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Probiotic antigenotoxic activity as a DNA bioprotective tool: a minireview with focus on endocrine disruptors

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One sentence summary: This review deals with the ability of probiotics and/or food associated bacteria to counteract the DNA damage induced by food and environmental genotoxic compounds with focus on endocrine disruptors.

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ABSTRACT

Nowadays, the interest in the role of dietary components able to influence the composition and the activity of the intestinal microbiota and, consequently, to modulate the risk of genotoxicity and colon cancer is increasing in the scientific community. Within this topic, the microbial ability to have a protective role at gastrointestinal level by counteracting the biological activity of genotoxic compounds, and thus preventing the DNA damage, is deemed important in reducing gut pathologies and is considered a new tool for probiotics and functional foods. A variety of genotoxic compounds can be found in the gut and, besides food-related mutagens and other DNA-reacting compounds, there is a group of pollutants commonly used in food packaging and/or in thousands of everyday products called endocrine disruptors (EDs). EDs are exogenous substances that alter the functions of the endocrine system through estrogenic and anti-estrogenic activity, which interfere with normal hormonal function in human and wildlife. Thus, this paper summarizes the main applications of probiotics, mainly lactobacilli, as a bio-protective tool to counteract genotoxic and mutagenic agents, by biologically inhibiting the related DNA damage in the gut and highlights the emerging perspectives to enlarge and further investigate the microbial bio-protective role at intestinal level.

Keywords: probiotics; antigenotoxicity; SOS-Chromotest; endocrine disruptors; zebrafish

INTRODUCTION

In recent years, the field of nutritional science has enjoyed great successes, defining the essential requirements for specific nutrients to assume during each phase of human life and for the maintenance of a health status and well-being. The quality of food and the magnitude of the diet-health relationship have now attracted extreme interest with important scientific discoveries and with commercial and technological implications. According to the World Health Organization (WHO), the state of health of the population is strongly influenced by the diet. The human gut hosts a complex ecosystem generated by the integrity and stable cooperation between immune cells, resident microbiota and gastro-intestinal (GI) epithelium (McCracken and Lorenz 2001). When this balance is altered, it may trigger a state of dysbiosis that can lead to systemic or chronic diseases, (Sekirov et al. 2010; Prakash et al. 2011; Abbasi et al. 2015, Federici et al. 2017). In this respect, the intestinal microbiota is considered a very metabolically active organ that can be affected by multiple factors such as host gene, age and delivery pattern, antibiotics and diet (Hasan...

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and Yang 2019). The latter relationship is still not completely understood, even though recent studies highlighted the contribution of particular nutrients or chemical components, and following the transfer of different microorganisms, in affecting the intestinal microbiota (Scott et al. 2013; David et al. 2014; Graf et al. 2015). Epidemiological and clinical-experimental evidences highlighted the crucial role played by the intestinal microbiota in the modulation of genotoxic and mutagenic risk in the GI tract (Burns and Rowland 2000; Davis and Milner 2009).

Therefore, the interest concerning dietary components (including food-borne microbes) that can modulate the intestinal microbiota and potentially confer benefits to the consumer is now growing (Gratz, Wallace and El-Nezami 2011; Claesson et al. 2012; Sharma and Shukla 2016). Probiotics and prebiotics represent the most used strategies for the production of functional foods and for the maintenance of the intestinal ecosystem equilibrium (Hill et al. 2014; Abuajah, Ogbonna and Osuji 2015; Gibson et al. 2017; Champagne, Gomes da Cruz and Daga 2018; Kerry et al. 2018). In particular, some microorganisms, mainly of the genera Lactobacillus and Bifidobacterium, that are present in food as probiotics, exert positive effects by protecting against intestinal pathogens, modulating the immune response, reducing the cholesterol levels and by improving the digestion of lactose (Shah 2007; Sekirov et al. 2010; Nagpal et al. 2012; Hill et al. 2014; Marco and Golomb 2016). The positive clinical outcomes are due to the ability of microorganisms to endure the harsh GI tract conditions and to persist stably herein. Recently, the microbial ability to make a protective role at GI level by counteracting the biological activity of genotoxic compounds is thought important for reducing gut pathologies and is considered a new tool for probiotics and functional foods (Trotta et al. 2012; Raman et al. 2013; Prete et al. 2017).

In this perspective, diet cannot be exclusively conceived as a source of nutrients for both humans and gut microbiota and a vehicle of potentially active microbes (O’Hara et al. 2006; Sekirov et al. 2010), but also as a supply of genotoxic compounds that can be commonly found in the gut. Recently, beside food-related mutagens, a group of pollutants commonly used in cosmetics, food packaging and/or in thousands of everyday products called EDs deserves great interest. EDs are exogenous substances or mixture that alter the functions of the endocrine system through estrogenic and anti-estrogenic activity, which are known to interfere with normal hormonal function in human and wildlife (Albert and Jégou 2014; Perugini et al. 2019; Zezza et al. 2019). Recently, the exposure of EDs as environmental stressors has been associated with metabolic disorders, such as diabetes and obesity (Le Magueresse-Battistoni et al. 2017), besides many others adverse effects in various organs including, in some cases, carcinogenic effects through DNA damage (Voss et al. 2005; Ventrice et al. 2013; Zarean et al. 2016). Up to date, the microbial antigenotoxic activity has been widely investigated against model genotoxins (i.e. 4-nitro-quinoline-1-oxide (4-NQO), methylmethanesulfonanidine (MMS)) and some food-related mutagens (i.e. aflatoxins, nitrosamines) by primary DNA damage in vitro assays, mainly SOS-Chromotest and Comet assay (Caldini et al. 2005; Caldini et al. 2008; Corsetti et al. 2008; Fang et al. 2008; Verdeneelli et al. 2010; Walla, Keshani and Kanwar 2014; Bocci et al. 2015; Pithva et al. 2015; Prete et al. 2017; Chandel et al. 2019; Janosch et al. 2019). However, only few studies have been focused on evaluating the genotoxic effects of EDs (Cabaton et al. 2009; Umbuzeiro, Machala and Weiss 2011; Martinez-Paz et al. 2013; Erkekoğlu and Kocer-Gumusel 2014) and their impact on gut homeostasis and immune response modulation and this field of research is now an emerging area of interest.

Furthermore, several studies showed validation in in vivo using murine models (Dominici et al. 2014) or by evaluating the antigenotoxicity using faecal water (FW) (Nowak, Slizewska and Otleswka 2015; Federici et al. 2017). About that, the use of zebrafish (Danio rerio) as in vivo model to evaluate different probiotic effects as well as to evaluate toxicity and genotoxicity of several compounds is an emerging tool (Avella et al. 2012; Zhang et al. 2012; Zhou et al. 2012; Caballero-Gallardo, Olivero-Verbel and Freeman 2016; Gioacchini, Rossi and Carnevali 2017; Dal Santo, Grotto and Boligon 2018). Several studies reported that administration of probiotic containing Lactobacillus rhamnosus modulates the composition of the gut microbiota, which subsequently changes host lipid and glucose metabolism, activates the endocannabinoid system, controls immune response and inflammation and restructures intestinal epithelial organization, affecting fish growth (Falcinelli et al. 2017; Gioacchini, Rossi and Carnevali 2017).

In particular, zebrafish is a well-characterized in vivo model, with a relatively short life cycle (few months) that allows full lifecycle tests (Ankley and Johnson 2004). In addition to that, the zebrafish genome can be considered approximately 70% equivalent of human orthologs, providing a further value as a model species for biomedical research (Howe et al. 2013). Zebrafish has emerged as an ideal in vivo model to study the aquatic toxicity of environmental chemicals including EDs (Chen et al. 2018a; Perugini et al. 2019).

Thus, this paper reviews the main applications of probiotics and food-associated microbes, mainly lactobacilli, as a bio-protective tool to counteract genotoxic and mutagen agents, by biologically inhibiting the related DNA damage in the gut. Starting with an overview of the main EDs, an emerging group of mutagens, and their adverse effects at intestinal level, the most widely applied in vitro models to investigate the microbial anti-genotoxic activity are described. The final part of the review highlights the innovative perspective to investigate the microbial bio-protective role at intestinal level, by using Danio rerio as a novel in vivo model to investigate probiotic effects.

**EMERGING ENVIRONMENTAL MUTAGENS: ENDOCRINE DISRUPTORS (EDs)**

In 2002, the WHO defined EDs as ‘an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations’ (WHO/UNEP 2012). Humans and animals may be exposed to a wide range of endocrine active substances through the diet as well as other sources. Endocrine disruption includes a variety of different mechanisms of toxicity that might affect different individual endpoints (Table 1). The molecular mechanisms by which EDs can exert their effects are various and include their ability: (i) to bind to estrogen receptors by triggering a cascade of molecular effects that ultimately modify gene expression; (ii) to alter the levels of endogenous hormones by changing the rate of their production; (iii) to mimic or inhibit the activity of endogenous hormones (Alofe, Kisanga and Inayat-Hussain 2019). A key characteristic of EDs action is a non-monotonic dose-response curve causing adverse effects at low doses but not necessarily at higher doses (Beausoleil et al. 2013). The majority of EDs are synthetic compounds released into the environment by anthropogenic activities, either alone or in combination, although some of them are naturally present as plant-derived phytoestrogens or fungus-derived mycoestrogens (Tavares et al. 2016; Darbre 2019).
Emerging evidence indicates an association between exposure to EDs and modifications of gut bacterial communities leading to increased susceptibility to pathogens and/or the occurrence of metabolic diseases (Velmurugan et al. 2017). Indeed, gut bacteria were demonstrated to be efficient in the biotransformation of multiple classes of contaminants modulating the EDs toxicological potential toward host organism (Evariste et al. 2019). Moreover, recent studies report that some EDs also induce genotoxic effects (Güzel and Ayaz 2019; Hercog et al. 2019).

Among different types of chemicals known as EDs, only a small fraction of them, such as many pesticides, have shown to interfere also with the metabolism of target organism (MacBean 2012). In addition, the relationship between the occupational exposure to various pesticides and the presence of DNA damage and oxidative stress in humans has been demonstrated (Jacobsen-Pereira et al. 2018). Pesticides can also act on gut microbiota composition, affecting their metabolites and destroying intestinal mucosa (Yuan et al. 2019).

Other types of EDs that represent a great concern for their mutagenic and genotoxic activity are synthetic chemicals, such as polychlorinated biphenyl (PCBs) and polybrominated diphenyl ethers (PBDEs), both widespread anthropogenic compounds commonly found in dietary sources (Liu et al. 2017; Dunnick et al. 2018). Recently, prenatal exposure to PBDEs and PCBs was associated with changes in gut microbiota of infants and children (Iszatt et al. 2019; Laue et al. 2019).

Heavy metals are naturally occurring elements that have wide range of industrial, domestic, agricultural, medical and technological applications (Tchounwou et al. 2012). Their distribution in the environment is raising concerns over their potential effects on human health, since sub-lethal levels of heavy metals increase mutation rates and enrich de novo mutations (Li et al. 2019). Heavy metals reveal also a direct impact on the gut microbiota that, in turn, have an essential role in limiting heavy metal body burden (Breton et al. 2013). Moreover, probiotic-based diet containing Lactobacillus plantarum strains showed a protective effect on the intestinal microbiota following exposure to waterborne cadmium (Zhai et al. 2017).

On the other hand, engineered nanomaterials (ENMs) are increasingly used in many applications across military, industrial and consumer field (Agans et al. 2019). The cytotoxicity and genotoxicity properties of ENMs are not well established as well as their interaction with gut microbiota and intestinal barrier. However, available data suggest that nanoparticles may affect the microbiota, and clinical disorders such as colitis, obesity and immunological dysfunctions might follow (Pietroiusti, Magrini and Campagnolo 2016).

Pharmaceuticals and personal care products (PPCPs) are a group of emerging environmental pollutants able to induce physiological effects in humans at very low doses (Ebele, Abdallah and Harrad 2017). Among these PPCPs, antimicrobial agents used for human and veterinary medicines or included in consumer products as preservatives, are continuously released in the environment. Although the exposure to some PPCPs (e.g. parabens) has been associated with metabolic syndrome in humans, the long-term effects on the host microbiome remain still unclear (Kim and Chevrier 2019). The effects of triclosan, largely used as antimicrobial agent, are widely investigated because its high detection levels in human fluids and its impact on the metabolism and the gut microbiota in animal models (Ma et al. 2020). Recently, dietary administration of L. plantarum ST-III has shown to alleviate intestinal damages and behavioural symptoms resulting from triclosan exposure (Zang et al. 2019).

Microplastics, plastics that break down into pieces smaller than 5 mm, are sometime included in the group of EDs because they seem to lead dysbiosis through mechanical disruption or acting as a vector of chemicals and other EDs (Fackelmann and Sommer 2019). Bisphenol A (BPA) is widely used for manufacturing plastic products and has been classified as an environmental obesogenic compound acting through several mechanisms

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**Table 1.** Principal EDs and their side effects on human health.

| EDs classes                  | Representative compounds*                        | Adverse side effects                                                                 | References                      |
|------------------------------|--------------------------------------------------|---------------------------------------------------------------------------------------|---------------------------------|
| Pesticides                   | Chlorpyrifos, Atrazine, Glyphosate                | Genotoxicity obesity, insulin resistance, gut microbiota dysbiosis                    | Jacobson-Pereira et al. 2018    |
| Polybrominated Diphenyl      | PBDE-47, PBDE-99, PBDE-153                        | Carcinogenicity                                                                        | Yuan et al. 2019                |
| Ethers (PBDEs)               |                                                   | Mutagenicity, obesity, metabolic syndrome, non-alcoholic fatty liver disease           | Dunnick et al. 2018             |
| Polychlorinated Biphenyls    | PCB-153, PCB-126, PCB-173                        | Mutagenicity, oxidative stress, immunosuppression                                      | Liu et al. 2017                 |
| (PCBs)                       |                                                   |                                                        | Wahlang et al. 2013             |
| Heavy metals                 | Cadmium, Lead, Arsenic                            |                                                        | Li et al. 2019                  |
| Engineered nanomaterials     | Graphene oxide, Titanium dioxide                  | Oxidative stress, gut microbiota dysbiosis, immunotoxicity                            | Zhang et al. 2017               |
| (ENMs)                       |                                                   |                                                        | Zheng et al. 2019               |
| Personal care products       | Methylparaben, Propylparaben, Butylparaben       | Estrogenic, anti-androgenic properties, obesity                                       | Chen et al. 2016                |
| (PCPs)                       |                                                   |                                                        | Nowak et al. 2018               |
| Phenolic derivates           | BPA, BPS, BPF                                    | Obesity, genotoxicity, oxidative stress                                                | Legeay and Faure 2017           |
| Phthalates                   | DEHP, DnHP                                       | Hormones disruption, genotoxicity                                                      | Karabulut and Barlas 2018       |
| Nitrosamines                 | NDMA, NDPA, NMOR                                 | Hormones disruption, obesity                                                          | Somade et al. 2016              |
|                             |                                                   |                                                        | Zhu et al. 2019                  |

*EDs noted as: 2,2′,4,4′-Tetra bromodiphenyl ether (PBDE-47), 2,2′,4,4′,5′-Pentabromodiphenyl ether (PBDE-99), 2,2′,4,4′,5,5′-Hexabromodiphenyl ether (PBDE-153), 2,2′,4,4′,5,5′-Hexachlorobiphenyl (PCB-138), 3,3′,4,4′-5-Pentachlorobiphenyl (PCB-126), 2,2′,3,3′,4,4′-Hexachlorobiphenyl (PCB-173), Bisphenol A (BPA), Bisphenol S (BPS), Bisphenol F (BPF), Di-(2-ethylhexyl) phthalate (DEHP), Di-n-hexyl phthalate (DnHP), N-nitrosodimethylamine (NDMA), N-nitroso-di-n-propylamine (NDPA), N-nitrosomorpholine (NMOR).
Phthalates are another group of chemicals used to make plastics more flexible and harder to break. They are used in a wide variety of products, such as vinyl flooring, adhesives, detergents, lubricating oils, automotive plastics, plastic clothes and personal care products (Monneret 2017). Postnatal exposure to phthalates results in a transient gut microbial dysbiosis in human new-borns (Yang et al. 2019). Di-(2-ethylhexyl) phthalate (DEHP) induced genotoxic, morphometric and hormonal effects in pre-pubertal male rats (Karabulut and Barlas 2018). However, the genotoxic potential and toxicological effects of other phthalates such as di-n-hexyl phthalate (DnHP), are not yet clarified even though one of the probable mechanism could be the generation of reactive oxygen species and thus the induction of oxidative stress, strictly related to the inflammatory diseases and cancer (Erkekoglu and Kocer-Gumusel 2014).

Finally, nitrosamines (NAs) are well established carcinogenic, mutagen and hepatotoxic agents which can be formed mostly through the oxidation of dialkylhydrazine intermediates, in a variety of food stuffs such as smoked fish, dried malt, beer, milk products, meat products and preserved fruit juices (Somade et al. 2016). They are able to interfere with thyroid and reproductive functions as well as to induce changes in gut microbiota leading to obesegenic microbiota profile at environmentally relevant levels (Somade et al. 2016; Zhu et al. 2019).

In recent years, EDs have been linked to a wide range of pathologies and long-term effects as decreased fertility or infertility, alteration in the normal onset of puberty and puberty-related development as well as the promotion of breast, testicle and prostate cancer (Wallace 2015). Furthermore, some EDs have been considered obesogens because they might also interfere with regulatory processes in metabolism and in the control of adipocyte function, resulting in an imbalanced regulation of body weight, which can lead to obesity (Nappi et al. 2016) metabolic syndromes and diabetes (Fénelchel and Chevalier 2017). At the end, it is important to highlight that EDs can be found in mixtures and a cumulative action can occur, thus their potential combined effects should be useful to understand their role in a wide range of pathologies.

**IN VITRO INVESTIGATIONS OF MICROBIAL ANTIGENOTOXIC ACTIVITY**

Experimental evidence for antigenotoxic activity of probiotics and/or food-borne microbes, mainly lactobacilli and bifidobacteria, has been primarily obtained by evaluating the microbial ability to inhibit DNA damage, the earliest event in carcinogenesis, in in vitro models (Hiramaya and Rafter 2000; Commnaye et al. 2005). To date, the most widely applied assays to evaluate bacterial genotoxic inhibition are represented by primary DNA damage in vitro assays, such as the SOS-chromotest with prokaryotic target and Comet assay by using eukaryotic cell lines as targets (Fig. 1). Among short-term mutagenic assays, the SOS-chromotest has been proposed as a screening tool to evaluate probiotic antigenotoxicity due to the practical advantages, such as sample preparation, rapid procedure, specificity in carcinogens recognition and concordance with other mutagenicity tests (e.g. Ames test). SOS-chromotest evaluates the DNA damage induced by genotoxic compounds on a prokaryotic tester organism (Escherichia coli PQ37). Briefly, E. coli PQ37 (p5A::lacZ, uvrA, rfa, Pho’+) carries a p5A::lacZ gene fusion, responsible for β-galactosidase (βG) induction in the presence of DNA damage, easily detectable through a colorimetric enzymatic assay (Quillardet and Hofnung 1993).

Several probiotics and/or food-associated bacterial strains, mainly lactic acid bacteria (LAB), have been investigated using SOS-Chromotest for their inhibition effects of model genotoxins and food-related mutagens of different nature in which a consistent decrease in biological activity was observed (Table 2).

Overall, the results highlighted a widespread inhibition activity among various LAB strains, but with a clear strain-dependent specificity and a specific bacteria-genotoxin interaction as a putative bioconversion mechanism responsible for genotoxin detoxification (Corsetti et al. 2008; Prete et al. 2017), also confirmed by gas chromatography-mass spectrometry (GC-MS) and Infrared-Raman analyses (Verdenelli et al. 2010, Bocci et al. 2015). Other studies were conducted on probiotics Bacillus spp. (Caldini, Trotta and Cenci 2002; Cenci et al. 2008), Streptococcus salivarius var. thermophilus and Enterococcus faecium strains (Raiipulis, Toma and Semonjovs 2005; Walia, Keshani and Kanwar 2014) and in yeasts strains (Trotta et al. 2012; Prete et al. 2017). Remarkably, most of the strains that are able to suppress the effects of genotoxic compounds have also displayed in vitro (Trotta et al. 2012) and in vivo (Deabes et al. 2012) antioxidant properties. Interestingly, most in vitro studies have underlined a strong antigenotoxic activity in many food-associated strains similar to that of probiotics, confirming that probiotic properties can be common features of food-borne microbes (Marco, Heeney and Bind 2017).

Similar results have also been reported by investigating primary DNA damage in in vitro intestinal cell lines (i.e. HT-29, Caco-2) or in in vivo test subjects by Comet assay, a single cell gel electrophoresis test (Table 2). This is an easy and highly sensitive cellular assay widely used in genotoxicity and DNA damage/repair studies for detecting single/double strand breaks, and a variety of DNA lesions (i.e. apyrimidinic/apurinic and excision repair sites) in individual mammalian cells (Singh et al. 1988). The comet assay has been used to investigate the antigenotoxic activity of some food-associated lactobacilli and bifidobacteria in the colon mucosa of rats treated with MNNG or 1,2-dimethylhydrazine (DMH), in which a dose-dependent protective effect of microbial viable cells was reported (Pool-Zobel et al. 1996). Similar results were also found for different LAB species against different genotoxins in which concordance of in vitro results obtained with SOS-Chromotest and Comet assay was also confirmed (Caldini et al. 2005; Chandel et al. 2019). Comet assay was also used to investigate prebiotics and probiotics antigenotoxicity on human genotoxic FW by using adenocarcinoma cell lines with interesting findings on the prebiotics protective effect against early DNA damage (Burns and Rowland 2004; Nowak, Slizewska and Otlewska 2015). Antigenotoxic activity was also reported for Gram-negative probiotic strains, E. coli Nissle 1917 (Janosch et al. 2019).

Furthermore, the capacity of probiotics to degrade and/or adsorb environmental chemicals such as EDs is also emerging. Yasushi et al. (2007) reported that lyophilized cells of LAB could remove BPA from the medium by adsorption mechanism and a similar property was also found in yeast Pichia pastoris (Mergler, Wolf and Zimmermann 2004). Recently, a panel of commercial probiotic strains, belonging to multiple species (i.e. Lactobacillus spp., Bifidobacterium spp. and Streptococcus thermophilus) has been investigated for their ability of detoxifying and degrading
environmental BPA (Solouki, Fazeli and Solouki 2018). Dairy lactobacilli also displayed in vitro binding activity towards pesticides (Trinder et al. 2015) and BPA (Zhu et al. 2017), confirming the bioremediation by using microbial cells as a low cost putative strategy to face increasing EDs exposure (Rai, Ganguli and Neogi 2017). Despite these efforts, the role of probiotics and foodborne microorganisms in degrading EDs and hence exhibiting protective effects remains unclear. Therefore, no investigations are available about the microbial antigenotoxic activity against EDs, thus we performed a preliminary study that enlarged the investigation of the antigenotoxic activity of two documented probiotics L. plantarum strains IMC510 and IMC513 (Prete et al. 2017; Garcia-Gonzalez et al. 2018; Prete et al. 2020) against two different EDs: BPA and Di-n-hexyl phthalate (DnHP),

Figure 1. Schematic representation of in vitro primary DNA damage assays used to assess microbial antigenotoxic activity. SOS-Chromotest is a rapid colorimetric assay that evaluates the DNA damage induced by genotoxic compounds through the measure of the SOS-response of a prokaryotic tester organism E. coli PQ37 (σE::lacZ, uvrA, rfa, Pho), by quantifying β-galactosidase production under the induction of the SOS repair system. Comet assay is based on the encapsulation of eukaryotic cells in a low-melting (LM) point agarose suspension, followed by electrophoresis of lysed cell in neutral or alkaline conditions. Electrophoresis results in comets-like DNA migration, visualized by fluorescence microscopy. Undamaged DNA preserves a highly compact structure appearing as head of the comet, whereas DNA damaged strands migrates to resemble a comet tail, whose intensity reflects the amount of DNA damages.
Table 2. Probiotics and food-associated bacteria evaluated for their antigenotoxic activity against different mutagenic compounds.

| Antigenotoxic bacteria | Methods | Genotoxic agents<sup>a</sup> | References |
|------------------------|---------|-----------------------------|------------|
| **Probiotics/human strains** |         |                             |            |
| B. breve, B. longum    | Comet assay | MNNG, DMH                  | Pool-Zobel et al. 1996 |
| L. acidophilus, Bifidobacterium spp. | Ames test | 4-NQO, MNNG, NF, NPD, AFB; MelIQ, PhilP, MeA,C | Lankaputhra and Shah 1998 |
| Bacillus spp. (probiotics) | SOS-Chromotest | 4-NQO | Caldini, Trotta and Cenci 2002 |
| Bifidobacterium sp. 420, Bifidobacterium lactis Bb-12, L. plantarum, Streptococcus thermophilus, L. bulgaricus, Enterococcus faecium | Comet assay | FW | Burns and Rowland 2004 |
| L. plantarum KL and 8014, L. casei Shiroti, L. acidophilus T20, Streptococcus salivarius var. thermophiles, B. lactis Bb-12 | SOS-Chromotest | Furazolidone | Raipulis, Toma and Semjonovs 2005 |
| Bacillus spp. (probiotics) | SOS-Chromotest | 4-NQO, MNNG, MelIQ, AFB1 | Cenci et al. 2008 |
| L. acidophilus J76, L. casei 5H10, B. longum, L. fermentum, L. plantarum J25, L. rhamnosus JS4, B. longum | SOS-Chromotest; Ames test | Heating cooking oils | Isidori and Parrella 2009 |
| L. rhamnosus IMC501 | GC-MS; SOS-Chromotest | 4-NQO | Verdenelli et al. 2010; Bocci et al. 2015 |
| L. casei LOCK 0900, L. paracasei LOCK 0919 | Comet assay | PhilP | Nowak, Sliżewska and Klewicka 2011 |
| L. rhamnosus 0900 and 0908, L. casei 0919, L. casei Shiroti, L. casei DN 114-001, L. johnsonii La1, L. rhamnosus GG, B. animalis spp. lactis Bb-12, L. delbrueckii 0987, L. mucosae 0988 | Comet assay | FW | Nowak, Sliżewska and Otłewa 2015 |
| L. rhamnosus Vc | SOS-Chromotest; Ames test | MNNG | Pithva et al. 2015 |
| L. plantarum IMC510, IMC513, WCFS1 | SOS-Chromotest | 4-NQO | Prete et al. 2017 |
| Escherichia coli Nissle 1917 | Comet assay, Ames test | 4-NQO | Janosch et al. 2019 |
| L. rhamnosus MD14, L. plantarum GMD, P. pentosaceus GMD17A | SOS-Chromotest; Comet Assay | DMH, 4-NQO | Chandel et al. 2019 |
| **Food-associated strains** | Comet assay | MNNG; DMH | Pool-Zobel et al. 1996 |
| L. acidophilus, L. gasseri, L. confusus, S. thermophilus | SOS-Chromotest | 4-NQO | Cenci et al. 2002 |
| L. delbrueckii subsp. bulgaricus, L. casei, L. rhamnosus, L. acidophilus, L. plantarum | SOS-Chromotest; Comet Assay, Ames test | 4-NQO, MNNG | Caldini et al. 2005 |
| L. casei, L. acidophilus, L. delbrueckii subsp. bulgaricus, L. rhamnosus, L. plantarum | SOS-Chromotest; Comet Assay | MNNG | Caldini et al. 2008 |
| Lactobacillus spp. | SOS-Chromotest | 4-NQO, MNNG | Corsetti et al. 2008 |
| L. casei | SOS-Chromotest | 4-NQO, MNNG | Fang et al. 2008 |
| L. salivarius FDB89 | SOS-Chromotest | 4-NQO | Darsanaki et al. 2012 |
| Lactobacillus spp. | Ames test | Sodium azide, NF | Wala, Keshani and Kanwar 2014 |
| E. faecium, Bacillus coagulans, L. plantarum | SOS-Chromotest, Ames test | 4-NQO, Furazolidone | |
| L. plantarum | Comet assay | AFB1 | Kurhan and Cakir 2016 |
| L. plantarum | SOS-Chromotest | 4-NQO | Prete et al. 2017 |
| LAB | SOS-Chromotest | DMH | Sharma, Chandel and Shukla 2020 |

<sup>a</sup>Genotoxic compounds noted as: methylxanthine (MNNG), 1,2-dimethylhydrazine (DMR), 4-nitroquinoline-1-oxide (4-NQO), 2-nitrofluorene (NF), 4-nitro-0-phenylenediamine (NPD), Aflatoxin-B (AFB), 2-amino-3-methyl-3H-imidazoquinoline (MeIQ), 2-amino-1-methyl-6-phenyl-1H-imidazo[4,5-b]pyridine (PhI), 2-Amino-3-methyl-3H-pyrido[2,3-b]indole (MeA,C), genotoxic faecal water (FW), Aflatoxin-B<sub>1</sub> (AFB<sub>1</sub>).

Commonly used in food packaging and/or in thousands of everyday products. As reported in Fig. 2, co-incubation of EDs with both L. plantarum probiotic strains showed appreciable genotoxicity inhibition (>75% BPA and > 50% DnHP), confirming the ability of L. plantarum strains to inhibit the biological activity of genotoxic compounds (Prete et al. 2019). Based on these preliminary results (data not shown), L. plantarum IMC 513 and DnHP, which induced the major genotoxicity on the tester strain, were selected as a combined treatment to use in an ongoing in vivo experimental trial with zebrafish.

In vivo studies have shown that some probiotics (e.g. Lactobacillus spp.) could be capable of countering the DNA damage produced by potent genotoxins with consequent reduction of preneoplastic state and protection against neoplastic activities of these compounds (Dominici et al. 2011). Among mechanisms by which probiotics may exert their beneficial effects, significant differences exist between different probiotic strains and model genotoxins, including direct and indirect modes of...
action. Indeed, probiotics can prevent the absorption of genotoxic xenobiotics in the intestine, reduce their bioavailability or could be responsible for the enzymatic inactivation of mutagens (e.g. scavenging). Moreover, Dominici and co-workers (2014) have demonstrated the capability of L. rhamnosus IMCS01 to modulate faecal microbial ecosystem in mice treated with PhIP, one of the most frequent heterocyclic amines found in cooked meat, and to reduce the activity of β-glucuronidase and β-N-acetyl-glucosaminidase. β-glucuronidase has the ability to reverse phase II detoxifying reactions in which chemicals (e.g. dietary carcinogens) undergo conjugation in the liver; as a consequence, conjugated carcinogens are not excreted and parent genotoxic xenobiotics are released in the colon lumen (Dominici et al. 2014). The pre-treatment of male albino mice with L. rhamnosus GG before intoxication with aflatoxins has ameliorated the oxidative status by decreasing lipid peroxidation, increasing superoxide dismutase activity and reducing glutathione contents (Deabes et al. 2012). Other mechanisms potentially involved in the protective role of Lactobacillus strains (L. salivarius REN) in rats exposed to 4-NQO could be related to the suppression of cell proliferation, induction of cell apoptosis and/or downregulation of cyclooxygenase-2 expression (Zhang et al. 2013). Rodent models have been also used to investigate the effects of BPA and ethinyl estradiol exposure on gut microbiota (Javurek et al. 2016), and the protective effects of L. casei and B. breve against BPA (Oishi et al. 2008).

As previously stated, mice and rats have been used extensively to study antigenotoxicity properties of probiotics. However, in vivo models prove to be very expensive compared to in vitro approaches since a large number of animals is requested and a copious amount of test compounds have to be expended.

Zebrafish, on the other hand, has emerged as an attractive alternative model for studying probiotic properties and genotoxic activities of several EDs. Zebrafish has offered an alternative to animal experiments in mammals based on the 3Rs-replacement, reduction, and refinement. It has been extensively used for testing different types of toxicity, including developmental toxicity, cardiotoxicity, nephrotoxicity, and hepatotoxicity (Chakravarthy et al. 2014). The attributes that make zebrafish an useful in vivo model have been related to ex vivo embryo fertilization and embryogenesis, optical transparency of embryos and larvae, rapid embryological development, cheap housing costs and genetic tractability (Lieschke and Currie 2007). Moreover, 71.4% of human genes have at least one zebrafish orthologue and, reciprocally, 69% of zebrafish genes have at least one human orthologue (Howe et al. 2013). More importantly, about 84% of human genes known to be associated with human diseases are also present in zebrafish (Howe et al. 2013). Zebrafish has also been used to study host-microbe interactions in the digestive system. Zebrafish colonization is characterized by a condition very similar to that of human, despite the relevant differences between human and zebrafish gut microbiota. The role of gut microbiota on host biology is similar between zebrafish and mammals and, in both species, intestinal microbiota participates in the education of the immune system, maturation of the gut, and promotion of nutrients metabolism in the host (Bates et al. 2007). The number of microbiota related studies in zebrafish are still limited but growing, in developmental and physiological microbiology, colitis models, effects of antibiotics and immune responses (Okazaki et al. 2019).

Moreover, the administration of probiotics seems to modulate the gut microbiota composition of zebrafish, leading to beneficial properties. L. rhamnosus has attenuated obese phenotype (Falcinelli et al. 2017), the induction of oocyte maturation, the increase of fecundity in female zebrafish as well as the acceleration of zebrafish backbone calcification (Avella et al. 2012; Gioacchini et al. 2012). A probiotic mixture of lactobacilli, bifidobacteria and Streptococcus thermophilus has activated the endocannabinoid system in zebrafish, leading to the modulation of immune cells function by inducing gene expression of toll-like receptors and other immune related molecules (Gioacchini, Rossi and Carnevali 2017).

Recently, the effects of several EDs on zebrafish gut microbiome have been reported. Gut microbiota dysbiosis in zebrafish has been associated to metals, pesticides, fungicides and microplastics exposure (Jin et al. 2017; Xia et al. 2018; Zhang et al. 2018; Qiao et al. 2019). Chronic co-exposure to titanium dioxide nanoparticles and bisphenol A have led to oxidative stress in zebrafish and this event has been correlated with higher abundance of gut pathogenic bacteria (Chen et al. 2018). Moreover, it has been also proved the protective role of bioactive compounds, identified in a plant species from South America, against oxidative stress and genotoxic activities induced by glyphosate-based herbicide in adult zebrafish (Dal Santo, Grotto and Boligon 2018).

Based on that, the use of zebrafish as an in vivo experimental model to evaluate the antigenotoxic properties of probiotics and/or food-associated bacteria could be a promising tool to investigate the probiotics bio-protective role at GI level against different food and environmental genotoxic compounds.

Figure 2. In vitro antigenotoxic activity of probiotics L. plantarum strains against bisphenol A (BPA) and di-n-hexyl phthalate (DnHP), evaluated by the SOS-Chromotest. Percent genotoxicity inhibition was calculated from residual activity (SOS induction factor) on supernatants in relation to that of a positive control. Data are shown as means ± SD from three replicates.
CONCLUSION

Nowadays, the interest in the diets or dietary components able to modulate the risk of genotoxicity and GI diseases is relevant, considering that we are everyday exposed to high level of exogenous and endogenous risk factors. Within the huge variety of genotoxic compounds that can be found in the gut, mainly related to Western diets and lifestyle, EDs are emerging as novel genotoxic agents that can be associated to a wide range of pathologies and long-term effects. Experimental evidences confirm the magnitude of the diet-health relationship and probiotics and/or food-associated microbes assume an interesting role as potential bio-protective tool in the modulation of food-related genotoxic and mutagenic risk at GI level. Protective effects of probiotics, prebiotics and food-associated microbes over EDs have also been touch on, although further in vivo studies are needed to elucidate the mechanisms underlying these genotoxic-preventing effects. In line with this, the use of zebrafish as experimental model may open new perspectives in deciphering effective intervention for inflammatory and cancer prevention.

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