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Probiotics and Oxidative Stress

Tiiu Kullisaar, Epp Songisepp and Mihkel Zilmer
University of Tartu, Bio-competence Centre of Healthy Dairy products LCC, Estonia

1. Introduction

A large number of reports about the health benefits of probiotics could be found in the PubMed database. Very little information is available about probiotics possessing physiologically relevant antioxidative properties. Quite scarce is information on the influence of probiotics on human body oxidative stress status and a limited number of clinical trials have been conducted on the effect of antioxidative lactic acid bacteria on human oxidative stress-driven cardiovascular disease-related aspects. In this chapter possibilities of antioxidative probiotics to influence on oxidative stress status in human body are discussed.

2. Short survey of probiotics

The potential life-lengthening properties of lactic acid bacteria (LAB) were proposed by Metchnikoff already at the beginning of the 20th century. The term "probiotic" is an etymological hybrid derived from Greek and Latin meaning "for life" (Hamilton-Miller et al., 2003). Today probiotics are defined as live microorganisms which, when consumed in appropriate amounts, confer a health benefit on the host (FAO/WHO, 2002). Genera most commonly used as probiotics are *Lactobacillus* and *Bifidobacterium*, but other LAB such as lactococci, streptococci, enterococci as well as propionibacteria, bacilli (e.g. *Bacillus subtilis*) and yeasts (e.g. *Saccharomyces boulardii*) are applied. However, probiotics are usually LAB. Introducing a new probiotic into the market involves a step-wise process in order to obtain a functional and safe product (Saarela et al., 2000; Vankerckhoven et al., 2008). Exact requirements are set for probiotic bacteria. Centuries-long use of LAB in the food industry has proven their safety. Nevertheless, it is compulsory to test the safety of each new potential probiotic. The recommendations include an absence of hemolytic activity and the transferable antibiotic resistance of the selected strain, while safety should be proven in various animal models (FAO/WHO, 2002; Vesterlund et al., 2007; Kõll et al., 2010). There is a necessity for pilot clinical trials on healthy volunteers to exclude the adverse effects of probiotic administration on gut health, biochemical and cellular indices of blood reflecting the proper functions of human organs (Reid, 2005; Rijkers et al., 2010). Probiotics must be able to resist stomach acid, bile and the effects of digestive enzymes. Thus, potential probiotic candidates will be selected mostly from human normal microflora. The ability to survive in the GI tract, adhere to intestinal epithelium and maintain its metabolic activity is directly related to the manifestation of probiotic properties in the human body. Probiotic properties are strictly strain-specific. Even the related microbial species may have very

different clinical effects. Thus, one cannot arbitrarily attribute the properties of one probiotic strain to another, even within the same species (Vaughan et al., 2005).

Probiotic effects have a dosage threshold. The minimum effective dose, which affects the intestinal environment and provides beneficial effects on human health, is considered to be 10^6-10^9 live microbial cells per day. The minimum dose depends on the particular strain and the type of foodstuffs (Reid, 2005, Williams, 2010, Champagne et al., 2011). Probiotics have been demonstrated to be effective in a variety of conditions including the relaxation of intestinal discomfort (bloating and pain), the alleviation of chronic intestinal inflammatory diseases, the prevention and treatment of pathogen-induced diarrhea, lowering lactose intolerance and food allergies, the lowering of cholesterol levels, the prevention of urogenital infections and the reduction of atopic diseases (Andersson et al. 2001; Chapman et al., 2011). The important area of human physiology that is relevant to functional food science according to the ILSI and FUFOSE (the European Commission Concerted Action on Functional Food Science in Europe) is, among others, the modulation of the defence against high-grade oxidative stress. The latter is one of the principal players in the pathogenesis of CVD and other diseases. Thousands of reports reflecting the abovementioned different health benefits of probiotics could be found in the databases. However, scarce information is available regarding probiotics possessing physiologically relevant antioxidative properties and a limited number of clinical trials on the effect of antioxidative LAB on human CVD-related aspects have been conducted.

3. Short survey of oxidative stress

A net of pro-oxidants and the potency of an antioxidant defence system normally balanced in the body. Principal pro-oxidants are reactive species (including free radicals) divided into reactive oxygen species (ROS) and reactive nitrogen species (RNS) and they mediate the main effects of other pro-oxidative factors (Sies, 1991; Halliwell & Gutterridge, 1999). In the organisms the crucial ROS are superoxide radical, hydroxyl radical, lipid peroxyl radical and non-radical hydrogen peroxide (the latter is produced from superoxide by superoxide dismutase) and the principal RNS are nitric oxide and non-radical peroxynitrite. The pathological efficiency of the hydroxyl radical is the most potent and it is rapidly generated via the Fenton cycle where free iron (a very potent pro-oxidant) reacts with hydrogen peroxide (Halliwell & Gutterridge, 1999). Most of the mentioned reactive species (RS) come from endogenous sources as by-products of normal essential metabolic processes, while exogenous sources involve exposure to cigarette smoke, environmental pollutants, radiation, drugs, bacterial infections, excess of food iron, dysbalanced intestinal microflora, etc. Several diseases are associated with the toxic effect of the transition metals (iron, copper, cadmium). Thus, abnormal formation of the RS can occur *in vivo* and that leads to the damage of lipids, proteins, nucleic acids and carbohydrates of cells and tissues. An excessive production of RS causes an imbalance in the pro-oxidants/antioxidants system. Any imbalance in favour of the pro-oxidants potentially leading to damage was termed 'oxidative stress' (Sies, 1991). Recently an additional adapted conception of oxidative stress (OxS) was advanced as "a disruption of redox signalling and control"(Jones, 2006), emphasizing an impact of the redox ratio as good tools for the quantification of OxS. It is remarkable that the glutathione redox ratio has a crucial impact concerning this conception. A large body of evidence confirms that high-grade OxS is one of the crucial players in the pathogenesis of disorders/diseases (cf

below). To maintain the physiological grade of OxS needed for a number's biofunctions like intracellular messaging, growth, cellular differention, phagocytosis, immune response, etc the human body has an integrated antioxidative defence system (IADS, Table. 1). Several antioxidative components for this human IADS are derived from foodstuffs and provided by GI microbiota. Interestingly, it became more and more apparent that the IADS of the host and GI microbiota are tightly linked and some specific strains with physiologically effective antioxidative activity may have a great impact on the management of the OxS level in the gut lumen, inside mucosa cells and even in the host blood, to support the functionality of the IADS of the human body. Thus, experiments to find out strains with physiologically relevant antioxidative properties/effects as well as trials (including special clinical trials) using capsules of such strains or foodstuffs enriched with antioxidative strains are needed. Unfortunately, scientific data on probiotic LAB with physiologically relevant antioxidative properties is very limited and the data of experiments/trials about both intestinal antioxidative protection/influence and systemic antioxidative protection/influence (effects of OxS-related indices) are scarce.

Oxidative stressors (pro-oxidants)	Integrated antioxidative defence system
Ischemia/reperfusion Smoking, Inflammation, xenobiotics PUFA megadoses Iron or copper excess Radiation, Exhaustive exercises Prolonged severe emotional stress	Vitamin E, C, Q, A Enzymes as antioxidants (SOD, GPx, CAT, HO1) Other antioxidants (GSH, plasma albumin, uric acid, Bilirubin, carotenoids, etc)

Table 1. A net effect of oxidative stressors and the potency of the integrated antioxidant defence system (IADS) of the body are normally balanced. An imbalance leads to potentially harmful oxidative stress. PUFA, polyunsaturated fatty acids; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase; HO1, haem oxygenase1; GSH, reduced glutathione.

However, as a certain progress has been made during recent years and we will give a summarized overview about probiotics and OxS.

4. Short survey of oxidative stress-related pathological states (CVD, metabolic syndrome, allergy, atopic dermatitis, radiation induced problems in the intestinal tract)

A large body of evidence exists that high-grade OxS has one of the crucial roles in the pathogenesis of disorders/diseases of the vascular system (atherosclerosis, myocardial infarction, stroke, peripheral artery disease), the nervous system (Alzheimer's disease, Parkinson's disease), the liver (cirrhosis, ethanol damage), the skin (dermatoses), the pancreas (diabetes mellitus), metabolic syndrome, obesity, premature ageing, the eyes (age-related macular degeneration, retinopathy), development of some tumors and the GI (inflammatory bowel disease, coeliac disease, etc), etc (Halliwell & Gutterridge, 1999; Stocker & Keaney, 2004; Kals et al., 2006; Stojiljkovic et al., 2007; Krzystek-Korpacka et al., 2008; Tsukahara, 2007; Suzuki et al., 2007; Vincent et al., 2007; Castellani et al., 2008). It has recently reviewed that harmful GI consequences of radiation therapy have OxS-related background (Spyropoulos

et al., 2011). Firstly, radiolysis of water molecules causes rapid production of ROS, secondly, an increase in oxygen radical production in the vascular wall has shown already 2h after irradiation with a more intense OxS observed at 6h, this second burst being produced mainly by infiltrating inflammatory cells (Molla & Panes, 2007).

Prolonged excessive ROS/RNS production can trigger chemical chain reactions with all major biomolecules such as DNA, proteins, and membrane lipids. DNA is affected with a variety of lesions like oxidized bases, stand brakes, as well as DNA–DNA and DNA–protein cross-links (Barker et al., 2005). Oxidatively damaged proteins are characterized by formation of carbonyl groups (Stadtman, 1992). Hydroxyl radicals depolymerize hyaluronic acid, degrade collagen, inactivate enzymes and transport proteins via sulfhydryl oxidation. RNS may induce nitration of protein tyrosine residues. Lipid peroxidation is the oxidative degradation of membrane lipids and oxidation that can cause severe impairment of membrane function through changes in membrane permeability and fluidity, its protein oxidation, ultimately leading to cell lysis (Halliwell & Gutterridge, 1999). Lipid peroxidation also damages blood lipoproteins. Therefore, prolonged high-grade OxS causes damages in biomolecules, cells, tissue and organ functionality. Reactive species-damage can be evaluated via markers for oxidized proteins (i.e. nitrated tyrosine, protein carbonyls); oxidized nucleic acid bases (8-oxo-2-deoxyguanosine), oxidized carbohydrates (glycated products) and oxidized lipids (F2-isoprostanes, oxidized low-density lipoproteins (oxLDL), etc). Additional approach for investigations of OxS is an assay of the capacity of IADS (i.e. assay of total antioxidative status or response (TAS, TAR), etc). All these markers are informative but they are not still ultimately accepted as new risk markers yet. However, recently pathogenetic relevance of isoprostanes and oxLDL has been accepted (Statements of European Food Safety Authority). A large number of articles shows that oxLDL level is associated with development of cardiovascular diseases (CVD). Thus, to describe both process and status of atherosclerosis common risk markers like low-density lipoprotein or LDL-cholesterol, HDL-cholesterol, fasting triglycerides (TG), plasma homocysteine as well as by new additional OxS- and inflammation-related indices (oxLDL, 8-isoprostanes, highly sensitive C-reactive protein) should be used. All these markers are also diet-related markers (Mensink et al., 2003). It is reviewed that OxS indices (oxLDL, urine 8-isoprostanes, etc) together with the increased inflammatory markers (white blood cells (WBCs), highly sensitive C-reactive protein have been shown to be characteristic of patients with atherosclerotic lesions of the vascular system (Stocker, Keaney, 2004). Consequently, probiotics with physiologically relevant multivalent antioxidative properties/effects expressed via a positive influence both on a GI and systemic OxS level may have impact concerning the pathogenesis of different disorders/diseases, particularly CVD.

5. Properties of probiotics necessary to have an influence on oxidative stress status

5.1 Role of probiotics in intestinal antioxidative protection (possible action mechanisms)

The most documented effects of LAB in humans are the stimulation of the immune system, the prevention and the reduction of the intensity and duration of diarrhea, and reduction of lactose intolerance (Wolvers et al., 2010). LAB also have some other beneficial effects such as vitamin synthesis, improvement of mineral and nutrient absorption, degradation of

antinutritional factors, and/or modulation of GI physiology and reduction of pain perception. Special probiotic strains may induce the expression of receptors on epithelial cells that locally control the transmission of nociceptive information to the GI nervous system (Rousseaux et al., 2007). Beneficial bacteria have enzymatic equipment which enables them to break down a wide variety of food constituents that cannot be metabolized by the host such as galactooligosaccharides, inulin, resistant starches, and antinutritional factors such as tannins or phytates responsible for the chelation of minerals including iron, zinc, magnesium and calcium (Gilman & Cashman, 2006; Songre-Quattara et al., 2008; Cecconi et al., 2009). They can also modify the host gut physiology by increasing the production of growth factors (Alberto et al., 2007). LAB may thus be of benefit to health and help protect against diseases, like CVD, diabetes, metabolic syndrome, etc. As far as OxS is at least one of the components of initiation and/or the development of the mentioned diseases thus any kind of agent which can prevent the development of harmful OxS has a principal impact. Probiotics involve LAB or bifidobacteria of human origin. They can during the consumption period adhere to the epithelial cells of GI modulating the human physiological status via the gut associated immune system and/or directly due to the expression of receptors of GI and/or systematically. LAB beneficial effects are strain-specific. *In vitro* and cellular models, the probiotic properties of lactobacilli have been limited to few parameters such as the ability to survive low (pH 2-3) and bile salts, to produce pathogen inhibitory compounds (including hydrogen peroxide), to compete with energy availability or adhesion sites, and to enhance immune response (Ryan et., 2008; Todorov et al., 2008; Pfeiler & Klaenhammer, 2009). Along with the probiotics themselves, there are metabiotics i.e, the metabolic by-products of probiotics. Metabiotics are beneficial in promoting a healthy GI by creating an environment most favorable to probiotics, by nourishing the enterocytes, reinforcing mucosal barrier function, by maintaining or supporting epithelial integrity or signaling the immune system to limit inflammatory responses both in the gut and through influencing T-cells throughout the body. The principal metabiotics are short-chain fatty acids but also other substances like polyamines (putrescine, spermidine, spermine) have an impact (Larqué et al., 2007). It has been demonstrated, that NO produced by LAB protects mucosa for damages and excessive permeability (Payne et al., 1993; Korhonen et al., 2001).

Since 1993 when Kaizu and co-workers discovered antioxidative activity of LAB, a few of them have had effects in clinical human trials (Kaizu et al., 1993). One of them is antioxidative-antiatherogenic and antimicrobial probiotic *Lactobacillus fermentum* ME-3 (LfME-3). Tartu University has patented this strain in Estonia, Russia, USA and Europe. LfME-3 (DSM 14241) is of human origin (Sepp et al., 1997) and has proven its safety as a probiotic exhibiting both antimicrobial and antioxidative benefits under different *in vitro* and *in vivo* conditions (Kullisaar et al., 2002, 2003; Truusalu et al., 2004; Songisepp et al., 2005; Järvenpää et al., 2007). What makes this strain such a powerful multivalent antioxidant? It is confirmed that *in vitro* the superoxide anion scavenging efficiency of LfME-3 was more than 80-100 times stronger as compared with trolox or ascorbic acid (Ahotupa, personal communication). LfME-3 expresses Mn-superoxide dismutase (MnSOD) activity, can effectively eliminate hydroxyl and peroxyl radicals, and has the complete glutathione system (reduced glutathione, glutathione peroxidase, GPx, glutathione reductase, GRed) necessary for glutathione recycling, transporting and synthesis (Kullisaar et al., 2002, 2010). Mn-SOD is very important in the control of LP. Manganese and Mn-SOD

activity of LAB (not possessing catalase) is important for their survival in the oxidative milieu (milk, host) created by the production of hydrogen peroxide (Sanders et al., 1995). It has been shown that some LAB (*L.gasseri*) engineered to produce SOD reduce the inflammation in the case of colitis in interleukin-10-deficient mice (Carroll et al., 2007).

Glutathione (*L*-gamma-Glu-*L*-Cys-Gly or GSH) is a major cellular non-enzymatic antioxidant. It eliminates lipid- and hydroperoxides, hydroxyl radical and peroxynitrite mainly via cooperation with Se-dependent glutathione peroxidase (Zilmer et al., 2005). The GI surface is an important host organism-environment boundary and the interactions of gut microbes inside the intestinal lumen and mucosal cells are important for the host. An impaired environment such as the imbalance of GI microbiota, but also the increase of LP and decrease of the reduced GSH both at the GI surface and in the GI cells, are the mighty modulators causing different unhealthy outcomes in the host. In this process the involvement of the glutathione system is crucial as GSH, besides its role as a crucial antioxidant, is the principal redox controller for a number of processes in cells. Glutathione-related data has impact for LfME-3 regarding at several aspects (cf. 5.2). Thus, confirmation of the presence of all glutathione system components in a specific concrete LAB gives very valuable information as it shows that a specific LAB strain has especially high oxygen and ROS tolerance under different stress conditions. An essential physiological trait for probiotics is tolerance to stress in the GI as well as during the production of functional foods (Ross et al., 2005). Beside that GSH has essential role in maintaining mucosal integrity. Studies have shown diminished GSH levels in inflammatory diseases of intestine and GSH supplementation has beneficial effect (Coskun et al., 2010).

Evidently some probiotics are able to promote an elevation of the level of beneficial bacteria in the GI. In experiments and clinical trials, the administration of the LfME-3 strain has led to the improvement of the GI microbial ecology. More than a 10-fold increase of total lactobacilli counts in comparison with the individually different initial count was registered in the collected faecal samples (Mikelsaar & Zilmer, 2009). The metabolites secreted by LfME-3 into the GI tract could be used as a substrate by other lactobacilli. Adding LfM-3 as a probiotic into a dairy product (yoghurt, cheese, milk) also suppressed the putative contaminants of food (*Salmonella spp., Shigella spp.*,). The secreted substantial amount of hydrogen peroxide and the production of NO by LfME-3 are the main antimicrobial agents (Mikelsaar & Zilmer, 2009. Animal studies have confirmed that the increase in total LAB counts as much as the specific LfME-3 strain antioxidative action in the GI eradicated live salmonellas and prevented the formation of typhoid nodules in experimental *Salmonella Typhimurium* infections, resembling typhoid fever in humans (Truusalu et al., 2004, 2008). It was the first time that the antibiotic therapy of an invasive infection like enteric fever was shown to be more effective if administered together with a probiotic.

5.2 Role of probiotics for systemic antioxidative defence (possible action mechanisms)

Such information is limited. However, some specific multifunctional probiotics may have an influence on systemic (blood) antioxidative defence and the OxS status of host. Thus, to characterize the role of high-grade OxS in the pathogenesis of CVD, we will give an overview about the possible action mechanisms of probiotics on OxS-related indices of CVD.

On the basis of simplified general understandings it can be speculated that there are several factors that may have an impact on OxS. This is only one of the examples. It can be speculated that the suppression of *Helicobacter pylori* infection by some LAB (Wang et al., 2004; Cruchet et al., 2003; Linsalata et al., 2004) may have a certain effect on the host OxS-related indices in blood. However, such approaches are actually only speculations. Why? An analysis of scientific literature allows one to conclude that for a real effect on the systemic OxS-related indices of a host, a specific probiotic strain should have multifunctional bioquality: a) to have positive effects on GI total lactobacilli counts; b) to be able to suppress putative contaminants of food; c) to have biovaluable different antioxidative properties; d) to have a positive effect on OxS-related CVD markers, like TG, oxLDL, etc. In section 5.1. it was explained that the probiotic LfME-3 carries first three types (a,b,c) of properties. Thus, these multifunctional properties of LfME-3 may protect the host against both food-derived infections and help in the prevention of the oxidative damage of food. For example, the antioxidative protection provided by the LfME-3 strain for the prevention of the oxidative spoilage of semi-soft cheeses was found out (Järvenpaa et al., 2007). Thus, points a, b and c have an impact on the role of probiotics for systemic antioxidant defence. However, it is crucial also to have data (according to point d) about the specific influence of probiotics on OxS-related CVD markers. Since LfME-3 has been carefully investigated, concerning the latter we will use gathered information as a model to discuss possible mechanisms on how probiotics may have an influence on the OxS-driven CVD risk markers of a host.

We repeatedly showed that administering a food products to humans comprising strain LfME-3 enhances the systemic antioxidative activity of sera (increases total antioxidative activity, TAA, or total antioxidative status, TAS), enhances the lag phase of LDL (increases oxi-resistance of LDL particles, i.e. suppresses production of atherogenic oxLDL) and decreases level of oxidized glutathione (pro-oxidant), oxLDL and BCD-LDL of sera (Kullisaar et al., 2003, 2011; Mikelsaar et al., 2007). Clinical trials showed that the strain LfME-3 alleviates inflammation and OxS-associated shifts in gut, skin and blood (Kullisaar et al., 2003, 2008; Kaur et al.2008). This realizes via complicated cross-talk between probiotic and host body cells via the integrated influence of several factors of strain LfME-3 like having complete glutathione system, the expression of antioxidative enzymes, the production of CLA and NO by strain LfME-3, etc (Mikelsaar & Zilmer, 2009; Kullisaar et al., 2010, 2011). This strain survives in different fermentation processes of milk due to its good tolerance to low temperature, acid and salt (Songisepp et al., 2004; Songisepp, 2005) and is able for temporal colonization of the GI tract of the consumer. All this is very important as the GI surface is a crucial host organism-environment boundary and the interactions of GI microbes inside the intestinal lumen and mucosal cells have impact for the metabolic activity both microbes and host cells. An impaired environment (the imbalance of GI microbiota, the increase of LP, decrease of the GSH) both at the intestinal surface and in the intestinal cells, are substantial modulators causing unhealthy outcomes in the host. In addition, data that these cellular modulators of the intestinal mucosal status can be repaired by applying of strain LfME-3 was confirmed by using a mouse model of experimental *S. Typhimurium* infection (Truusalu et al., 2004, 2008). Concerning this process the involvement of the glutathione system is crucial as GSH, in addition its role as a crucial cellular antioxidant, is the principal redox controller for a number of cellular processes. Glutathione-related information has impact for LfM-3 regarding next information: a) a recent adapted conceptions of OxS is advanced as "a disruption of redox signalling and control" (Jones, 2006) or "steady-state ROS" (Lushchak,

2011) that emphasize an impact of GSH and its redox ratio for the quantification of OxS and the signalling role of GSH, described previously (Karelson et al., 2002; Zilmer et al., 2005); and b) there exists the possibility for the effective participation of LfM-3 in both enzymatic and non-enzymatic glutathione-driven protection as this strain carries all components needed for functionality of complete glutathione system (Kullisaar et al, 2010). It is interesting to add that recently it has been shown that just *L. fermentum* as a species significantly counteracted the depletion of colonic GSH content induced by some inflammatory processes (Peran et al., 2007) that also supported our understandings. There exists also a correlation between the glutathione redox ratio and DNA oxidative damages (de la Asuncion et al., 1996). Thus, consumption of multivalent probiotic LfME-3, which produces glutathione and has complete glutathione redox cycle enzymes (GPx and GRed), may contribute to the reduction of lipid hydroperoxides in the GI tract and in hepatocytes and prevent them from entering the circulation (Kullisaar et al., 2010). This may lead to an improvement of systemic picture in the host organism.

Data showed that the improvement of the intestinal extra- and intracellular environment yielded beneficial changes of some general (systemic) biochemical indices of the host organism. The administration of LfME-3 to healthy volunteers and atopic adults results in a reduction of LP and a counterbalance of the glutathione system both in blood and in skin. In addition, in several trials LfME-3 has beneficial effect on the blood LDL fraction: the prolongation of its resistance to oxidation, the lowering of the content of oxLDL (a potent inflammatory and atherogenic factor) and BDC-LDL and the enhancement of the TAS of sera (Kullisaar et al., 2003, 2011; Songisepp et al., 2005; Mikelsaar et al., 2008). In trial on elderly persons the lower content of oxLDL was significantly predicted by the higher count of live lactobacilli in the GI tract. Evidently, both the number special antioxidative characteristics of strain LfME-3 and the increase in lactobacilli counts induced by administration of LfME-3 are responsible for such effect on host lipoprotein circulation/metabolism. As we mentioned before, the status of OxS and blood lipoproteins are both related to the pathogenesis of different diseases, including inflammation-related diseases and CVD. Dzau et al (2006) presented in *Circulation* the pathophysiological continuum showing that traditional CVD risk factors all promote OxS and endothelial dysfunction as the first steps in a cascade of pathological events. Elevated OxS leads to the overproduction of oxLDL and the latter has accepted as one of the new systemic markers of the development of CVD (Bonaterra et al., 2010). The higher levels of circulating oxLDL are strongly (much more than LDL-cholesterol) associated with an increased incidence of metabolic syndrome already in people who are currently young and healthy according to a large population-based study (Holvoet et al., 2008). Next, oxLDL is an important determinant of structural changes of the arteries already in asymptomatic persons (Kals et al., 2006; Kampus et al., 2007). An increased production of atherogenic and inflammatory oxLDL within the vessel wall suppresses immunity-related cells, including regulatory T cells (George, 2008) exerting antiatherogenic and antiallergic effects.

The influence of strain LfM-3 on host systemic OxS markers has been showed also via the decline of the values of isoprostanes and 8-OHdG in urine (Kullisaar et al., 2003, 2008; Songisepp et al., 2005). These indices are very informative for systemic OxS burden (Halliwell & Gutteridge, 1999). Evidently the systemic antioxidative effect of strain LfME-3 begins from the alleviation of the OxS- and inflammation-related abnormalities in the GI cells that lead to the assembling of particles of chylomicrons, LDL and HDL with a higher

bioquality (with lower levels of harmful oxidation products) and higher concentrations of antioxidant factors/enzymes. The higher bioquality of assembled lipoprotein particles leads to improvement of their metabolism/circulation in the host body. This is one of the explanations why strain LfME-3 exerted the prolonged resistance of the blood lipoprotein fraction to oxidation, lowered the level of oxLDL and enhanced the TAC of sera in both healthy and problematic consumers (Kullisaar et al., 2003, 2006, 2008, 2011; Songisepp et al., 2005). Recently it was showed that administration of strain LfME-3 alleviated the postprandial elevation of TG levels in the blood, and improves HDL bioquality (elevates of paraoxonase level in HDL particles) (Kullisaar et al., 2006; 2008; 2011). The antioxidant activity of HDL can be expressed via several mechanisms (Bruckert & Hansel B, 2009). Paraoxonase (PON), an antioxidant enzyme associated with HDL, hydrolyzes oxidized phospholipids and inhibits the LDL oxidation that is an important step in atherogenesis. In animals, the addition of oxidized lipids to the circulation reduces PON activity, and diets rich in oxidized fat accelerate the development of aterogenesis (Sutherland et al., 1999). Removal and inactivation of lipid peroxides accumulating during LDL oxidation may be the central mechanism accounting for HDL antioxidative properties and when HDL particles have poor bioquality (low antioxidant properties and anti-atherosclerotic potency), they may have even inflammatory effect (Navab et al., 2006). The increase in PON activity after LfME-3 consumption shows that protection of LDL particles against oxidative modification by ROS is improved. PON inhibits atherogenesis by hydrolyzing lipid hydroperoxides and cholesterol ester hydroperoxides, reducing peroxides to the hydroxides, and hydrolyzing homocycteine thiolactone which prevents protein homocycteinylation (Beltowski et al., 2003; Durrington et al., 2005). Therefore, an elevation of PON activity should decrease the level of oxLDL. Antioxidant action of HDL is noted as one of the principal mechanisms mediating its cardioprotective effect (Hansel et al., 2006). It should be noted that HDL-associated antioxidant activity information is also supported both by data of anti-inflammatory effects of strain LfM-3 on the liver (Truusalu, et al., 2008) and by a hepato-protective role for PON against inflammation and liver disease mediated by OxS (Marsillach et al., 2009). Next, it is accepted that postprandial abnormal events are crucial concerning the development of CVD (Lopez-Miranda et al., 2006). Recently a postprandial decrease of three different OxS-related parameters (oxLDL, BCD-LDL, Beta2-GPI-OxLDL) was established (Kullisaar et al., 2011). Thus, the foodstuffs enriched with LfME-3 substantially improves postprandial indices both of lipid/lipoproteins and OxS (Kullisaar et al., 2006; 2008; 2011). The beneficial influence of such enriched food on the postprandial lipid metabolism and OxS is important as many links between OxS and metabolic syndrome occur during the postprandial period. These include an excessive and prolonged elevation of blood TG levels, impairment of the endothelial function, an intestinal overproduction of chylomicrons, a redundant load for insulin production, the elevation of levels of atherogenic oxLDL and possible disturbances in the antioxidative activity of HDL (Bae et al., 2001; Jackson et al., 2007; Perez-Martinez et al., 2009; Hopps et al.,2010). To summarize, a positive modulation of the postprandial situation, including postprandial OxS, is an important target for dietary preventive actions concerning cardiovascular diseases.

6. Possibilities of the oxidative stress-targeted administration of probiotics

6.1 Functional food and capsules

Functional foods are foods or dietary components (incl. probiotics) that may provide a health benefit beyond basic nutrition. Probiotic products may be conventional foods

consumed for nutritional purposes, but also for the probiotic effect or "medical foods" - the primary purpose is that food formulation is a delivery vehicle for probiotics or metabiotics (beneficial by-products of probiotics). Probiotics are also available as dietary supplements in capsule, powder or liquid extract form. In functional food products no more than two probiotic strains are used in combination as a rule. Probiotic dietary supplements can consist of one single strain or mixed cultures of two or even more strains. There is some evidence that multi-strain probiotic mixtures could be more effective than single strains, including strains that are components of the mixtures themselves (Chapman et al., 2011).

Many functional foods can be found in a form of synbiotics. Synbiotics have been defined as mixtures of probiotics and prebiotics (dietary fiber) (Schrezenmeir & de Vrese, 2001; Saulner et al., 2007). One of the main benefits of synbiotics is the increased persistence of probiotics in the GI tract. Probiotic dietary supplements (capsules, powders and chewing tablets) often additionally contain amino acids, vitamins and/or prebiotics. Probiotic functional foods could be fermented or non-fermented foods. Traditionally dairy products are the carriers of probiotics. A large variety of probiotic dairy products with particular functional properties are available on the market worldwide. Fermented dairy products, especially yoghurts and yoghurt-like products are most widely used. There is a technological reason for using dairy products as probiotic carriers: dairy products have been optimized for the survival of starter cultures (mostly LAB) and are relatively easily adapted to grant the survival of probiotic strains as well. Besides, dairy products have other advantages over other formulations. Dairy foods are refrigerated. Probiotic bacteria in cultured dairy products benefit, as they remain the most stable in a refrigerated storage condition.

Cheese is used as a probiotic vehicle to a lesser extent than fermented milk products (Songisepp et al., 2004; Ross et al., 2005, Ibrahim et al., 2010). Cheese (especially cheddar) may offer certain advantages over other probiotic products such as yogurt or milk. The cheese is a protective environment for the microbes, as the anaerobic conditions, relatively high fat content and buffering capacity of the cheese matrix helps to protect the probiotic strain in the product. The longer cheese is aged for, the higher density of probiotic microbes and metabiotics it will contain. Although the sensitivity of probiotics to physical and chemical stress, heat and acidity makes the product development challenging for other type of food products, probiotics in addition to dairy have been applied in nontraditional foods such as chocolate, energy bars, juices, smoothies, vegetables, breakfast cereals and even meat products like salami etc (Saarela et al., 2000, Siro et al., 2008).

The physiological state of the bacteria in a functional product is an important factor for the survival of the probiotic strain in the product, but most important is the manifestation of functional/health promoting properties in the human body after ingestion. There is a crucial difference between functional food and dietary supplements concerning the physiological state of the probiotic culture. Microbes are often freeze dried by the process of lyophilization before being manufactured as a dietary supplement (free-flowing powders, capsules, tablets). The dryness keeps the probiotic in a quiescent state during storage, while in food products the bacteria are in a vegetative state with a potentially active metabolism. Besides, dried probiotic cultures may have undergone several stressful processes during their production that damage the cells and may affect their viability (Champagne et al., 2011). Milk as a delivery vehicle has a dual effect on the probiotic additive: the buffering capacity of milk protects the viability of the strain against the stomach's acidic conditions. In addition

to the protective effect, which affects the survival of the ingested probiotic, milk contains lactose, minerals, vitamins and bioactive peptides, which enhance the metabolic activity of the ingested probiotic strain in the GI tract.

6.2 Special clinical trial with lactobacillus strains concerning oxidative stress

Probiotics have been advocated for the prevention and treatment of a wide range of diseases, and there is a growing evidence for their efficacy in some clinical scenarios. Probiotics are now widely used in many countries by consumers and in clinical practice. Given the increasingly widespread use of probiotics, a thorough understanding of their benefits is imperative. The properties of different probiotic species vary and can be strain-specific. Therefore, the effects of one probiotic strain should not be generalized to others without confirmation in separate studies. The proposed health benefits of probiotics have undergone increasingly rigorous scientific evaluation in recent years, and there is now strong evidence for their use in treating and preventing some human diseases.

A meta-analysis of randomized controlled trials (RCTs) has shown that many probiotics are effective in preventing antibiotic-associated diarrhoea (McFarland, 2006; Ruszczynski et al., 2008). A separate meta-analysis of RCTs has shown a variety of probiotics to be effective in the treatment of infective diarrhea in both adults and children (Allen et al., 2011) acute waterly diarrhoea (Dutta et al., 2011), *C. difficile* diarrhoea (Plummer et al., 2004), ulcerative colitis and necrotizing enterocolitis (Sari et al., 2011). There is also support from RCTs for the efficacy of a probiotic mix in patients with inflammatory bowel disease (Kajander et al., 2007; Hovyeda et al., 2009). Nevertheless the evidence to date suggests that the major clinical effects of probiotics are seen in prevention GI disorders, probiotic therapy has also been explored in non-GI diseases, including the treatment of atopic eczema in children and adults (Kalliomaki et al., 2001, 2007; Kaur et al., 2008). A specialprobiotic, LfME-3, offers a good potential also in cardiovascular health management. LfME-3 an antioxidative-antiatherogenic and antimicrobial probiotic decreases OxS level in human body. The foodstuffs enriched with this probiotic decrease the level of oxidized LDL, increases the level of HDL, modulates postprandial lipid profile and OxS, and decreases the level of 8-isoprostanes in urine (the markers of systemic OxS) and body overall OxS-load, indicating an atherogenic potential (Kullisaar et al., 2002, 2003, 2011; Songisepp et al., 2005; Mikelsaar, Zilmer, 2009, Table 2).

Intense physical activity increases oxygen consumption and inflammation induced by tissue damage and the probiotic consumption decreased the OxS level (Martarelli et al., 2011). The emerging evidence of a role for GI microbiota on central nervous system functions suggests that the oral intake of probiotics may have beneficial consequences on mood and psychological distress by the competitive exclusion of deleterious GI pathogens, decreases in proinflammatory cytokines and communication with the CNS, leading to changes in neurotransmitter level or function (Logan; Katzman, 2005; Messaoudi et al., 2011). Probiotics are widely used to promote host health. Despite the huge amount of *in vitro* and *in vivo* studies (including cell culture, animal and human studies) we still lack data on the exact mechanisms involved. Our recent results by using MALDI-TOF spectrometry proteomic analysis confirmed that the concentration of glutathione in the blood of the probiotic LfME-3 users increases substantially; that is in good correlation with earlier results. Thus, new proteomic and metabolomic data about LAB and the relation between the colonic microbiota

and host status could give new information regarding the mechanism of probiotic beneficial effects, including the effects on the OxS status of a host organism.

It has been demonstrated that functional food products with special *Lactobacillus sp.* strains have the potential to lower blood pressure (Naruszewicz et al., 2002). We demonstrated that semi-hard Edam-type cheese comprising the strain *L. plantarum* TENSIA (DSM 21380, property of Bio-Competence Centre of Healthy Dairy Products LLC) helps to maintain normal systolic and diastolic blood pressure in healthy adults and elderly subjects, thus supporting the functions of the cardiovascular system (Songisepp et al. 2009). Lately we have found that a 3-week consumption of 50g of probiotic cheese comprising *L. plantarum* TENSIA (daily dose 10^{10} of probiotic viable cells per serving) decreased both diastolic (diapason of change: -3.6±7.1 (median -2.3; p=0.01) and systolic (diapason of change: -4.4±8.2 (median -4.0, p=0.01) blood pressure in adult subjects with high normal blood pressure (130-139.5 mmHg).

Marker	oxLDL	HDL-Chol	BCD-LDL	Glutathione redox ratio (GSSG/GSH)	Total anti-oxidative activity	8-iso-prostanes
Number of participants	169	63	106	54	130	63
Decrease* or increase** of level compared to baseline	*16% p<0.03	**7% p<0.003	*17% p<0.02	*33% p<0.03	**20% p<0.005	*26% p<0.03

Table 2. Effects of foodstuffs enriched with probiotic LfME-3 on both oxidative stress-related indices and HDL-cholesterol level of human body. oxLDL, oxidized low-density lipoprotein; BDC-LDL, baseline conjugated diene in LDL; GSSG, oxidized glutathione; GSH, reduced glutathione; HDL-Chol, high-density lipoprotein cholesterol

In the elderly, the consumption of the same amount of probiotic cheese in a somewhat lower daily dose of probiotic viable cells per serving (10^8) decreased both diastolic (diapason of change -4.0 ± 5.2mmHg, median -5mm Hg; p= 0.004) and systolic (diapason of change: -5.9±13.4, median -12 mmHg; p=0.038).

It is repeatedly declared that new approaches in global CVD risk reduction are needed (Elliott, 2008). It is stated that for the prevention of CVD risk the anti-inflammatory agents and antioxidants are considered as a possible "third great wave"' (Bhatt, 2008). Evidently, the prevention complexes of several diseases could become more successful by including probiotics with a special multivalent (including antioxidative properties) biopotency.

7. References

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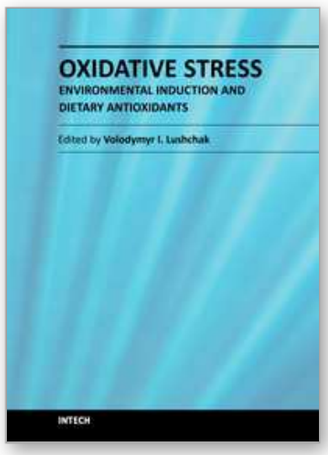

Oxidative Stress - Environmental Induction and Dietary Antioxidants

Edited by Dr. Volodymyr Lushchak

ISBN 978-953-51-0553-4
Hard cover, 388 pages
Publisher InTech
Published online 02, May, 2012
Published in print edition May, 2012

This book focuses on the numerous applications of oxidative stress theory in effects of environmental factors on biological systems. The topics reviewed cover induction of oxidative stress by physical, chemical, and biological factors in humans, animals, plants and fungi. The physical factors include temperature, light and exercise. Chemical induction is related to metal ions and pesticides, whereas the biological one highlights host-pathogen interaction and stress effects on secretory systems. Antioxidants, represented by a large range of individual compounds and their mixtures of natural origin and those chemically synthesized to prevent or fix negative effects of reactive species are also described in the book. This volume will be a useful source of information on induction and effects of oxidative stress on living organisms for graduate and postgraduate students, researchers, physicians, and environmentalists.

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
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