more difficult due to fast growth, high photosynthetic efficiency and cheap labor as in Spirulina cultivation. Anabaena and Spirulina are the sources of commercial proteins and vitamins. Especially, the cyanobacteria have the ability of undergoing high degree of O_2 reduction by utilizing 50% of photosynthetic electrons, but only 15% is utilized by plants [54]. Some of the previous reports of superoxide dismutase enzymes from cyanobacteria are given in table.

However, SODs from several alternative sources rather than Bovine and recombinant may be effective drug in the near future (Table 1). For example, SODs from *Humicola lutea*, yeast and *Debaryomyces hansenii* shown to have protective activity over myeloid Graffi tumor, infection of influenza virus, adjuvant arthritis and carrageenan induced edema in mice [55-57]. The SOD formulations from various sources is the need of the hour research in the pharmaceutical applications due to different properties of different forms of the enzyme.

S.no.	Algal species	References
1.	*Plectonema boryanum*	[58]
2.	*Plectonema boryanum* UTEX 485	[59]
3.	*Anabaena cylindrica*	[60]
4.	*Nostoc commune*	[61]
5.	*Microcystis aeruginosa*	[62]
6.	Nostoc PCC 7120	[63]
7.	*Anabaena variabilis* Kutz	[64]

Table 1: The Superoxide dismutase enzyme (SOD) reported from cyanobacteria.

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