

Microorganisms as a Source of Antioxidant Compounds

Subjects: Biology

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Microorganisms are a diverse group of microscopic organisms including archaea, bacteria, fungi, protozoa, algae, and viruses. Microbial diversity produces a massive pool of unique chemicals, which have become a valuable source for innovative biotechnology. Microorganisms can be used as a source of antioxidants with the advantage of fast growth under controlled conditions.

Keywords: oxidative stress ; natural antioxidant ; astaxanthin ; mycothiol

1. Actinomycetes

The actinomycetes are gram-positive, aerobic, filamentous, and spore-forming bacteria, with a superior reputation in producing different kinds of metabolites with a broad spectrum of biological activities, including antioxidant, antifungal, antibacterial, and insecticidal activities.

Mycothiol (MSH), a principal “sugar” thiol present in the cell wall of actinomycetes, serves as the glutathione (GSH) analogue. Actinomycetes lack the enzymes for GSH biosynthesis, but as a substitute, they utilize alternative low molecular weight thiols (LMW), such as bacillithiol (BSH) and MSH, ergothioneine (ESH) ^[1]. MSH maintains the intracellular redox homeostasis, allowing the appropriate working of many biological processes, counting enzyme activation, DNA synthesis, and cell-cycle regulation. MSH is a potent antioxidant, which acts as an electron donor/acceptor and assists as a cofactor in detoxifying free radicals, xenobiotics, and alkylating agents. Unlike GSH, MSH has two sugar components viz. *N*-glucosamine, inositol, and cysteine components as an alternative to the two amino acids, glycine, and glutamic acid ^[1].

1.1. Biosynthesis of MSH

MSH biosynthesis involves five enzymes, and the substrates *myo*-inositol-1-phosphate (Ins-P), UDP-*N*-acetyl glucosamine (UDP-GlcNAc), and Cysteine (Cys) represented in **Figure 1**. Firstly Ins-P is conjugated with UDP-GlcNAc to yield *N*-acetyl glucosamine *myo*-inositol-1-phosphate (GlcNAc-Ins-P) via enzyme glycosyltransferase MshA. The MSH phosphatase MshA2 dephosphorylated GlcNAc-Ins-P, which is further deacetylated by the metal-dependent deacetylase MshB generating glucosamine inositol [1-*O*-(2-amino-1-deoxy- α -D-glucopyranosyl)-*D*-*myo*-inositol]. The Cys ligase MshC inserted Cys to yield Cys-GlcN-Ins. Finally, the Cys amino group is acetylated by the MSH acetyltransferase MshD to produce MSH ^[2].

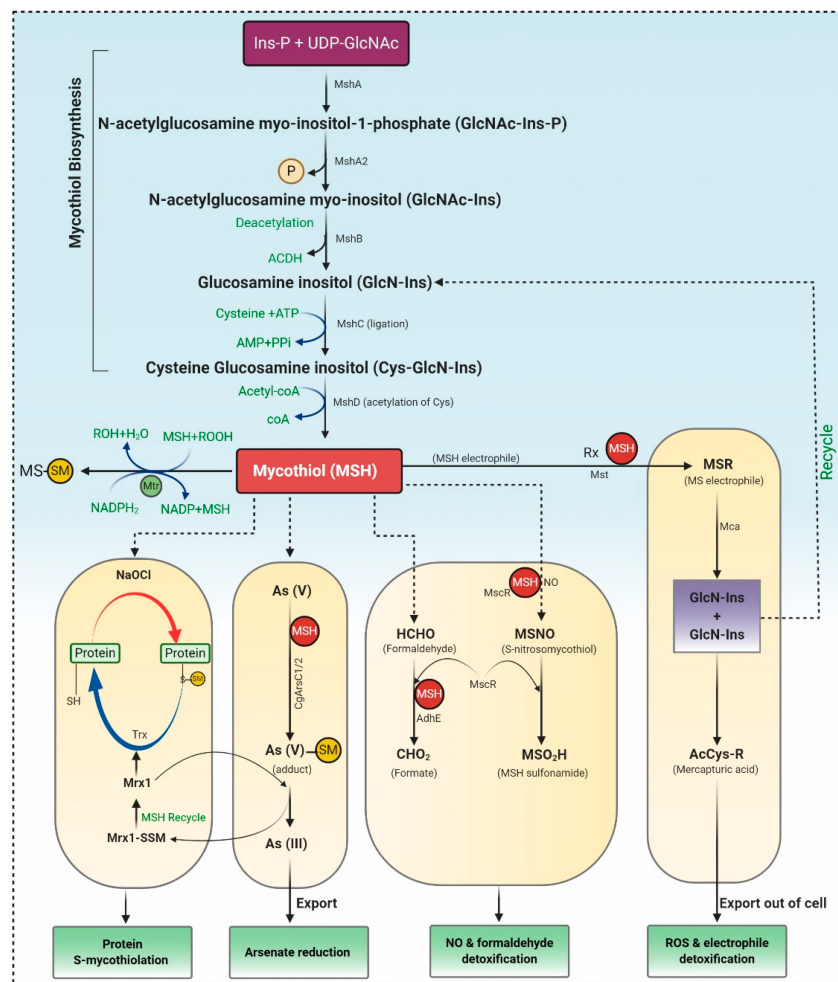


Figure 1. MSH biosynthesis and regulation in actinomycetes. Synthesis of MSH is catalyzed by five enzymes, including MshA, MshA2, MshB, MshC, and MshD. 1) Under Reactive Oxygen Species (ROS), MSH is oxidized to MSSM, which is further reduced by Mtr. 2) S-mycothiolation and protein regeneration occur via Mrx1 (mycoredoxin 1) /MSH/Mtr and thioredoxin/Thioredoxin reductase (Trx /TrxR) pathway. 3) Arsenate reductases CgArsC1/CgArsC2 along with MSH and Mrx1 reduced As (V) to As (III), which is exported through ABC transporter. 4) MSH acts as thiol cofactor for alcohol dehydrogenase MscR and formaldehyde dehydrogenase AdhE and is involved in NO and formaldehyde detoxification. 5) Mycothiol amidase (Mca) and mycothiol-S-transferases are involved in ROS and xenobiotic detoxification.

1.2. The Catalytic Action of the Mycothiol Disulfide Reductase (Mtr)

During the oxidative stress, MSH is oxidized to mycothiol disulfide (MSSM), which is further reduced by the NADPH-dependent Mtr. Mtr is the key enzyme involved in retaining MSH levels, which reduces the oxidized MSH disulfide. Mtr is a homodimeric flavoprotein disulfide isomerase that requires a cofactor, viz. FAD (flavin adenine dinucleotide). The oxidized Mtr (Mtrox) comprises a disulfide bridge between Cys39 and Cys44, which further reduces by accepting electrons from NADPH through FAD cofactor to produce reduced Mtr, as depicted in **Figure 1**. MSSM is confronted via the exchange of Cys39, resulting in the generation of Cys39-SSM, i.e., reduced MSSM and discharge of the first MSH moiety. Consequently, the disulfide bond in Cys39-SSM is condemned by the thiolate present at Cys44 forming Mtrox [3][4].

1.3. Mycothiol-Dependent Detoxification of Electrophiles

MSH S-transferases (Mst) conjugate with MSH electrophiles (RX), creating MS-electrophiles (MSR), MSH S-conjugate amidase (Mca) cleave an amide bond of MSR to form a glucosaminyl inositols (GlcN-Ins) and mercapturic acid (AcCys-R). AcCys-R or MSH-S-associates of antibiotics or toxins are expelled from cells via ABC transporters, whereas GlcN-Ins is salvaged back to mycothiol (**Figure 1**) [3][4].

1.4. Detoxification of Nitric Oxide (NO)

Detoxification of NO requires MSH-dependent enzyme nitrosothiol reductase (MscR) that exhibits S-nitrosomycothiol (MSNO) reductase activity, thereby generating MSH sulfonamide (MSO₂H). Both MSH-dependent formaldehyde dehydrogenase AdhE and MscR oxidize formaldehyde to formate. In *Corynebacterium's glutamicum*, maleylpyruvate is a ring fission output of gentisic acid and is converted to fumarylpyruvate through gentisate pathway by the MSH-dependent maleylpyruvate isomerase (**Figure 1**) [4][5].

1.5. Detoxification of Arsenate

Arsenate detoxification is catalyzed by the arsenate reductases (CgArsC1/CgArsC₂), which associate arsenate As (V) to MSH, generating As (V)-SM adduct, that is later on reduced by mycoredoxin-1 (Mrx1), forming Mrx1-SSM intermediate and As (III). As (III) is disseminated from the cells using two arsenite permeases of the Acr3 family. However, Mrx1-SSM depends on MSH for the regenerating Mrx1 [3][6].

1.6. Protein S-Mycothiolation under NaClO and H₂O₂ Stress

Proteins are S-mycothiolated and recreated by the Mrx1/MSH/Mtr and Trx/TrxR pathways during NaOCl and H₂O₂ stress. These proteins control the activity of mycothiol peroxidase (Mpx), thioredoxin peroxidase (Tpx), and methionine sulfoxide reductase A (MsrA) in vitro [3]. Mpx and MsrA result in the formation of intramolecular disulfides and S-mycothiolations and involve both the Trx and Mrx1 pathways for reformation [7]. The Mrx1/Mtr/MSH pathways are likewise involved in reducing the peroxiredoxin AhpE in *Mycobacterium tuberculosis* (Figure 1). Therefore, it can be concluded that mycothiol is a promising and suitable candidate for antioxidant therapeutics. Only a few studies investigated the antioxidant compounds from actinomycetes. Yang et al. [8] reported the production of 1"-O-methyl-8-hydroxymethyl-daidzein, an isoflavone from *Streptomyces* sp. YIM 65408, with ROS scavenging activity. Zhou et al. [9] reported antioxidant, 2,6-dimethoxy terephthalic acid (IC₅₀ 4.61mg/mL) from endophytic *Streptomyces* sp. YIM66017.

Several compounds such as antiostatins A1 to A4 and B1 to B4 reported from *S. cyaneus*, and carbazoquinocins A to F isolated from *S. violaceus*, were composed of carbazole comprising an o-quinone. Neocarazostatins A to C purified from the mycelium of *Streptomyces* sp. confirmed antioxidant activity [10]. Therefore, carbazole compounds produced by *Streptomyces* sp. contributed to be the major class of antioxidants. Tan et al. [11][12] demonstrated antioxidant activity of *Streptomyces* sp. MUM212 and *Streptomyces* sp. MUM265, concluded that both strains contain phenolic compounds that inhibit lipid peroxidation and reduce ROS due to their hydrogen-donating and electron transferring capabilities. Praptiwi et al. [13] reported organofluorine, a potent antioxidant from *Streptomyces* sp. strain TC1. Therefore, it can be considered that microbes are a potential candidate for production of bioactive compounds.

2. Archaea

Archaea specifically grow in harsh environmental conditions such as significantly higher or lower temperature, pH, salinity, etc. Halophilic archaea are a more promising candidate for producing carotenoids, thereby they show red and orange colored colonies. A universal purpose of carotenoids is their antioxidant activity leading to the protection of cells against oxidative stress, thus benefiting human health. Most haloarchaea biosynthesized bacterioruberin (BR), a C₅₀ carotenoid, and its precursor bis-anhydro bacterioruberin (BABR), 2-isopentenyl-3,4-dehydrorhodopin (IDR), and mono-anhydro bacterioruberin (MABR) are represented in Table 1 [14]. BR present in the cell membrane helps halo archaeal cells acclimatize to hypersaline environments, resulting in stabilizing the cell membrane under such stress. Rodrigo et al. [15] investigate and described the haloarcheal BR production and its applications in biomedicine. In contrast, Giani et al. [14] elaborated BR and its derivatives' production at mid-and large-scale and discussed its recent biotechnology and biomedicine applications.

Table 1. Characterization of bacterioruberin, and its most abundant derivatives.

Common Name	Molecular Formulae	Scientific Name	Producers	Mode of Action	References
Bacterioruberin (BR)	C ₅₀ H ₇₆ O ₄	2,2'-bis (3- hydroxy-3-methylbutyl)-3,4,3',4'-tetrahydro-1,2,1',2'-tetrahydro-γ,γ-carotene-1,1'-diol	<i>Haloarcula japonica</i> , <i>Halobacterium salinarum</i> , <i>Halorubrum sodomense</i> and <i>Haloarcula vallimortis</i> and <i>Halorubrum</i> sp. TBZ126	Protection against oxidative stress by arachidonic acid and H ₂ O ₂	[14][15]
Mono-anhydrobacterioruberin (MABR)	C ₅₀ H ₇₄ O ₃	30-(2-hydroxypropan-2-yl)-2,6,10,14,19,23,27,33-octamethyl3-(3-methylbut-2-en-1-yl) tetratriaconta 4,6,8,10,12,14,16,18,20,22,24,26,28-tridecaene-2,33-diol	<i>Haloferox volcanii</i>	ROS scavenging activity	[14][15]

Common Name	Molecular Formulae	Scientific Name	Producers	Mode of Action	References
Bis-anhydrobacterioruberin (BABR)	C ₅₀ H ₇₂ O ₂	2,6,10,14,19,23,27,31-octamethyl3,30-bis (3-methylbut-2-en-1-yl)dotriaconta 4, 6, 8, 10, 12,14,16,18,20,22,24,26,28-tridecaene-2,31-diol	<i>Haloferax volcanii</i>	ROS scavenging activity	[15]

BR acts as a “rivet” and affects membrane fluidity by imitating as a water barricade and permitting permeability to oxygen and other molecules [16]. In BR, 13 pairs of conjugated double bonds are present compared to the nine pairs of conjugated double bonds present in β-carotene. This transformation makes BR a better ROS scavenger as compared to β-carotene [17]. BR offers resistance to γ irradiation, intense light, and DNA damage caused by UV irradiation, radiography, and H₂O₂ exposure [18]. Carotenoids from *Halorubrum* sp. BS2 reported having extraordinary antioxidant capacity compared to ascorbic acid [19]. The antioxidant capabilities of carotenoids produced by *Haloterrigena turkmenica*, *Haloferax volcanii*, *Halococcus morrhuae*, *Halogramum rubrum*, and *Halobacterium salinarum* were significantly higher than β-carotene [14]. Hyperthermophilic archaea are furnished with a wide range of antioxidant enzymes that play a crucial role in protecting the living cells from oxidative damage. These enzymes, along with protein disulfide oxidoreductase, thioredoxin, and thioredoxin reductase, act as a core of the antioxidant system and maintain redox homeostasis. As demonstrated by recently published data, the mainstream of aerobic hyperthermophilic archaea utilizes peroxiredoxins (Prxs) and thiol-dependent peroxidases to scavenge peroxides. LMW thiols act as cofactors in the detoxification of xenobiotic compounds. Newton and Javor [20] reported γ-glutamylcysteine (γGC), the first LMW thiol isolated from halophilic archaeon (haloarchaeon) *Halobacterium salinarum*. Later on, it was reported from other haloarchaea, including *Halobacterium saccharovororum*, *Haloarcula (Halobacterium) marismortui*, *Haloarcula californiae*, *Halococcus* sp. LS-1), and *Haloferax (Halobacterium) volcanii* [21][22]. Bacterioferritin comigratory protein 1 (Bcp1) belongs to the Prx family, isolated from hyperthermophilic archaeon *Sulfolobus solfataricus*, which has been characterized as an antioxidant that inhibits H₂O₂-induced apoptosis in H9c2 rat cardiomyoblast cells [23]. A 22-kDa protein, structurally similar to a class of DNA-binding protein (Dps) was isolated from hyperthermophilic acidophile *Sulfolobus solfataricus*, demonstrated to protect nucleic acids by defending DNA from oxidative damage and eliminating constituents that contribute to the formation of hydroxyl radicals [24].

3. Bacteria

Bacteria are closely associated with all life forms on earth and possess the ability to produce ample extracellular metabolites. In bacteria, carotenoids are produced by the extremophiles, including *Thermus filiformis*, *Micrococcus freudenreichii*, *Flavobacterium* sp., *Serratia marcescens*, *Agrobacterium* sp., *Pseudomonas aeruginosa*, *Rheinheimera* sp., and *Chromobacterium* sp. *Thermophilic* bacterium produces all-trans-zeaxanthin, zeaxanthin monoglucoside, thermobiszeaxanthins, and thermozeaxanthins [12]. Carotenoid ingestion might decrease the threat of diseases linked with oxidative stress; therefore, it acts as a proficient scavenger of ROS, RNS, singlet oxygen species (¹O₂), and nonbiological radicals [25].

Correa et al. [26] analyzed that when Antarctica bacteria belonging to the *Pedobacter* genus were exposed to cold temperatures and high UV radiation, they had developed an important antioxidant system and produce a variety of pigments that belongs to the carotenoids group and are capable of preventing oxidative damage. The antioxidant capacity of a mix of pigments viz. yelcho2, β-Carotene, and α-Tocopherol, was analyzed using three different methods viz. DPPH, ROS detection, and an oxygen electrode. In December 1988, the Marine Biotechnology Institute Co., Ltd. (MBI, Kamaishi, Japan) was established and began the isolation of novel or rare marine bacteria; many among them have been revealed to yield dicyclic or monocyclic C40 carotenoids, along with several acyclic C30 carotenoids [27]. MBI reported that *Paracoccus* sp. strain N81106 [28], *Brevundimonas* sp. strain SD212 [29], and *Flavobacterium* sp. PC-6 [30] produces astaxanthin glucoside, 2-hydroxyastaxanthin and 4-ketonostoxanthin 3'-sulfate, respectively. These are novel dicyclic C40 carotenoids with β-carotene (β, β-carotene) skeleton. The carotenoid biosynthesis gene cluster responsible for astaxanthin manufacturing was reported from the marine bacterium *Agrobacterium aurantiacum* [9]. This carotenoid gene cluster consists of five carotenogenic genes viz. *crtB*, *crtW*, *crtZ*, *crtY*, and *crtl* with the same orientation. Mishra et al. [31] investigated the capability of a semiquinone glucoside derivative (SQGD) from a *Bacillus* sp. INM-1 towards SOD, catalase, GSH, GST antioxidant enzymes. There was a significant increase in SOD (35%) activity and GST level (0.46 ± 0.03 μmol/min/mg of protein) in mice's kidney after SQGD treatment compared to untreated control mice within 12–72 h.

Probiotics contain billions of bacteria that are capable of fermenting foods and beverages and are also used as a supplement due to their numerous beneficial activities including antioxidant properties. They accumulate in the GI tract

and produce metabolites, which possess antioxidant activities. Probiotic bacteria have unique antioxidant enzyme systems that stimulate the host antioxidant system. Research on pigs exposed that dietary *Lactobacillus fermentum* elevated the serum SOD, glutathione peroxidase (GPx) and hepatic CAT, and Cu and Zn-SOD than the control group. Aarti et al. [32] reported the hydrogen peroxide resistant activity of *Lactobacillus brevis* LAP2, isolated from *Hentak*, a fermented fish food from Manipur, India. Son et al. [33], isolated *Lactobacillus plantarum* Ln4 and G72, from kimchi, exhibited (40.97%) DPPH, ROS scavenging, and β -carotene oxidation-inhibitory activities (38.42%). Another strain, SC61 of *Lactobacillus plantarum*, isolated by Son et al. [34] from jangajji, a Korean traditional fermented food, expresses a high level of IL-1 β , IL-6, and TNF- α . These studies did not explore how the probiotic bacteria communicate with the signaling pathways, which is elaborated in **Table 2** and **Figure 2**. In 2018, Ayyanna and colleagues assessed probiotic strains *Lactobacillus mucosae* AN1 and *Lactobacillus fermentum* SNR1 for a significant decrease in rat paw edema, induced by Freund's adjuvant and carrageenan. This decrease in inflammation is due to the high expression of IL-10, an anti-inflammatory cytokine, inhibiting prostaglandins synthesis, thereby preventing autoimmune and inflammatory diseases [35].

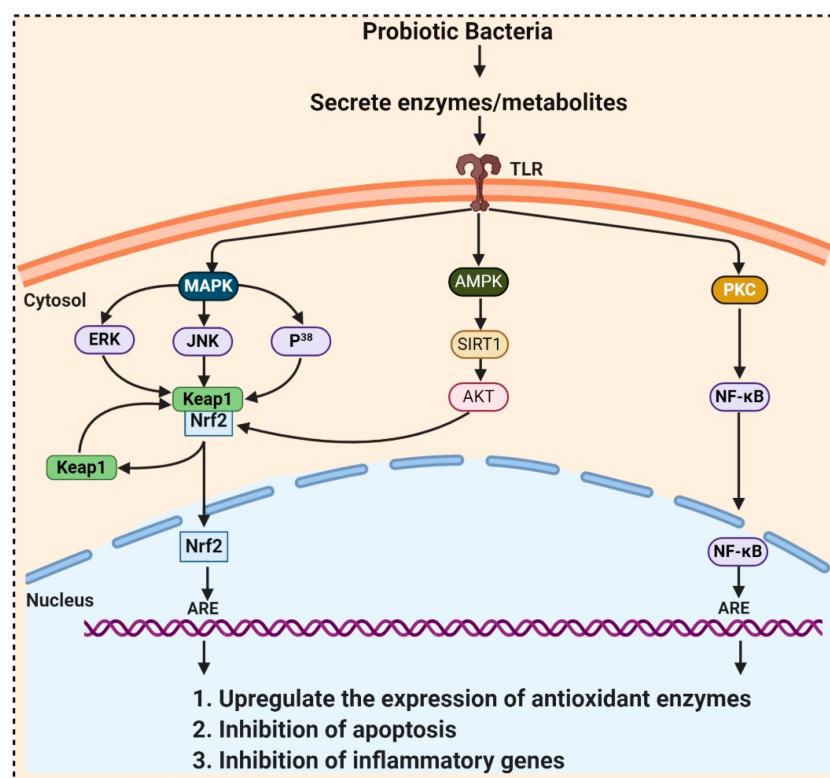


Figure 2. Probiotic activates metabolites and enzymes that enter the cell through toll-like receptors (TLR) and induced Nrf2-Keap1-ARE pathway. During ROS attack, the redox-sensitive cysteine residues of Keap1 react and disrupt the functional conformation of Keap1, thereby activating Nrf2. Nrf2 translocates towards the nucleus and binds to antioxidant response element (ARE) sequences, activating the transcription of ARE-driven genes, encoding antioxidant enzymes, and detoxifying proteins. ROS mediates the expression of redox-sensitive transcription factor NF κ B and further expression of inflammatory cytokines. ROS also activates SIRT1 (Silent information regulator T1), mediated Adenosine monophosphate (AMP)-activated protein kinase (AMPK) pathway.

Table 2. Some antioxidant probiotic bacteria with the signaling pathways used to activate antioxidant enzymes and molecules.

Probiotic Bacteria	Pathway	Model Used	Effects	References
Probiotic Formulation SLAB51, <i>Bacillus. Longum</i> , <i>Lactobacillus acidophilus</i>	Sirtuin-1 (SIRT1)	Alzheimer's disease (AD) (3xTg-AD) mice model	Increases antioxidant enzymes activity	[36]
<i>L. plantarum</i> LP6 <i>L. plantarum</i> C88 <i>L. rhamnosus</i> GG	PKC (protein kinase C)	Caco-2 cells Caco-2 cell Caco-2 cell	Enhanced activities of SOD and CAT Inhibit malondialdehyde (MDA) formation, raised SOD Ameliorate the oxidative stress-induced disruption of intestinal epithelial tight junction	[37][38] [39]

Probiotic Bacteria	Pathway	Model Used	Effects	References
<i>L. brevis</i> SBC8803	Integrin–p38 MAPK (mitogen activated protein kinase)	Caco2/BBE cells	Induce cytoprotective heat shock proteins (HSP27) maintaining intestinal homeostasis	[40]
<i>L. plantarum</i> , <i>L. rhamnosus</i> , <i>Bacillus subtilis</i> JH642	p38 MAPK	RAW 264.7 cells Caco2 _{bbe} cells	Decreases p38, JNK, ERK1/2 phosphorylation Increases p38 phosphorylation	[41] [42]
<i>Bacillus amyloliquefaciens</i> SC06		IPEC-1 cell line	Increase CAT and glutathione S- transferase (GST) expressions	[43]
<i>Clostridium butyricum</i> MIYAIRI 588	Nrf2-Keap1-ARE	rat smice	Activation of ARE-dependent genes i.e., GSTs, and TRX	[44]
<i>L. Plantarum</i> FC255			Elevate the activities of SOD and GP _x	[45]
<i>Lactobacillus casei</i> Bacillus LBP32	NF-κB	mice RAW 264.7 macrophages	Reduce cytokine production Reduce LPS-induced intracellular ROS accumulation	[46] [47]
<i>L. plantarum</i> CLP-0611		mice	Induced expression of M2 macrophage	[48]

Kullisaar et al. [49] reported that *Lactobacillus fermentum* E-3 and E-18 express Mn-SOD that resists oxidative stress. Probiotic bacteria's ability to locally deliver SOD exposed a novel perspective to bowel diseases caused by ROS production [49]. LeBlanc and colleagues' demonstrated that the engineered *Lactobacillus casei* BL23 produce SOD when given to mice with Crohn's disease and mice showed a quicker recovery from weight loss, improved gut enzyme activities, and a lesser extent of intestinal inflammation than the control mice [50]. CAT also participates in cellular antioxidant defense by decomposing H₂O₂, but LAB is usually CAT-negative. LeBlanc et al. [51] evidenced that *Lactococcus slactis* produced CAT and prevented 1,2-dimethylhydrazine (DMH)-induced colon cancer in mice. It was also proved that engineered *Lactobacillus casei* BL23 produce CAT and decrease or prevent the brutality of intestinal pathologies instigated by ROS. An in vitro study by Wang et al. [43] reported that *Bacillus amyloliquefaciens* SC06 raised CAT and GST gene expressions and activity in intestinal porcine epithelial cells-1 (IPEC-1). It is still doubtful whether those in vitro and in vivo results are conveyable to humans because maximum probiotics cannot be colonized in the gut and are eliminated immediately after consumption.

4. Fungi

Whenever there is a discussion about fungal metabolites, the story of penicillin has been told many times. Alexander Fleming, in 1929 discovered that mold juice' from *Penicillium notatum* had antibacterial action and named this biological activity "Penicillin" [52]. In the past few years, researchers instigated to look for a fungal strain that could be grown quickly and produce bioactive metabolites in submerged culture. Later on, *Penicillium chrysogenum* was selected for large-scale production of *Penicillium* [35]. A wide range of primary and secondary metabolites including flavonoids, phenols, steroids, alkaloids, xanthenes, etc. produced by the most diverse group of fungi, have antioxidant activities, and are highly exploited by the therapeutical, pharmacological, and medicinal sector. Maximum therapeutical and pharmaceutical investigations focused on the species belonging to Ascomycota and Basidiomycota [53], which include endophytic fungi, exhibiting higher antioxidant potential. A wide range of natural products including antioxidants, anticancerous, antiviral, anti-insecticidal, immune-suppressant, antimycobacterial, antimicrobial, and antimalarial, has been reported from endophytic fungi [34].

The discovery of antioxidant compounds, pestacin and isopestacin from *Pestalotiopsis microspore*, has opened a new direction towards investigating the antioxidant potential of endophytic fungi [32][54]. Zeng et al. [55] explored 49 endophytic fungi belonging to the family Xylariaceae, for in-vitro antioxidant activities. Among them, endophytic fungi from *Scapania verrucosa* were reported as a budding and novel source of natural antioxidants. Prihantini et al. [56] isolated seven fungal strains of the genus *Pestalotiopsis* from the leaves and stems of *Elaeocarpus sylvestris*. *Pestalotiopsis* sp. EST 02 and *Pseudocercospora* sp. ESL 02 possesses terreic acid and 6-methylsalicylic acid, having antioxidant activity. Nuraini et al. [57] reported that *Aspergillus minisclerotigens* AKF1 and *Aspergillus oryzae* exhibited antioxidant ability with an IC₅₀ 142.96µg/mL and 145.01µg/mL, respectively. GC-MS analysis confirmed that the most active compound isolated from AKF1 and DK7 was dihydropyran and 4H-Pyran-4-one,5-hydroxy-2-(hydroxymethyl-(CAS) Kojic acid, respectively.

Exopolysaccharides and flavonoids produced by *Fusarium oxysporum* isolated by Caicedo et al. [58] also exhibit such properties. Methanolic extracts from *Monascus purpureus*, OYRM1, *P. 32783*, *P. salmoneo-stramineus*, and *T. versicolor*

inhibit linoleic acid oxidation by at least 53% [59]. Total 42 fungal endophyte strains related to a medicinal plant, *Nerium oleander* L. (Apocynaceae) were isolated and screened for antioxidant and antimicrobial capacity. Out of these 42 isolated only *Chaetomium* sp. inhibit xanthine oxidase activity with an IC50 of 109.8 lg/mL, had an antioxidant activity with the highest level of phenolics [60].

5. Microalgae

More than 28,000 potential compounds are reported from microalgae, including hundreds of new compounds discovered every year [61]. Most of them have been described from *Chordata* (including ascidians) and *Porifera* (sponges), but they are hard to cultivate, and to attain a sustainable supply of the compounds of interest is problematic. In recent times, high interest is paid in analyzing microalgae's biotechnological potential as they have short generation time, easier to cultivate, and signify an ecofriendly approach but a less explored resource for drug discovery. Microalgae are photosynthetic eukaryotes comprising one of the key members of freshwater and marine phytoplankton and are exceptional cradles of pigments, carotenoids, ω -3 fatty acids, lipids, sterols, and other biomolecules as depicted in **Figure 3** [43]. More than 7000 species are enlisted as "Green microalgae" and they all grow in diverse habitats. Among them, *Haematococcus pluvialis* is the most important commercial microalgae and is also the richest source of natural astaxanthin which is regarded as "super anti-oxidant" [49]. Apart from β -carotene, astaxanthin has received considerable attention lately. Astaxanthin (3,3'-dihydroxy- β , β '-carotene-4,4' -dione), a marine xanthophyll carotenoid was first isolated by Kuhn and Soerensen from a lobster [62]. Astaxanthin was initially used as a colorant for aquaculture, besides being approved as a coloring agent for food supplements since 1991. Due to emerging health benefits, demand for astaxanthin has increased rapidly in medicine, food industries, cosmetics, etc. In 2016 market share of astaxanthin was USD 512.8 million, which increased at a CAGR of 6.73% by 2017 and was expected to reach USD 814.1 million and USD 2.57 billion by 2022 and 2025, respectively, worldwide [63][64]. The same is the case with carotenoids, whose market demand was 1.24 billion USD (B\$) in 2015 and is expected to rise to 1.6 B\$ by 2023 with a CAGR of 3.5%. The demand for lutein in 2015 was 135 M\$, which might escalate up to 200 M\$ by 2024, with a CAGR of 5.3%. The hike in CAGR is due to the increasing demand for lutein-rich dietary supplements [65].

Carotenoids	Astaxanthin (<i>Haematococcus pluvialis</i> , <i>H. lacustris</i> , <i>Monoraphidium</i> sp, <i>Neosporangiococcus</i> spp. and <i>S. obliquus</i>) β -Carotene (<i>Dunaliella salina</i> and <i>Scenedesmus</i> sp.) Diatoxanthin (<i>Phaeodactylum tricornutum</i>) Zeaxanthin; lutein (<i>Chlamydomonas reinhardtii</i>) Fucoxanthin (<i>Isochrysis</i> spp., <i>Nitzschia</i> spp., <i>P. parvum</i>)
Polyphenols	Flavonoids (<i>Chlorella pyrenoidosa</i> and <i>Chlamydomonas eugametos</i>) Isoflavonoids (<i>Spongiochloris</i> and <i>Scenedesmus</i>) Phenolic Polymers (<i>Spirulina maxima</i>) Phenolic acids (<i>C. nivalis</i>)
Sterols	Cholesterol (<i>Chlorella vulgaris</i> , <i>Isochrysis galbana</i> and <i>Schizochytrium aggregatum</i>) Ergosterol (<i>Chlorella vulgaris</i> , <i>Dunaliella salina</i> , <i>D. tertiolecta</i> and <i>Schizochytrium aggregatum</i>) Sitosterol (<i>Chrysoderma</i> sp., <i>Chrysomeris</i> , <i>Chrysoswaernella</i> and <i>Cyanophora paradoxa</i>) Desmosterol (<i>S. marinoi</i> and <i>Cyclotella cryptica</i>) Clinosterol (<i>Pavlova lutheri</i>)
Vitamins	Vitamin A (<i>D. salina</i> , <i>C. nivalis</i> , <i>Chlorococcum</i> spp., <i>Chlamydocapsa</i> spp., <i>C. acidophila</i> , <i>C. sorokiniana</i> , <i>D. salina</i> , <i>P. obovatus</i> and <i>Tetraselmis wettsteinii</i>) Vitamin C (<i>C. reinhardtii</i> , <i>Chlorella</i> spp., <i>C. vulgaris</i> , <i>D. tertiolecta</i> , <i>P. moriformis</i> , and <i>S. quadricauda</i>) Tocopherol (<i>C. reinhardtii</i> , <i>C. sorokiniana</i> , <i>C. pyrenoidosa</i> , <i>C. vulgaris</i> , <i>D. tertiolecta</i> , <i>S. acutus</i> , <i>T. suecica</i>) Vitamin B3 (<i>Chlamydomonas</i> spp., <i>Chlorella</i> spp., <i>C. eugametos</i> , <i>S. acutus</i> , and <i>S. quadricauda</i>)
Others	MAAs (mycosporine-like amino acids) (<i>Karlodinium micrum</i> , <i>Oxyrrhis marina</i> and <i>Heterocapsa triquetra</i>) DMS (dimethylsulphide) (<i>Scripsiella trochoidea</i> , <i>Pleurochrysis carterae</i> , <i>Chlorella</i> sp. and <i>Tetraselmis helgolandica</i>) Sulfated Carbohydrates (<i>Cylindrotheca closterium</i> , <i>Chlorella stigmatophora</i> and <i>Cochlodinium polykrikoides</i>)

Figure 3. Some antioxidant compounds reported from microalgae.

Polyene chain along with conjugated double bonds permit astaxanthin to pass through the lipid bilayer and allow the quenching of singlet oxygen and ROS removal [49][66].

The presence of hydroxyl and keto moieties on each ionone ring provides a unique structure to astaxanthin, permitting it to pass through the lipid bilayer membrane and allowing it to protect the cell membrane [49]. Unlike many antioxidants, which quench and scavenge ROS and other free radicals including, superoxide anion, hydrogen peroxide, singlet oxygen, etc.) either in the inner (e.g., vitamin E and β -carotene) or the outer side of the bilayer membrane (e.g., vitamin C), astaxanthin can act as both the inner and outer layers of the cellular membrane [67]. Many studies demonstrated that astaxanthin had 500 times higher antioxidant capacity than vitamin E, 65 times more potent than vitamin C, 54 times stronger than β -carotene, and ten times more potent than β -carotene, zeaxanthin, lutein, canthaxanthin. At present, approximately 95% of synthetic astaxanthin is available in the market, whereas only <1% natural astaxanthin derived from *H. pluvialis* is commercialized [68]. Astaxanthin upregulates the expression of Nrf2, whereas it simultaneously

downregulates the Kelch-like ECH-associated protein-1 (Keap1) expression. In the presence of oxidative stress, Nrf2 dissociates from Keap1, translocates into the nucleus, binds to the ARE, and induces the expression of Phase II enzymes, as shown in **Figure 4**. Astaxanthin prevents lipid peroxidation by activating antioxidant enzymes involved in lipid metabolism, namely thioredoxin reductase (TrxR) and paraoxonase-1 [69][70][71]. Pongkan et al. [72] depicted higher levels of mtROS production, mitochondrial depolarization, and swelling in mitochondria isolated from the ischemic myocardium of mice astaxanthin treatment decreases mtROS production, mitochondria depolarization, and swelling.

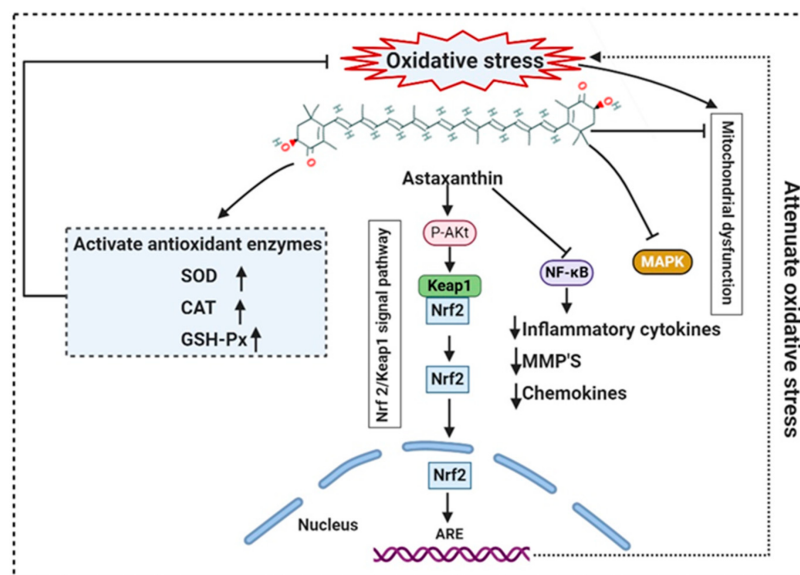
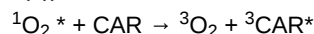


Figure 4. Mode of action of astaxanthin by inhibiting oxidative stress-induced mitochondrial dysfunction and activating transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2). Astaxanthin suppresses nuclear expression of NF-κB and MAPK to reduce downstream production of proinflammatory cytokines. NF-κB: Nuclear factor-κB; MMP: matrix metalloproteinase; MAPK: mitogen-activated protein kinase; ARE: Antioxidant response elements.

Synthetic astaxanthin, synthesized through Wittig reaction between asta-C₁₅ -triarylphosphonium salt and the C₁₀-dialdehyde [73], but it has 20 times lesser antioxidant capacity as compared to its natural counterpart, and until today it has not been permitted for human consumption [74]. However, *H. pluvialis* has been approved as a color additive and dietary-supplement in the USA, Japan, and some European countries. Astaxanthin from *H. pluvialis* had been granted “GRAS” status (Generally Recognized As Safe) by US FDA (Food and Drug Administration) [75]. The European Food Safety Authority (EFSA) recommended the use of 0.034 mg/kg of the bodyweight of astaxanthin. Spiller and Dewell [76] reported that 2–4 mg of a daily dose of astaxanthin is safe whereas not toxicity was reported up to 6mg/day consumption. Recently, Algatechnologies, Mera Pharmaceuticals Inc, Cyanotech Corporation, Fuji Chemical Industry Co. Ltd., etc. are involved in the commercial production of astaxanthin from *H. pluvialis*. Its global market is predicted to GRASP \$2.57 billion by 2025 [63]. L-Ascorbic acid (vitamin C), present both in cytosol and chloroplast, reduce many ROS and act as a scavenger for hydroxyl radicals, superoxide, and lipid hydroperoxides. The maximum concentration of vitamin C was reported from *Chlorella* sp. [36] and *Dunaliella* sp. [37], *Skeletonema costatum*, and *Chaetoceros calcitrans*. Glutathione (GSH), a tripeptide (Glu-Cys-Gly), acts as a sequence breaker of ROS reactions and ascorbate regeneration. α-tocopherol is the most active antioxidant synthesized in the chloroplasts of *Dunaliella tertiolecta* and *Tetraselmis suecica*. The lipophilic carotenoids, synthesized in diatoms and dinoflagellates allow quenching of singlet oxygen [77], leading to the formation of a carotenoid triplet state (Equation (1)).



Lutein is a xanthophyll that defends long-chain polyunsaturated fatty acids. *Muriellopsis* sp. accumulate high lutein levels, whereas, in *Scenedesmus*, lutein and β-carotene production are accelerated by increasing both the pH and temperature throughout the cultivation [39]. The commercial source of lutein is *Muriellopsis* sp.

β-carotene, isolated from the *Dunaliella salina*, is commercially produced for its antioxidant properties. Marennine is isolated from the marine diatom *Haslea ostrearia* also has antioxidant and antimicrobial activities [78]. Apart from diatoms, additional microalgae, including flagellates, dinoflagellates, and green algae, have also been scrutinized for potential biotechnological applications [79]. Carotenoid, fucoxanthin reported from brown seaweeds, and some diatom species have antioxidant, antidiabetic, antiangiogenic, anti-inflammatory, anticancer, and antimalarial activities. Methanolic extract of *Spirulina maxima*, and *Chlorococcum minutum* NIOF17/002 exhibit antioxidant activity with the IC₅₀ of 0.18 mg/mL and 3.92 to 9.83 mg/mL of ascorbic acid, respectively [80][81].

6. Protozoa

Protozoa are regarded as the first and simplest organism of the animal kingdom discovered by Anton van Leeuwenhoek. Most protozoan species are unicellular, free-living, motile, and microscopic, whereas some species are mutualistic and parasitic. More than 10,000 protozoan species are described as parasitic [82]. *Entamoeba histolytica*, *Trypanosoma*, *Giardia*, *Leishmania*, *Trichomonads*, and *Plasmodium* are usually anaerobic but sometimes found to be microaerobic or microaerophilic and produce H_2O_2 as a product of metabolism. Some intracellular protozoans are manifest to the toxic oxygen metabolites produced by the host's effector immune cells. Protozoa either lack or deficient in antioxidant enzyme systems including CAT, GSH, and SOD, necessary for the detoxification of H_2O_2 . However, they have alternative detoxification mechanisms. A higher concentration of NADH-dependent oxidase and a lower concentration of NADH-dependent peroxidase activities were perceived to accomplish this task. *Entamoeba histolytica*, *Trichomonas vaginalis*, and *Tritrichomonas foetus* were reported to encompass NADH oxidase activity. Since protozoa are known to be parasitic, very few reports are available regarding their antioxidant activity. Mastronicola et al. [83] explored the antioxidant defense systems in *Giardia intestinalis*, which lacks conventional antioxidant enzymes; despite this, it can rely on a well-organized antioxidant system to survive the nitrosative and oxidative stress conditions. Joardar & Babu [84] reviewed the most recent work on parasitic thiol-based enzymatic antioxidant thioredoxin reductase (TrxR), and discuss how to treat filariasis, helminths, and other parasitic infections. Jeelani and Nozaki [85] reported that *Entamoeba histolytica*, an intestinal parasite, causes amebic dysentery, lacks eukaryotic antioxidative defense systems but owns an efficient thioredoxin system, composed of thioredoxin and thioredoxin reductase, both are crucial for antioxidant activity and sustaining cellular redox balance [85].

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