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

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*Corresponding author: University of Teramo, Via Balzarini 1, 64100, Teramo. Tel: +39 3498769926; Fax: +39 0861266896; E-mail: rprete@unite.it**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), methylnitronitrosoguanidine (MNNG)) 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; Verdenelli et al. 2010; Walia, 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; Erkekoglu 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 vivo* using murine models (Dominici et al. 2014) or by evaluating the antigenotoxicity using faecal water (FW) (Nowak, Slizewska and Otlewska 2015; Federici et al. 2017). About that, the use of zebrafish (*Danio rerio*) as *in vivo* model to validate 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 life-cycle 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 antigenotoxic 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).

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

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

^aEDs noted as: 2,2',4,4'-Tetrabromodiphenyl 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-153), 3,3',4,4',5-Pentachlorobiphenyl (PCB-126), 2,2',3,3',4,5,6-Heptachlorobiphenyl (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).

Emerging evidence indicates an association between exposure to EDs and modifications of gut bacterial communities leading to increase 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 type 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 (Jacobson-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

(Legeay and Faure 2017). Recently, BPA and its analogues bisphenol S, bisphenol F and bisphenol AF, showed cytotoxic, genotoxic and oxidative potential activities (Maćczak et al. 2017; Hercog et al. 2019). Moreover, dietary BPA intake influences the gut microbiota composition and functions that could be an important factor to develop of metabolic syndrome (Lai et al. 2016).

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 obesogenic 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 (Fénichel 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; Commane 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 (*sfIA::lacZ*, *uvrA*, *rfa*, *Pho^c*) carries a *sfIA::lacZ* gene fusion, responsible for β -galactosidase (BG) 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 (Raipulis, Toma and Semjonovs 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* (Deabas 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 Binda 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 and 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

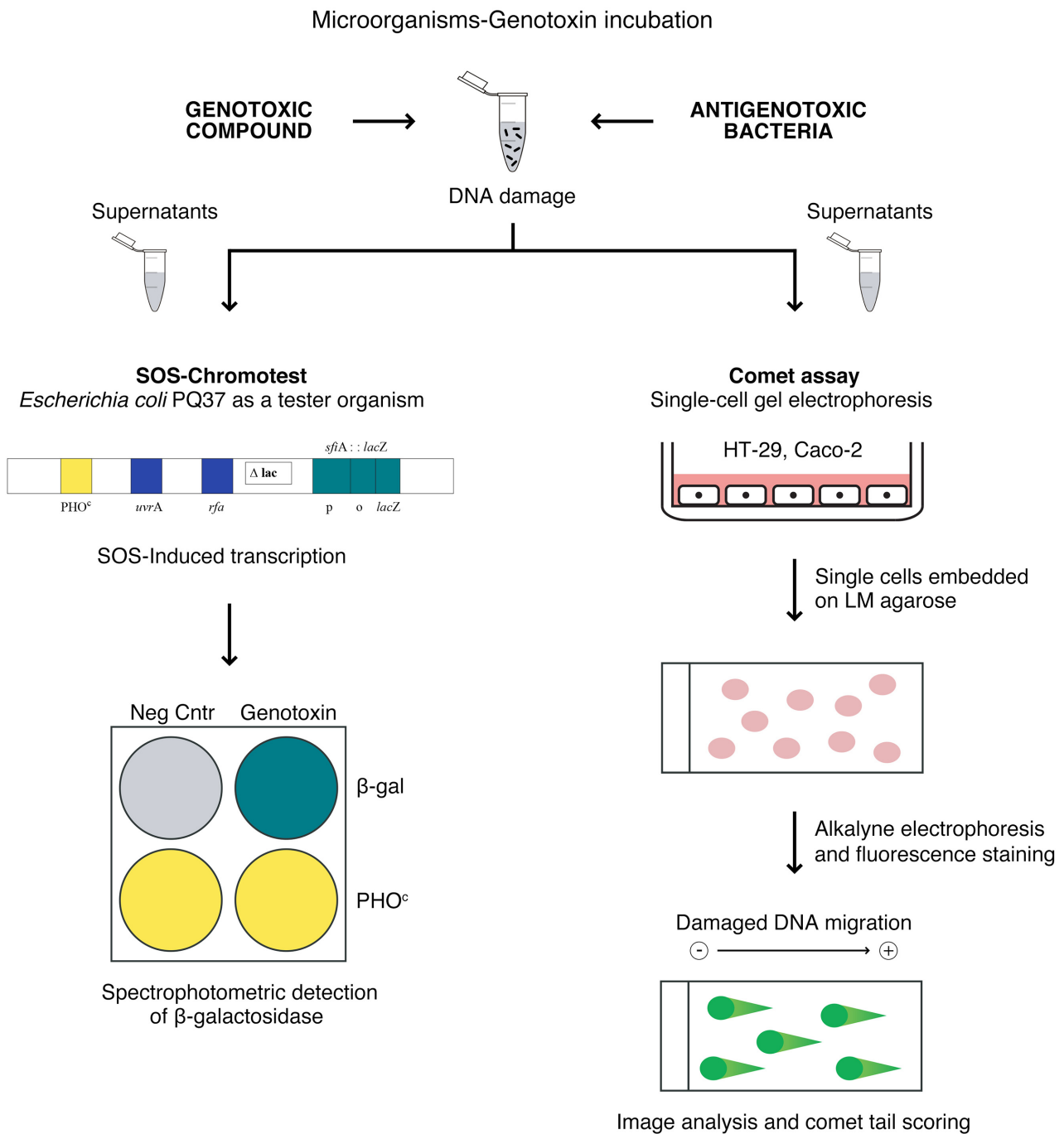


Figure 1. Schematic representation of *in vitro* primary DNA damage assays used to assess microbial antigenotoxic activity. SOS-Chromotest is a rapid colorimetric assay that evaluates the DNA damage induced by genotoxic compounds through the measure of the SOS-response of a prokaryotic tester organism *E. coli* PQ37 (*sfiA::lacZ*, *uvrA*, *rfa*, *Pho^c*), 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.

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 food-borne microorganisms in degrading EDs and hence exhibiting

protective effects remains unclear. Therefore, no investigations are available about the microbial antigenotoxicity 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),

Table 2. Probiotics and food-associated bacteria evaluated for their antigenotoxic activity against different mutagenic compounds.

Antigenotoxic bacteria	Methods	Genotoxic agents ^a	References
Probiotics/human strains			
<i>B. breve</i> , <i>B. longum</i>	Comet assay	MNNG; DMH	Pool-Zobel et al. 1996
<i>L. acidophilus</i> , <i>Bifidobacterium</i> spp.	Ames test	4-NQO; MNNG; NF; NPD; AFB; MeIQ, PhIP, MeAαC	Lankaputhra and Shah 1998
<i>Bacillus</i> spp. (probiotics)	SOS-Chromotest	4-NQO	Caldini, Trotta and Cenci 2002
<i>Bifidobacterium</i> sp. 420, <i>Bifidobacterium lactis</i> Bb-12, <i>L. plantarum</i> , <i>Streptococcus thermophilus</i> , <i>L. bulgaricus</i> , <i>Enterococcus faecium</i>	Comet assay	FW	Burns and Rowland 2004
<i>L. plantarum</i> KL and 8014, <i>L. casei</i> Shirota, <i>L. acidophilus</i> T20, <i>Streptococcus salivarius</i> var. <i>thermophilus</i> , <i>B. lactis</i> Bb-12	SOS-Chromotest	Furazolidone	Raipulis, Toma and Semjonovs 2005
<i>Bacillus</i> spp. (probiotics)	SOS-Chromotest	4-NQO, MNNG, MeIQ, AFB1	Cenci et al. 2008
<i>L. acidophilus</i> J76, <i>L. casei</i> 5H10, <i>L. bulgaricus</i> , <i>L. fermentum</i> , <i>L. plantarum</i> J25, <i>L. rhamnosus</i> J54, <i>B. longum</i>	SOS-Chromotest; Ames test	Heating cooking oils	Isidori and Parrella 2009
<i>L. rhamnosus</i> IMC501	GC-MS; SOS-Chromotest	4-NQO	Verdenelli et al. 2010; Bocci et al. 2015
<i>L. casei</i> LOCK 0900, <i>L. paracasei</i> LOCK 0919	Comet assay	PhIP	Nowak, Slizewska and Klewicka 2011
<i>L. rhamnosus</i> 0900 and 0908, <i>L. casei</i> 0919, <i>L. casei</i> Shirota, <i>L. casei</i> DN 114-001, <i>L. johnsonii</i> La1, <i>L. rhamnosus</i> GG, <i>B. animalis</i> ssp. <i>lactis</i> Bb-12, <i>L. delbrueckii</i> 0987, <i>L. mucosae</i> 0988	Comet assay	FW	Nowak, Slizewska and Otlewska 2015
<i>L. rhamnosus</i> Vc	SOS-Chromotest; Ames test	MNNG	Pithva et al. 2015
<i>L. plantarum</i> IMC510, IMC513, WCFS1	SOS-Chromotest	4-NQO	Prete et al. 2017
<i>Escherichia coli</i> Nissle 1917	Comet assay, Ames test	4-NQO	Janosch et al. 2019
<i>L. rhamnosus</i> MD14, <i>L. plantarum</i> GMD, <i>P. pentosaceus</i> GMD17A	SOS-Chromotest; Comet Assay	DMH, 4-NQO	Chandel et al. 2019
Food-associated strains			
<i>L. acidophilus</i> , <i>L. gasseri</i> , <i>L. confusus</i> , <i>S. thermophilus</i>	Comet assay	MNNG; DMH	Pool-Zobel et al. 1996
<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>L. casei</i> , <i>L. rhamnosus</i> , <i>L. acidophilus</i> , <i>L. plantarum</i>	SOS-Chromotest	4-NQO	Cenci et al. 2002
<i>L. casei</i> , <i>L. acidophilus</i> , <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>L. rhamnosus</i> , <i>L. plantarum</i>	SOS-Chromotest; Comet Assay; Ames test	4-NQO, MNNG	Caldini et al. 2005
<i>Lactobacillus</i> spp.	SOS-Chromotest	4-NQO, MNNG	Caldini et al. 2008
<i>L. casei</i>	SOS-Chromotest	4-NQO, MNNG	Corsetti et al. 2008
<i>L. salivarius</i> FDB89	SOS-Chromotest	4-NQO	Fang et al. 2008
<i>Lactobacillus</i> spp.	Ames test	Sodium azide, NF	Darsanaki et al. 2012
<i>E. faecium</i> , <i>Bacillus coagulans</i> , <i>L. plantarum</i>	SOS-Chromotest, Ames test	4-NQO, Furazolidone	Walia, Keshani and Kanwar 2014
<i>L. plantarum</i>	Comet assay	AFB1	Kurhan and Cakir 2016
<i>L. plantarum</i>	SOS-Chromotest	4-NQO	Prete et al. 2017
LAB	SOS-Chromotest	DMH	Sharma, Chandel and Shukla 2020

^aGenotoxic compounds noted as: methylnitro nitrosoguanidine (MNNG), 1,2-dimethylhydrazine (DMH), 4-nitro-quinoline-1-oxide (4-NQO), 2-nitrofluorene (NF), 4-nitro-*O*-phenylenediamine (NPD), Aflatoxin-B (AFB), 2-amino-3-methyl-3H-imidazoquinoline (MeIQ), (2-amino-1-methyl-6-phenyl-1H-imidazo[4,5-b]pyridine (PhIP), 2-Amino-3-methyl-9H-pyrido[2,3-b]indole (MeAαC), genotoxic faecal water (FW), Aflatoxin-B₁ (AFB1).

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 trail with zebrafish.

IN VIVO STUDIES: ZEBRAFISH AS A MODEL FOR ANTIGENOTOXICITY

In vivo studies have shown that some probiotics (e.g. *Lactobacillus* spp.) could be capable of counteracting 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

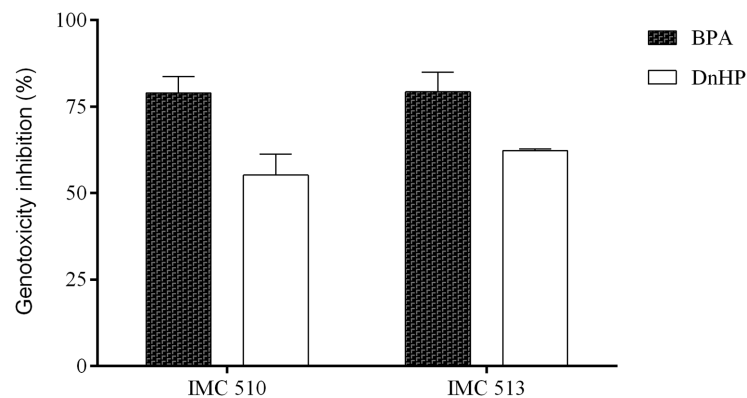


Figure 2. *In vitro* antigenotoxic activity of probiotics *L. plantarum* strains against bisphenol A (BPA) and di-n-hexyl phthalate (DnHP), evaluated by the SOS-Chromotest. Percent genotoxicity inhibition was calculated from residual activity (SOS induction factor) on supernatants in relation to that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

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* IMC501 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 (Deabas 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* 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 microbiome 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. 2018b). 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.

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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REFERENCES

- Abbasi MH, Zahedi M, Darvish Moghadam S et al. Small bowel bacterial overgrowth in patients with irritable bowel syndrome: the first study in Iran. *M Middle East J Dig Dis* 2015;7:36–40.
- Abujah CI, Ogbonna AC, Osuji CM. Functional components and medicinal properties of food: a review. *J Food Sci Technol* 2015;52:2522–9.
- Agans RT, Gordon A, Hussain S et al. Titanium dioxide nanoparticles elicit lower direct inhibitory effect on human gut microbiota than silver nanoparticles. *Toxicol Sci* 2019;172:411–6.
- Albert O, Jégou B. A critical assessment of the endocrine susceptibility of the human testis to phthalates from fetal life to adulthood. *Hum Reprod Update* 2014;20:231–49.
- Alofe O, Kisanga E, Inayat-Hussain SH. Determining the endocrine disruption potential of industrial chemicals using an integrative approach: public data bases, *in vitro* exposure, and modelling receptor interactions. *Environ Int* 2019;131:104969.
- Ankley GT, Johnson RD. Small fish models for identifying and assessing the effects of endocrine-disrupting chemicals. *ILAR J* 2004;45:469–83.
- Avella MA, Place A, Du SJ et al. *Lactobacillus rhamnosus* accelerates zebrafish backbone calcification and gonadal differentiation through effects on the GnRH and IGF systems. *PLoS One* 2012;7:e45572.
- Bates JM, Akerlund J, Mittge E et al. Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. *Cell Host Microbe* 2007;2:371–82.
- Beausoleil C, Ormsby JN, Gies A et al. Low dose effects and non-monotonic dose responses for endocrine active chemicals: science to practice workshop: workshop summary. *Chemosphere* 2013;93:847–56.
- Bocci A, Sebastiani B, Trotta F et al. *In vitro* inhibition of 4-nitroquinoline-1-oxide genotoxicity by probiotic *Lactobacillus rhamnosus* IMC501. *J Microbiol Biotechnol* 2015;25:1680–6.
- Breton J, Daniel C, Dewulf J et al. Gut microbiota limits heavy metals burden caused by chronic oral exposure. *Toxicol Lett* 2013;222:132–8.
- Burns AJ, Rowland IR. Anti-carcinogenicity of probiotics and prebiotics. *Curr Issues Intest Microbiol* 2000;1:13–24.
- Burns AJ, Rowland IR. Antigenotoxicity of probiotics on faecal water-induced DNA damage in human colon adenocarcinoma cells. *Mutat Res* 2004;551:233–43.
- Caballero-Gallardo K, Olivero-Verbel J, Freeman JL. Toxicogenomics to evaluate endocrine disrupting effects of environmental chemicals using the zebrafish model. *Curr Genomics* 2016;17:515–27.
- Cabaton N, Dumont C, Severin I et al. Genotoxic and endocrine activities of bis(hydroxyphenyl)methane (bisphenol F) and its derivatives in the HepG2 cell line. *Toxicol* 2009;255:15–24.
- Caldini G, Trotta F, Cenci G. Inhibition of 4-nitroquinoline-1-oxide genotoxicity by *Bacillus* strains. *Res Microbiol* 2002;153:165–71.
- Caldini G, Trotta F, Corsetti A et al. Evidence for *in vitro* antigenotoxicity of cheese non-starter lactobacilli. *Antonie Van Leeuwenhoek* 2008;93:51–59.
- Caldini G, Trotta F, Villarini M et al. Screening of potential lactobacilli antigenotoxicity by microbial and mammalian cell-based tests. *Int J Food Microbiol* 2005;102:37–47.
- Cenci G, Caldini G, Trotta F et al. *In vitro* inhibitory activity of probiotic spore-forming bacilli against genotoxins. *Lett Appl Microbiol* 2008;46:331–7.
- Cenci G, Rossi J, Trotta F et al. Lactic acid bacteria isolated from dairy products inhibit genotoxic effect of 4-nitroquinoline-1-oxide in SOS chromotest. *Syst Appl Microbiol* 2002;25:483–90.
- Chakravarthy S, Sadagopan S, Nair A et al. Zebrafish as an *in vivo* high-throughput model for genotoxicity. *Zebrafish* 2014;11:154–66.
- Champagne CP, Gomes da Cruz A, Daga M. Strategies to improve the functionality of probiotics in supplements and foods. *Curr Opin Food Sci* 2018;22:160–6.
- Chandel D, Sharma M, Chawla V et al. Isolation, characterization and identification of antigenotoxic and anticancerous indigenous probiotics and their prophylactic potential in experimental colon carcinogenesis. *Sci Rep* 2019;9:14769.
- Chen L, Guo Y, Hu C et al. Dysbiosis of gut microbiota by chronic coexposure to titanium dioxide nanoparticles and bisphenol A: implications for host health in zebrafish. *Environ Pollut* 2018a;234:307–17.
- Chen L, Hu C, Lok-Shun Lai N et al. Acute exposure to PBDEs at an environmentally realistic concentration causes abrupt changes in the gut microbiota and host health of zebrafish. *Environ Pollut* 2018b;240:17–26.
- Chen M, Yin J, Liang Y et al. Oxidative stress and immunotoxicity induced by graphene oxide in zebrafish. *Aquat Toxicol* 2016;174:54–60.

- Claesson MJ, Jeffery IB, Conde S et al. Gut microbiota composition correlates with diet and health in the elderly. *Nature* 2012;**488**:178–84.
- Commane D, Hughes R, Shortt C et al. The potential mechanisms involved in the anti-carcinogenic action of probiotics. *Mutat Res* 2005;**591**:276–89.
- Corsetti A, Caldini G, Mastrangelo M et al. Raw-milk traditional Italian ewe cheeses as a source of *Lactobacillus casei* strains with acid-bile resistance and antigenotoxic properties. *Int J Food Microbiol* 2008;**125**:330–5.
- Dal Santo G, Grotto A, Boligon AA. Protective effect of *Uncaria tomentosa* extract against oxidative stress and genotoxicity induced by glyphosate-Roundup® using zebrafish (*Danio rerio*) as a model. *Environ Sci Pollut Res* 2018;**25**:11703–15.
- Darbre PD. The history of endocrine-disrupting chemicals. *Curr Opin Endocr Metab Res* 2019;**7**:26–33.
- Darsanaki RK, Issazadeh K, Reza Majid M et al. Antimutagenic activity of *Lactobacillus* spp. isolated from fresh vegetables against sodium azide and 2-Nitrofluorene. *J Pure Appl Microbiol* 2012;**6**:1677–82.
- David LA, Maurice CF, Carmody RN et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014;**505**:559–63.
- Davis CD, Milner JA. Gastrointestinal microflora, food components and colon cancer prevention. *J Nutr Biochem* 2009;**20**:743–52.
- Deabes MM, Darwish HR, Abdel-Aziz KB et al. Protective effects of *Lactobacillus rhamnosus* GG on Aflatoxins-induced toxicities in male Albino Mice. *J Environ Anal Toxicol* 2012;**2**:2–9.
- Dominici L, Moretti M, Villarini M et al. In vivo antigenotoxic properties of a commercial probiotic supplement containing bifidobacteria. *Int J Probiotics and Prebiotics*, 2011;**6**:3–4.
- Dominici L, Villarini M, Trotta F et al. Protective effects of probiotic *Lactobacillus rhamnosus* IMC501 in mice treated with PhIP. *Microbiol Biotechnol* 2014;**24**:371–8.
- Dunnick JK, Pandiri AR, Merrick BA et al. Carcinogenic activity of pentabrominated diphenyl ether mixture (DE-71) in rats and mice. *Toxicol Rep* 2018;**5**:615–24.
- Ebele AJ, Abdallah MAE, Harrad S. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerg Contam* 2017;**3**:1–16.
- Erkekoglu P, Kocer-Gumusel B. Genotoxicity of phthalates. *Toxicol Mech Methods*. 2014;**24**:616–26.
- Evariste L, Barret M, Mottier A et al. Gut microbiota of aquatic organisms: a key endpoint for ecotoxicological studies. *Environ Pollut*. 2019;**248**:989–99.
- Fackelmann G, Sommer S. Microplastics and the gut microbiome: how chronically exposed species may suffer from gut dysbiosis. *Mar Pollut Bull* 2019;**143**:193–203.
- Falcinelli S, Rodiles A, Hatef A et al. Dietary lipid content reorganizes gut microbiota and probiotic *Lactobacillus rhamnosus* attenuates obesity and enhances catabolic hormonal milieu in zebrafish. *Sci Rep* 2017;**7**:5512.
- Fang W, Lu J, Ai-Ping L et al. Analysis of antigenotoxicity of *Lactobacillus salivarius* by high performance liquid chromatography. *Chin J Anal Chem* 2008;**36**:740–4.
- Federici E, Prete R, Lazzi C et al. Bacterial composition, genotoxicity, and cytotoxicity of fecal samples from individuals consuming omnivorous or vegetarian diets. *Front Microbiol* 2017;**8**:300.
- Fénelon P, Chevalier N. Environmental endocrine disruptors: New diabetogens? *C R Biol* 2017;**340**:446–52.
- Garcia-Gonzalez N, Prete R, Battista N et al. Adhesion properties of food-associated *Lactobacillus plantarum* strains on human intestinal epithelial cells and modulation of IL-8 release. *Front Microbiol* 2018;**9**:2392.
- Gibson GR, Hutkins R, Sanders ME et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* 2017;**14**:491–502.
- Gioacchini G, Giorgini E, Merrifield DL et al. Probiotics can induce follicle maturational competence: the *Danio rerio* case. *Biol Reprod* 2012;**86**:65–71.
- Gioacchini G, Rossi G, Carnevali O. Host-probiotic interaction: new insight into the role of the endocannabinoid system by in vivo and ex vivo approaches. *Sci Rep* 2017;**7**:1261.
- Graf D, Di Cagno R, Fak F et al. Contribution of diet to the composition of the human gut microbiota. *Nutrients* 2015;**7**:17–44.
- Gratz SW, Wallace RJ, El-Nezami HS. Recent perspectives on the relations between fecal mutagenicity, genotoxicity, and diet. *Front Pharmacol* 2011;**2**:4.
- Güzel BD, Ayaz TB. In vitro genotoxic and cytotoxic effects of some paraben esters on human peripheral lymphocytes. *Drug Chem Toxicol* 2019;**42**:386–93.
- Hasan N, Yang H. Factors affecting the composition of the gut microbiota, and its modulation. *Peer J* 2019;**7**:e7502.
- Hercog K, Maisanaba S, Filipič M et al. Genotoxic activity of bisphenol A and its analogues bisphenol S, bisphenol F and bisphenol AF and their mixtures in human hepatocellular carcinoma (HepG2) cells. *Sci Total Environ* 2019;**15**:267–76.
- Hill C, Guarner F, Reid G et al. Expert consensus document: the international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 2014;**11**:506–14.
- Hiramaya K, Rafter J. The role of probiotic bacteria in cancer prevention. *Microb Infect* 2000;**2**:681–6.
- Howe K, Clark MD, Torroja CF et al. The zebrafish reference genome sequence and its relationship to the human genome. *Nature* 2013;**496**:498–503.
- Isidori M, Parrella A. Genotoxicity of aqueous extract from heated cooking oils and its suppression by lactobacilli. *Food Sci Tech Int* 2009;**15**:0267273.
- Iszatt N, Janssen S, Lenters V et al. Environmental toxicants in breast milk of Norwegian mothers and gut bacteria composition and metabolites in their infants at 1 month. *Microbiome* 2019;**7**:34.
- Jacobsen-Pereira CH, dos Santos CR, Maraslis FT et al. Markers of genotoxicity and oxidative stress in farmers exposed to pesticides. *Ecotox Environ Safe* 2018;**148**:177–83.
- Janosch D, Dubbert S, Eiteljörge K et al. Anti-genotoxic and anti-mutagenic activity of *Escherichia coli* Nissle 1917 as assessed by in vitro tests. *Benef Microbes* 2019;**10**:449–61.
- Javurek AB, Spollen WG, Johnson SA et al. Effects of exposure to bisphenol A and ethinyl estradiol on the gut microbiota of parents and their offspring in a rodent model. *Gut microbes* 2016;**7**:471–85.
- Jin C, Luo T, Zhu Z et al. Imazalil exposure induces gut microbiota dysbiosis and hepatic metabolism disorder in zebrafish. *Comp Biochem Phys C* 2017;**202**:85–93.
- Karabulut G, Barlas N. Genotoxic, histologic, immunohistochemical, morphometric and hormonal effects of di-(2-ethylhexyl)-phthalate (DEHP) on reproductive systems in prepubertal male rats. *Toxicol Res* 2018;**7**:859–73.
- Kerry RG, Patra JK, Gouda S et al. Benefaction of probiotics for human health: a review. *J Food Drug Anal* 2018;**26**:927–39.

- Kim J, Chevrier J. Exposure to parabens and prevalence of obesity and metabolic syndrome: an analysis of the Canadian Health Measures Survey. *Sci Total Environ* 2019;**713**:135116.
- Kurhan S, Cakir I. DNA-bioprotective effects of lactic acid bacteria against Aflatoxin B1. *Curr Res Nutr Food Sci* 2016;**4**:87–91.
- Lai KP, Chung YT, Li R et al. Bisphenol A alters gut microbiome: comparative metagenomics analysis. *Environ Pollut* 2016;**218**:923–30.
- Lankaputhra WE, Shah NP. Antimutagenic properties of probiotic bacteria and of organic acids. *Mutat Res* 1998;**397**:169–82.
- Laue HE, Brennan KJ, Gillet V et al. Associations of prenatal exposure to polybrominated diphenyl ethers and polychlorinated biphenyls with long-term gut microbiome structure: a pilot study. *Environ Epidemiol* 2019;**3**:pe(039).
- Legeay S, Faure S. Is bisphenol A an environmental obesogen? *Fund Clin Pharmacol* 2017;**31**:594–609.
- Le Magueresse-Battistoni B, Labaronne E, Vidal H et al. Endocrine disrupting chemicals in mixture and obesity, diabetes and related metabolic disorders. *World J Biol Chem* 2017;**8**:108–11.
- Lieschke GJ, Currie PD. Animal models of human disease: zebrafish swim into view. *Nat Rev Genet* 2007;**8**:353–67.
- Liu Y, Hu K, Jia H et al. Potent mutagenicity of some non-planar tri- and tetrachlorinated biphenyls in mammalian cells, human CYP2E1 being a major activating enzyme. *Arch Toxicol* 2017;**91**:2663–76.
- Li X, Gu AZ, Zhang Y et al. Sub-lethal concentrations of heavy metals induce antibiotic resistance via mutagenesis. *J Hazard Mater* 2019;**369**:9–16.
- MacBean C. *The Pesticide Manual: A World Compendium*. British Crop Production Council 16th Edition. MacBean C (ed.) 2012.
- Marco ML, Golomb BJ. Fermented foods, *Lactobacillus*, and health. *Microbe* 2016;**11**:349–54.
- Marco ML, Heeney D, Binda S. Health benefits of fermented foods: microbiota and beyond. *Curr Opin Biotechnol* 2017;**44**:94–102.
- Martinez-Paz P, Morales M, Martinez-Guitarte J-L et al. Genotoxic effects of environmental endocrine disruptors on the aquatic insect *Chironomus riparius* evaluated using the comet assay. *Mutat Res* 2013;**758**:41–47.
- Ma Y, Guo Y, Ye H et al. Perinatal triclosan exposure in the rat induces long-term disturbances in metabolism and gut microbiota in adulthood and old age. *Environ Res* 2020;**182**:109004.
- Maćczak A, Cyrkler M, Bukowska B et al. Bisphenol A, bisphenol S, bisphenol F and bisphenol AF induce different oxidative stress and damage in human red blood cells (in vitro study). *Toxicol in Vitro* 2017;**41**:143–9.
- McCracken VJ, Lorenz RG. The gastrointestinal ecosystem: a precarious alliance among epithelium, immunity and microbiota. *Cell Microbiol* 2001;**3**:1–11.
- Mergler M, Wolf K, Zimmermann M. Development of bisphenol A-adsorbing yeast by surface display of the *Kluyveromyces* yellow enzyme on *Pichia pastoris*. *App Microbiol Biotechnol* 2004;**63**:418–21.
- Monneret C. What is an endocrine disruptor? *C R Biol* 2017;**340**:403–5.
- Nagpal R, Kumar A, Kumar M et al. Probiotics, their health benefits and applications for developing healthier foods: a review. *FEMS Microbiol Lett* 2012;**334**:1–15.
- Nappi F, Barrea L, Di Somma C et al. Endocrine aspects of environmental “obesogen” pollutants. *Int J Environ Res Public Health* 2016;**13**:765.
- Nowak A, Slizewska K, Klewicka E. Effect of probiotic lactobacilli on faecal enzyme and genotoxic activity in human faecal water in the presence of the carcinogen PhIP in vitro. *Int J Dairy Technol* 2011;**65**:300–7.
- Nowak A, Slizewska K, Otlewska A. Antigenotoxic activity of lactic acid bacteria, prebiotics, and products of their fermentation against selected mutagens. *Regul Toxicol Pharmacol* 2015;**73**:938–46.
- Nowak K, Ratajczak-Wrona W, Górska M et al. Parabens and their effects on the endocrine system. *Mol Cell Endocrinol* 2018;**474**:238–51.
- O’Hara AM, O’Regan P, Fanning A et al. Functional modulation of human intestinal epithelial cell responses by *Bifidobacterium infantis* and *Lactobacillus salivarius*. *Immunology* 2006;**118**:202–15.
- Oishi K, Sato T, Yokoi W et al. Effects of probiotics, *Bifidobacterium breve* and *Lactobacillus casei*, on bisphenol A exposure in rats. *Biosci Biotechnol Biochem* 2008;**72**:1409–15.
- Okazaki F, Zang L, Nakayama H et al. Microbiome alteration in type 2 diabetes mellitus model of zebrafish. *Sci Rep* 2019;**9**:867.
- Perugini M, Merola C, Amorena M et al. Sublethal exposure to propylparaben leads to lipid metabolism impairment in zebrafish early-life stages. *J App Toxicol* 2019; ACCEPTED MANUSCRIPT; DOI:10.1002/jat.3921.
- Pietroiusti A, Magrini A, Campagnolo L. New frontiers in nanotoxicology: gut microbiota/microbiome-mediated effects of engineered nanomaterials. *Toxicol Appl Pharm* 2016;**299**:90–95.
- Pithva SP, Ambalam PS, Ramoliya JM et al. Antigenotoxic and Antimutagenic Activities of probiotic *Lactobacillus rhamnosus* Vc against N-Methyl-N’-Nitro-N-Nitrosoguanidine. *Nutr Cancer* 2015;**67**:1142–50.
- Pool-Zobel BL, Neudecker C, Domizlaff I et al. *Lactobacillus* and *bifidobacterium*-mediated antigenotoxicity in the colon of rats. *Nutr Cancer* 1996;**26**:365–80.
- Prakash S, Rodes L, Coussa-Charley M et al. Gut microbiota: next frontier in understanding human health and development of biotherapeutics. *Bio Tar Ther* 2011;**5**:71–86.
- Prete R, Garcia-Gonzalez N, Perugini M Società Italiana di Microbiologia Agro-Alimentare e Ambientale, SIMTREA et al. In vitro antigenotoxic activity of probiotic *Lactobacillus plantarum* IMC 510 and IMC 513 against two different endocrine disruptors: bisphenol A and di-n-hexyl phthalate. Microbial Diversity as a source of novelty: function, adaptation and exploitation. *Proceedings of the 5th International Conference on Microbial Diversity, Catania, Italy, 25th- 27th September 2019*, Eds: Società Italiana di Microbiologia Agro-Alimentare e Ambientale, SIMTREA, ISBN 978-88-943010-1-4. Session IV, PS4-9, 2019, pp. 342–3.
- Prete R, Long SL, Lopez Gallardo A et al. Beneficial bile acid metabolism from *Lactobacillus plantarum* of food origin. *Sci Rep* 2020;**10**:1165.
- Prete R, Tofalo R, Federici E et al. Food-Associated *Lactobacillus plantarum* and yeasts inhibit the genotoxic effect of 4-Nitroquinoline-1-Oxide. *Front Microbiol* 2017;**8**:2349.
- Qiao R, Deng Y, Zhang S et al. Accumulation of different shapes of microplastics initiates intestinal injury and gut microbiota dysbiosis in the gut of zebrafish. *Chemosphere* 2019;**236**:124334.
- Quillardet P, Hofnung M. The SOS chromotest: a review. *Mutat Res* 1993;**297**:235–79.
- Rai P, Ganguli A, Neogi SB. Endocrine disrupting chemicals: current trends, attributable effects on women health and future perspectives. *Int J Basic Appl Biol* 2017;**4**:198–205.

- Raipulis J, Toma MM, Semjonovs P. The effect of probiotics on the genotoxicity of furazolidone. *Int J Food Microbiol* 2005;**102**:343–7.
- Raman M, Ambalam P, Kondepudi KK et al. Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer. *Gut Microbes* 2013;**4**:181–92.
- Scott KP, Gratz SW, Sheridan PO et al. The influence of diet on the gut microbiota. *Pharm Res* 2013;**69**:52–60.
- Sekirov I, Russell SL, Antunes LC et al. Gut microbiota in health and disease. *Physiol Rev* 2010;**90**:859–904.
- Shah NP. Functional cultures and health benefits. *Int Dairy J* 2007;**17**:1262–77.
- Sharma M, Chandel D, Shukla G. Antigenotoxicity and cytotoxic potentials of metabiotics extracted from isolated probiotic, *Lactobacillus rhamnosus* MD 14 on Caco-2 and HT-29 human colon cancer cells. *Nutr Cancer* 2020;**72**:110–9.
- Sharma M, Shukla G. Metabiotics: one-step ahead of probiotics; an insight into mechanisms involved in anticancerous effect in colorectal cancer. *Front Microbiol* 2016;**7**:1940.
- Singh NP, McCoy MT, Tice RR et al. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res* 1988;**175**:184–91.
- Solouki S, Fazeli MR, Solouki S. Efficiency of multispecies probiotic supplements in bio-removal of bisphenol A: an in vitro study. *App Food Biotechnol* 2018;**5**:37–45.
- Somade OT, Ugbaja RN, Adeyi OE et al. Thyroid and reproductive hormones disruption as well as kallikrein-3 level in dimethyl nitrosamine-induced toxicity: effects of ascorbate treatment in male wistar rats. *Biochim Open* 2016;**3**:40–46.
- Tavares RS, Escada-Rebello S, Correia M et al. The non-genomic effects of endocrine-disrupting chemicals on mammalian sperm. *Reproduction* 2016;**151**:R1–R13.
- Tchounwou PB, Yedjou CG, Patlolla AK et al. Heavy metal toxicity and the environment. In: *Molecular Clinical and Environmental Toxicology*. Basel: Springer, 2012, 133–64.
- Trinder M, Bisanz JE, Burton JP et al. Probiotic lactobacilli: a potential prophylactic treatment for reducing pesticide absorption in humans and wildlife. *Benef Microbes* 2015;**6**:841–7.
- Trotta F, Caldini G, Dominici L et al. Food borne yeasts as DNA-bioprotective agents against model genotoxins. *Int J Food Microbiol* 2012;**153**:275–80.
- Umbuzeiro G, Machala M, Weiss J. Diagnostic tools for effect-directed analysis of mutagens, AhR agonists, and endocrine disruptors. In: Brack W. (eds) *Effect-Directed Analysis of Complex Environmental Contamination. The Handbook of Environmental Chemistry* Springer 2011; DOI:10.1007/978-3-642-18384-3.4.
- Velmurugan G, Ramprasath T, Gilles M et al. Gut microbiota, endocrine-disrupting chemicals, and the diabetes epidemic. *Trends Endocrinol Metab* 2017;**28**:612–25.
- Ventrice P, Ventrice D, Russo E et al. Phthalates: European regulation, chemistry, pharmacokinetic and related toxicity. *Environ Toxicol Pharmacol* 2013;**36**:88–96.
- Verdenelli MC, Ricciutielli M, Gigli F et al. Investigation of the antigenotoxic properties of the probiotic *Lactobacillus rhamnosus* IMC 501[®] by gas chromatography-mass spectrometry. *Ital J Food Sci* 2010;**22**:473–8.
- Voss C, Zerban H, Bannasch P et al. Lifelong exposure to di-(2-ethylhexyl)-phthalate induces tumors in liver and testes of Sprague-Dawley rats. *Toxicology* 2005;**206**:359–71.
- Wahlang B, Falkner KC, Gregory B et al. Polychlorinated biphenyl 153 is a diet-dependent obesogen that worsens nonalcoholic fatty liver disease in male C57BL6/J mice. *J Nutr Biochem* 2013;**24**:1587–95.
- Walia S, Keshani SS, Kanwar SS. Exhibition of DNA-bioprotective activity by microflora of traditional fermented foods of North-Western Himalayas. *Food Res Int* 2014;**55**:176–80.
- Wallace DR. Nanotoxicology and Metalloestrogens: Possible Involvement in Breast Cancer. *Toxics* 2015;**3**:390–413.
- WHO/UNEP, State of the science of endocrine disrupting chemicals - 2012, Bergman A. et al. (eds) 2012. Available from: http://www.who.int/iris/bitstream/10665/78101/1/9789241505031_eng.pdf.
- Xia J, Lu L, Jin C et al. Effects of short term lead exposure on gut microbiota and hepatic metabolism in adult zebrafish. *Comp Biochem Phys C* 2018;**209**:1–8.
- Yang YN, Yang YCS, Li IH et al. Phthalate exposure alters gut microbiota composition and IgM vaccine response in human newborns. *Food Chem Toxicol* 2019;**132**:110700.
- Yasushi E, Kimura N, Ikeda I et al. Adsorption of bisphenol A by lactic acid bacteria, *Lactococcus* strains. *App Microbiol Biotechnol* 2007;**74**:202–7.
- Yuan X, Pan Z, Jin C et al. Gut microbiota: an underestimated and unintended recipient for pesticide-induced toxicity. *Chemosphere* 2019;**227**:425–34.
- Zang L, Ma Y, Huang W et al. Dietary *Lactobacillus plantarum* ST-III alleviates the toxic effects of triclosan on zebrafish (*Danio rerio*) via gut microbiota modulation. *Fish Shellfish Immun* 2019;**84**:1157–69.
- Zarean M, Keikha M, Poursafa P et al. A systematic review on the adverse health effects of di-2-ethylhexyl phthalate. *Environ Sci Pollut Res Int* 2016;**23**:24642–93.
- Zeza D, Tait S, Della Salda L et al. Toxicological, gene expression and histopathological evaluations of environmentally realistic concentrations of polybrominated diphenyl ethers PBDE-47, PBDE-99 and PBDE-209 on zebrafish embryos. *Ecotoxicol Environ Saf* 2019;**183**:109566.
- Zhai Q, Yu L, Li T et al. Effect of dietary probiotic supplementation on intestinal microbiota and physiological conditions of Nile tilapia (*Oreochromis niloticus*) under waterborne cadmium exposure. *Antonie Van Leeuwenhoek* 2017;**110**:501–13.
- Zhang M, Wang F, Jiang L et al. *Lactobacillus salivarius* REN inhibits rat oral cancer induced by 4-nitroquinoline 1-oxide. *Cancer Prev Res* 2013;**6**:686–94.
- Zhang R, Pan Z, Wang X et al. Short-term propamocarb exposure induces hepatic metabolism disorder associated with gut microbiota dysbiosis in adult male zebrafish. *Acta Biochim Biophys Sin* 2018;**51**:88–96.
- Zhang W, Liu W, Zhang J et al. Characterisation of acute toxicity, genotoxicity and oxidative stress posed by textile effluent on zebrafish. *J Environ Sci* 2012;**24**:2019–27.
- Zhang Z, Zheng Z, Cai J et al. Effect of cadmium on oxidative stress and immune function of common carp (*Cyprinus carpio* L.) by transcriptome analysis. *Aquat Toxicol* 2017;**192**:171–7.
- Zheng M, Lu J, Lin G et al. Dysbiosis of gut microbiota by dietary exposure of three graphene-family materials in zebrafish (*Danio rerio*). *Environ Pollut* 2019;**254**:112969.
- Zhou Z, Wang W, Liu W et al. Identification of highly-adhesive gut *Lactobacillus* strains in zebrafish (*Danio rerio*) by partial *rpoB* gene sequence analysis. *Aquaculture* 2012;**370–371**:150–7.
- Zhu J, Kong Y, Yu J et al. Consumption of drinking water N-Nitrosamines mixture alters gut microbiome and increases the obesity risk in young male rats. *Environ Pollut* 2019;**248**:388–96.
- Zhu YT, Yang CX, Lou BB et al. Efficiency of dairy strains of lactic acid bacteria to bind bisphenol A in phosphate buffer saline. *Food Control* 2017;**73**:1203–9.