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Probiotic treatment of Inflammatory Bowel Disease: Its extent and intensity

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Abstract

Free radicals (reactive oxygen species, superoxides and hydroxyl radicals) lead to the development of oxidative stress because of imbalance in the amount of antioxidants. Continued development of oxidative stress leads to chronic diseases in humans. The instability in the antioxidant activities and accumulation of oxidative stress due to free radicals may occur in diseases like inflammatory bowel disease (IBD). Antioxidants are substances that inhibit or delay the mechanism of oxidation of molecules mediated by free radicals andalso transform into lesser-active derivatives. Probiotics are defined as live microorganisms that show beneficial effects on inflamed intestine and balance the inflammatory immune responses in the gut. Probiotic strains have been reported to scavenge hydroxyl radicals and superoxide anions thatare abundantly produced during oxidative stress. The most widely studied probiotic strains are *Streptococcus*, *Bifidobacterium* and *Lactobacillus*. Probiotics cultured in broth have shown some amount of antioxidant activities. Fermented milk and soy milk, which possessstarter microorganisms (probiotics), tends to increase the antioxidant activities many-fold. This review aims to discuss the *in vivo* and *in vitro* antioxidant activities of specific probiotics with various assays with respect to IBD.

Key Words: Oxidative stress; Inflammatory bowel disease; Probiotics; Therapy; Anti-oxidative activity

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Core tip: Inflammatory bowel diseases (IBDs) are degenerative diseases that causechronic inflammation in the intestine. The most prevalent therapy for IBD is conventional antibiotic therapy. Keeping the adverse effects of antibioticsin mind, researchers have shownthat *Streptococcus, Lactobacillus* and*Bifidobacterium*are some of the most efficient antioxidative agentswith respect to *in vitro* and *in vivo* activities. Probiotics individually or in combination play an important role in regulating superoxide dismutaseactivity, which is always dysregulated due to oxidative stress caused in IBD. The mechanism of antioxidation of probiotics using NRf2-antioxidative response element pathway, nuclear factor-κB and protein kinase C pathway may be activated to contribute to the reduction of oxidative-stress-induced IBD. The review focuses on the antioxidative activities of the specific bacterial strains as therapeutic molecules in IBD. Multiple combinations of probiotic strains havestill not been adequately studied. We are currently researching the antioxidative effect of *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*in combination.

INTRODUCTION

Inflammatory bowel disease (IBD) is an umbrella term used to describe chronic inflammation in the human digestive tract. IBDs are characterized by diarrhea, rectal bleeding, abdominal pain, fatigue and weight loss. IBDs are prevalent in western countries, although they are on the rising track in the asian countries, which mimics the prevelance American and European countries. When the burden of IBD is compared betweeneastern and western countries, the prevalence of IBD in India, which is one of

the eastern countries, is found to be the highest. The imbalance in pro-oxidants and antioxidants in the gut leads to inflammation. Despite having antibiotic medication, the prevalence ofIBD is still high worldwide. Thus, there is a need toinvestigatesmall molecule therapeutic approaches to stop the increasein the number of cases of IBD.In humans, reactive oxygen species (ROS) function as regulators and mediators to ensure correct cell functioning[1,2]. Overproduction of ROS can easily induce damage to proteins, nucleic acids or lipids through free radical reactions. Therefore, in the event of excess ROS production, protective antioxidant mechanisms are required to prevent oxidative stress[1,2]. ROS include superoxides, nitric oxides (NO), hydroxyl radicals, singlet oxygen and hydrogen peroxide (H_2O_2) that contributes to cellular damage, leading to inflammation. IBD is known for the occurrence of oxidative stress. Ulcerative colitis (UC), which is one typeof IBD, leads to the increased generation of highly toxic ROS that exceeds the capacity of the limited intestinal antioxidative defense system[3,4]. Oxidative stress in IBD is the key factor for progression of inflammation and is identified by the increased production of ROS, decreased antioxidant molecules and enzymes (β-carotene, vitamin C and vitamin E) and enhanced lipid peroxidation in the intestine[5].

In the inflammatory processes, intestinal cells of inflamed tissue in response to chemical agents or pathogens, produce high levels of ROS and superoxide anions[6]. Exposure to antigens for a short period of time does not cause any harm because of the adequate first-line defense system producingantioxidative enzymes for protection[6]. However, in chronic intestinal inflammation, there is persistent high ROS production. This process damages the intestinal epithelial barrier, enhances inflammation and injures the intestinal epithelium[6]. Lipid peroxidation is another process that involves a source of secondary free radicals, which directly interact with other biomolecules. The lipid peroxidation depends on the number of double bonds; therefore, polyunsaturated fatty acids are the most susceptible to oxidation. Lipid peroxidation occurs on polyunsaturated fatty acids located on thecell membrane[7]. Superoxide anion radicals, H_2O_2 and hydroxyl radicals secreted by neutrophils and other phagocytes, causes cell

membrane to be impaired, eventually leading to cell death by lipid peroxidation[6]. Enhanced free radicals in the gut can exert peroxidation of membrane phospholipids of intestinal epithelial cells, resulting in the release of toxic products like malondialdehyde (MDA) that can cause damage and cellular stress. MDA is the key breakdown product of lipid peroxides, which is present in the plasma of IBD patients[6]. Increased level of MDA in plasma of Crohn's disease (CD) patientsis considered to be anoxidative stress marker[6]. Decreased superoxide dismutase (SOD)-2 expression is one of the identification markersin colitis-induced mice.

The current preferred therapies for IBD include 5-aminosalicylate, steroids, corticosteroids and azathioprine[8]. The limitations of IBD therapy include the clinical adverse effects of antibiotics, corticosteroids and immunomodulators,which revolves around nausea, vomiting, stomach pain, diarrhea, headaches, respiratory infections, acne, weight gain, insomnia, dizziness,muscle or joint cramps and pathological side effects, causing some pathogenic bacteria to become resistant in IBD. Surgery isgenerally costly and unaffordable to many people inremote areas. Also it can cause harm to many organs. Thus, the literature reviews have confirmed the apparent need for improvised treatment using small molecules, like probiotics[9]. Nowadays, 60%–80% of the world population relies on alternative medication to cure IBD.Probiotics are preferably of human origin: they have to be safe for the host, genetically stableand capable of surviving throughout the gastrointestinal tract. Probiotics are generally applicable for viable cells, whereas, postbiotics are soluble factors (either secreted by live bacteria or released after bacterial cell lysis), which are beneficial to human hosts. Probiotics have recently emerged as one of the powerful novel therapeutic small molecules against IBD.The have been shown to have a positive effect on oxidative stressby promoting the potency of theantioxidative defense system,and in turn may lower the risk of several inflammatory disorders such asIBD. Various known probiotics play an important role in antioxidative activity. Probiotics could be a possible intervention for reducing ROS and lipid peroxidation and thereby increasing SOD activity. Our goal was to review on the oxidative stress during IBD and *in vivo* and *in*

vitro antioxidative activities of probiotics. Antioxidative activities of probiotics like *Streptococcus*, *Bifidobacterium* and *Lactobacilli* against oxidative stress in IBD are the main focus of the review.

MECHANISM OF OXIDATIVE REACTION INSIDE A CELL

Oxidative stress occurs due to an imbalance between free radical production and antioxidant defense, resulting in hydroxylation of DNA, denaturation of proteins, peroxidation of lipid, and apoptosis, ultimately compromising cell viability[10]. An excess of oxidative stress can lead to the oxidation of lipids and proteins, which is associated with changes in their structure and function. $H_2O_2$is formed by dismutation of superoxidesor direct reduction of oxygen. H_2O_2 can penetrate most of the cell membranes and react with iron in the cell to form hydroxyl radicals. Therefore,hydrogen peroxides are more cytotoxic than superoxide anion radicals. The oxidative modification of lipids, proteins, nucleic acids and carbohydrates isinduced and mediated by both free radicals and nonradical activities of reactive species[7,11].Superoxidesareunreactive molecules but undergo dismutation or enzymatic catalysis to form H_2O_2[7,11].Hydroxyl radicals are thought to initiate ROS and remove hydrogen atoms. This form of radical is extremely reactive and attack most cellular components[7,11](Figure 1).

MECHANISM OF ANTIOXIDANT MOLECULES

To neutralize the damaging effect of oxidative stress, we need supplements that possess some antioxidative activities. Antioxidants are proteins or enzymes in nature. Antioxidants inhibit cellular damage mainly through their radical scavenging properties[12]. The principle micronutrients that can scavenge free radicals are vitamin E, Vitamin C and β-carotene. Humans cannot produce these antioxidant micronutrients. So, they must be supplied through thediet[7].SODcatalyzes the breakdown of superoxide anions into oxygen and H_2O_2using Zn/Cu, Fe/Mn and Ni as cofactors[10,13]. Only a few species of *Lactobacillus*, *Lactobacillus casei*, *Lactobacillus paraplantarum*,

Lactobacillus bucneri and *Lactobacillus sakei* exhibit SOD activity. Catalases are the common enzymes found in all living organisms, which are frequently used by cells to catalyze the decomposition of H_2O_2 to water and less reactive gaseous oxygen[10].

The nicotinamide adenine dinucleotide phosphate (NADP) oxidase/NADP peroxidase enzyme system prevents oxygen accumulation in bacterial cells by formation of H_2O_2 followed by water. This maintains an intracellular redox balance[10,14]. Antioxidants work by scavenging free radicals, preventing production of free radicals and improving levels of endogenous antioxidants. Scavenging antioxidants remove active species rapidly, before they react with biologically essential molecules in the body. This antioxidants function by scavenging active free radicals before they attack biologically essential molecules by donating hydrogen atoms to give stable compounds.

PROBIOTICS AS ANTIOXIDANT SMALL MOLECULES

When the antioxidant capacity of damaged mucosa is compromised, various natural substances can act as antioxidant molecules to inhibit ROS generation, cell damages and improve the activity of antioxidative enzymes in cells. A food can be considered as functional, when it is demonstrated to provide nutritional effects for health and well-being and reduction of the risk of disease. Ingredients that make foodsfunctional are: dietary fibers, vitamins, minerals, antioxidants and essential fatty acids. One of the novel approaches as therapy against oxidative stress are the development of probiotics[16,17]. Probiotics are the functional foods that possess antioxidant properties[7,15]. Several studies have highlighted that the ability of probiotics are to enhanceantioxidant properties. For probiotics growth, milk can be used as a substrate for starter microorganisms. Naturally, milk hasits own antioxidant activities due to the presence of bioactive compounds of whey proteins, caseins, lactoferrin, urate, ascorbate, α-tocopherol, β-carotene as well as enzymes like SOD, catalase and glutathione peroxidase. Fermented milk with probiotic microorganisms hasfurther improved antioxidant potential[18]. Furthermore, the fermentation of soyabean extract using probiotic cultures of lactic acid bacteria possesses superoxide radical scavenging and

reducing activities. Soybeans contain SOD, which possesses the superoxide anion scavenging effect. Soymilk obtained from soybean is also expected to possess SOD. The fermented soymilk has an increased superoxide-anion-scavenging effect due to the production of secretory byproducts in the presence of lactic acid bacteria[19].

MODES OF ANTIOXIDATIVE ACTIONS OF PROBIOTICS

Probiotics can directly act to neutralize oxidants by the production of antioxidant enzymes. The antioxidant mechanism of probiotics could be assigned to ROS scavenging, chelation of metal ions, enzyme inhibition and their reducing ability. Probiotics have an antioxidant effect by scavenging of oxidants or by prevention of generation of free radicals in the intestine. Probiotics can upregulate the intracellular activity of SOD, catalase and glutathione peroxidase to protect the cells from intracellular damage. Pro-oxidative metal ions are capable of initiating decomposition of H_2O_2 into radicals and triggering lipid peroxidation. Certain chelators are normally detected in probiotics, stating the chelating capacity of probiotics[8,18]. According to reviews, *Lactobacillus rhamnosus* and *Lactobacillus paracasei* have significantly inhibited the production of hydrogen peroxide, whereas, *L. casei* also possess high antioxidant activity *via* chelating Fe^{2+}[10,21]. Different *in vitro* and *in vivo* studies have reported that probiotic bacteria can protect against oxidative stress through regulation of the Nrf2 (Nuclear factor erythroid 2-related factor 2)–Keap1–antioxidant response element (ARE) pathway, protein kinaseC (PKC) pathway and nuclear factor (NF)-κB pathway[7,10,22].

The Nrf2–Keap1–ARE system transmits signal into the nucleus. Under normal conditions, Keap1 is associated with Nrf2. However, in ROS infiltration in cells, the bond between Keap1 and Nrf2 is cleaved and Nrf2 eventually enters the nucleus and binds to ARE and enhances the production of the antioxidative enzymes production[7,10,23]. ROS activates NF-κB, entailing expression of inflammatory cytokines. NF-κB responds to oxidative stress. Thus, the probiotic formulations (*Lactobacillus sp.*,*Bifidobacterium sp.* and *Streptococcus sp.*) are able to inhibit NF-κB activation in colonic epithelial cells[10,24](Figures 2 and 3).

PKCs are the family of protein kinases that are the target for redox modifications. Administration of *L. plantarum*improved the oxidative stress in a rat model of obstructive jaundice by strengthening the expression and activity of the PKC pathway[10,24,25].

IN VITRO AND *IN VIVO* ANTIOXIDATIVE ACTIVITY

Not all the probiotics have antioxidant activity due to high strain heterogeneity. *Bacillus proteolyticus*showsthe highest 1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radical scavenging activity[26]. Wang *et al* reported that *Bacillus amyloliquefaciens* could significantly increase the antioxidative capacity of epithelial cells to reduce induced oxidative stress in pigs[26]. *Bacillus subtilis* and *L.casei* can scavenge free radicals (*in vitro*) and reduce oxidative damage by improving lipid metabolism followed by reduction in lipid peroxidation. *Streptococcus thermophilus* (YIT 2001) showed the highest *in vitro* antioxidative activity against lipid peroxidation[27]. *Lactobacillus* and *S. thermophilus* showed the highest TAA_{LA} (total antioxidant activity against linoleic acid oxidation) and TAA_{AA} (total antioxidant activity against ascorbate auto-oxidation). The cell-free extracts and intact cells of *Lactobacillus acidophilus* (ATCC4356) demonstrated an increased inhibition of linoleic acid peroxidation from 38% to 48%. This indicates astrong antioxidative activity[14]. *Bifidobacterium longum* was also investigated for inhibition of lipid peroxidation activity.

In-vitro cell based antioxidative activity

Stress induced HT29 cells, *i.e.*, H_2O_2-stimulated HT29 cells showed a reduced amount of intracellular SOD, catalase and increased ROS activity. The cultured cells were treated with probiotics for 24h. The supernatant of the cells was collected to study the presence of the antioxidative enzyme activity of SOD and catalase[28]. The *Bifidobacterium bifidum* treated cell line showed increased catalase activity. SOD and catalase production by *B. bifidum* can decrease oxidative stress. Moreover, *in vitro* studies showed that strains like *L. acidophilus* and *Lactobacillus delbrueckii* displayed highest superoxide anion radical

dismutation. *L. plantarum* showed increased ability to degrade chemically pure H_2O_2 and demonstrated the highest catalase activity[29]. SOD activity was found in *Lactococcus, S.thermophilus* and *Bifidobacterium*, with significantly higher activity in *Lactococcus* than in *S. thermophilus*[14]. SOD activity of cell-free extracts of the above-mentioned probiotics wasstudied by the amount of inhibition of reduction of nitroblue tetrazolium[14]. Greatest SOD activity was demonstrated by *Lactococcus* strains. Glutathione was analyzed in deproteinized bacterial cell-free extract using a commercial kit that showed thatthe *Lactococcus* group hadthe highest inhibitory effect[14]. However, *S.thermophilus, Lactococcuslactis* and *Bifidobacteriumanimalis* also containedrelevant amounts of intracellular reduced and oxidized forms of glutathione. Total glutathione measurement was carried out in presence of glutathione reductase and NADP[14].

Invivo probiotic antioxidative activity

In an animal model of IBD, it was observed that *L. acidophilus* with dismutase-like activity wasmore effective than *L. plantarum*in suppressing the inflammatory process[29]. *In vivo* studies have also revealed that *L.plantarum* 0B and *L. acidophilus* has the highest catalase activity and highest dismutase-like activity respectively. Male Wister rats were administered probiotic formulation (mixture of *B.animalis, L. acidophilus*DSMZ 23033and*Lactobacillus brevis* DSMZ 23034) after acclimatization of rats in cages. After 18 d of probiotics supplementation, blood plasma was collected to study the antioxidant status[14]. Reactive oxygen metabolite(ROM) concentration of plasma was evaluated as studied by d-ROM test. Plasma total antioxidant activity (TAA) was spectrometrically measured in the presence of 2-binamine-di-3-ethylbenzothiazolin-6-sulfonic acid (ABTS) radical by evaluating the decoloration and reduction of radical cations of ABTS[14]. Plasma ROM concentration was inversely related to the dose of administered probiotics[14]. TAA was significantly related to the dose of administered probiotics. In another study, oral administration of *Bifidobacterium breve*yakult appeared to prevent

transepidermal water loss and significantly suppress oxidation of lipids, proteins and H_2O_2 levels[31].

The antioxidant activity of buffalo milk fermented with *B.bifidum* and *L.acidophilus* was evaluated. Control groups included mice fed with standard dahi without probiotic enrichment and another with fermented milk. Catalase and SOD activity in blood was analyzed[27,31]. SOD activity in red blood cellsincreased exclusively after probiotic dahi administration. Dahi supplemented by *L.casei* NCDC19 and *L.acidophilus* NCDC14 inhibited lipid peroxidation and maintained the activity of glutathione peroxidase, SOD and catalasein streptozotocin-induced oxidative stress inrats[32,33]. *Lactobacillusfermentum*(Lf1) was studied to assess its antioxidative properties, andconfirmed the enhanced expression of NRF2 and MDA inhibition in HT29 cells under stress[34]. In another study it was shown that *S.thermophilus* YIT2001 decreased the amount of lipid peroxide in colonic mucosa and improved the symptoms of DSS-induced colitis in mice[27].

QUANTIFIABLE PARAMETERS THAT INDICATE ANTIOXIDATIVE ACTIVITY

Scavenging activity of ROS is one of the antioxidative properties of probiotics. The Reactive Oxygen Species are used to include both oxygen centered radicals and nonradical derivatives of oxygen. There is the scavenging activity of probiotics for two of the most important ROS, hydroxyl radicals and H_2O_2.

DPPH RADICAL SCAVENGING ACTIVITY

To evaluate the antioxidative activity of probiotics, DPPH solution was mixed in methanol and probiotic sample and incubated at 37°C for 30 min in the dark. The DPPH radical scavenging activitywascalculated by measuring the absorbance of the sample and blank at 517nm.The radical scavenging activity was calculated as follows: [1-(A_{517} (sample)/A_{517} (blank)]× 100%. According to Das and Goyal, DPPH radical scavenging activitywashigher in *L. plantarum* and *L. acidophilus*. Scavenging activity of *Bacillus* rangedfrom 46% to 190%. *B.proteolyticus* showed the highest DPPH radical scavenging

activity, whereas, *B.amyloliquefaciens* had the weakest DPPH radical scavenging activity[36]. Probiotic strains such as*S.thermophilus* and *L.delbrueckii*can scavenge ROS, hydroxyl radicals and H_2O_2[37]. Cell-free supernatants of *Lactobacillus*exhibitstrong DPPH radical scavenging activity[37]. Moreover, the crude peptides extracted from *L. acidophilus, L. casei* and *L. paracasei*have radical scavenging activities forDPPH*in vitro.*

LIPID PEROXIDATION INHIBITION

To study the effectiveness of antioxidants, inhibition of lipid peroxidation is commonly studied. Bacterial strains (*L. acidophilus and B.longum*) and the intracellular cell-free extract indicated an inhibitory rate on linoleic acid peroxidation that ranged from 33% to 46%[38]. *L. acidophilus* and*B.longum* demonstrated a high antioxidative activity for inhibiting lipid peroxidation. Inhibitory rate of different strains of *L.acidophilus*rangedfrom 34.9% to 46.3%[37]. Cell-free supernatants of *Lactobacillus*show higher inhibitory effect thanMRSbroth cell culture. Intact cells or intracellular cell-free extracts of *L. acidophilus* and *B.longum*wereinvestigated for their antioxidative effects, which demonstrated thatinhibition of linoleic acid peroxidation rangedfrom 38% to 48%[34,39]. *Levilactobacillus brevis* exhibited greater radical scavenging activity and lipid peroxidation inhibitory activity than *Pediococcuspentasaceus*[35]. Manystudies related to lipid peroxidation have chosen linoleic acid as the source ofunsaturated fatty acids. Unsaturated fatty acids such aslinoleic acid, methyl linoleate and arachidonic acid are typically used. The protocol forlipid peroxidation assay using linoleic acid has beenstandardized to study the inhibition of linoleic acid peroxidation. Egg homogenate is generally not used for lipid peroxidation inhibition studies in the presence of probiotics. Thus, lipid peroxidation assay using egg homogenate can be used to investigate the inhibition of lipid peroxidation by probiotics.

REDUCING ACTIVITY

Reducing power is based on the kinetics of reduction of Fe^{3+} to Fe^{2+} to prevent the oxidation reaction[37]. Ferric-reducing antioxidant power allows estimation ofthe ability

to reduce pro-oxidant metal ions. The fermented black soybean broths of *B.subtilis* have shown a potent reducing power as compared to positive controls *i.e.*, α-tocopherol andbutylated hydroxytoluene[39]. Cell-free supernatants of *Lactobacillus*strains showed significantly higher reducing powerthan MRS broth containing *Lactobacillus*[38]. Ferric ion reducing antioxidant power assay was performed for the fermented milk with *Lactobacillus sp., S. thermophilus* and *Bifidobacterium sp.* in the presence of green tea supplementation[15]. Fermented milk with 15% green tea infusion (GTI) shows the highest anti-oxidative power as compared to 10% or 5% GTI[15].

SUPEROXIDE ANION SCAVENGING ACTIVITY

Superoxides are radicals with free electrons located on oxygen[16]. These radicals initiate lipid oxidation as the superoxides and H_2O_2 are precursors of singlet oxygen and hydroxyl radicals[17]. Assayscan measure the ability to scavenge superoxide anion radicals. *S.thermophilus* containing fermented milk accounts for the highest superoxide anion scavenging effect as compared to *L. acidophilus*. Archibald and Fridovichshowed that *S. thermophilus* was able to produce SOD,while*L.acidophilus* was not. Fermented soy milk with *L. acidophilus*+ *Bifidobacteriuminfantis*, *L. acidophilus*+ *B. longum*, *S. thermophilus*+ *B. infantis*, or *S. thermophilus*+ *B. longum* shows higher superoxide anion scavenging activity than reducing activity[17]. Thecell-free supernatant of *L. plantarum* and *L.acidophilus* showed a potent inhibitory superoxide radical scavenging activity with increasing concentration compared to ascorbic acid[40]. Xing*et al*[36] had studied an enhanced superoxide radical scavenging activity in co-fermentation conditions in milk (with *B.infantis, L. plantarum, B. animalis* and *S. thermophilus*). *S. thermophilus* exhibited only 58.34% activity, whereas co-fermentationincreasedthe superoxide scavenging activity to 65%.

SCAVENGING OF HYDROGEN PEROXIDE ACTIVITY

H_2O_2can be generated in biological system in oxidative stress conditions. Being a non-radical oxygen containing reactive agent, it can form hydroxyl radicals (the most highly

oxygen radical known). Soymilk fermented with *Bifidobacterium* alone accumulated the largest amount of H_2O_2, whereas, fermented soymilk with *Bifidobacterium* and lactic acid bacteria simultaneously reduced H_2O_2[17].

HYDROXYL RADICAL SCAVENGING ACTIVITY

Among reactive oxygen species, hydroxyl radicals are the most reactive species. It can react with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to the cells. Venkatesan *et al* stated that different concentrations of probiotic species of *Bifidobacterium* and *Lactobacillus* showed strongest radical scavenging activities.The hydroxyl radical scavenging activity of cell-free supernatant of *L. plantarum* and *L.acidophilus* showed potent hydroxyl radical scavenging activity when compared to positive control ascorbic acid. These two specific strains have shown a better DPPH and hydroxyl radical scavenging activity.The radical scavenging activity was calculated as follows: [A(sample)-A(control)/A(blank)-A(Control)]× 100%.Cell-free supernatants of various *Lactobacillus* strains (*L. rhamnosus, L. casei, L. plantarum, L. reuteri, L. acidophilus, Lactobacillus fermenti* and *Lactobacillus parciminis)* were studied through *in vitro* cell-free hydroxyl radical assay. It was concluded that all the *Lactobacillus*strains showed a better scavenging than hydroxyl radical scavenging activities.

ASSESSING THE POTENCY OF PROBIOTICS AS ANTIOXIDANTS

Generally, antioxidants are molecules thatinteract with the free radicals generated in the cells and terminate the chain reaction before damage is done to the vital molecules. In recent years, researchers have witnessed a beneficial effect of probiotics, especially in regulating the oxidative stress in IBD[32]. *Lactobacillus*, *Streptococcus* and *Bifidobacterium* have been shownto have antioxidative activity that can easily scavenge oxidative stress inducing molecules inside a cell.

CONCLUSION

From this review, it can be concluded that, in IBD, high levelsof oxidative stress induceintestinaltissue damage. Oxidative stress is defined as an imbalance between pro-oxidants and antioxidants, and is tightly associated with the exacerbation of IBD. This disturbs the cellular homeostasis by causing cell injury and increased permeability of the mucosal barrier. Probiotics are equipped with antioxidative defense mechanisms, not only to protect theirown survival but also to confer protection to the host cell against oxidative stress during colitis.Probiotics are used to combat IBD by reducing ROS generation and lipid peroxidation and by increasing production of antioxidant enzymes (SOD, catalases and peroxidases)[40]. The most common strains studied, *Bifidobacterium* and *Lactobacillus*, are reported to secrete SODand antioxidant molecules thatcanalleviate oxidative stress in inflamed intestine[41]. Accumulation of probiotic strains in inflamed colon results in some protective effects like, metal-chelating activities, antioxidant enzymes (SOD), eventually showing free-radical scavenging activities by restoring the gut microbiota during colitis. Different *in vitro* studies have suggested that combination of probiotics in fermented milk improve its antioxidative activity[40]. An enhanced superoxide radical scavenging activity of soy milk containing *Bifidobacterium* was observed. Multiple *in vivo* and *in vitro* studies have demonstrated that *Lactobacillus*, *Streptococcus* and *Bifidobacterium* possess outstanding antioxidant activities like DPPH, hydroxyl, superoxide radical scavenging and reducing activities. The important mechanism of antioxidant activities is used by probiotics to reduce oxidative stress, which includes, redox signaling of Nrf2 leading to increase in antioxidant enzyme levels and scavenging of ROS. Moreover, it can also be concluded that multiple probiotic strains in combination is much more effective than single probiotic strain with respect to antioxidative studies. Antioxidant probiotic strains can be selected and investigated as promising candidate for the prevention and control of oxidative stress caused in IBD. Thus, to develop a novel probiotic combination product with the potential for preventing the oxidative stress, there remains a need to search for particular probiotic strains that can be effective in mitigation of oxidative stress in IBD.The mechanism of the reviewed probiotic strains (*Streptococcus, Lactobacillus* and

Bifidobacterium) bywhich they regulate the oxidative stress based cellular cascade in IBD conditions, needs to be investigated in detail and validate these antioxidative properties in specific *invivo* models. Likewise, our novel combination probiotics (*S.thermophilus*, *L.acidophilus* and *B.bifidum*) areunder investigationwith respect to theirantioxidative properties.

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