*Arch. Biol. Sci.*, Belgrade, 64 (3), 1197-1206, 2012 DOI:10.2298/ABS1203197J

# PROTECTIVE EFFECT OF PROBIOTIC BACTERIA AGAINST CADMIUM-INDUCED GENOTOXICITY IN RAT HEPATOCYTES *IN VIVO* AND *IN VITRO*

ADEL M. JAMA[1], DRAGANA MITIĆ-ĆULAFIĆ[2], S. KOLAREVIĆ[2], SINIŠA F. ĐURAŠEVIĆ[1] and JELENA KNEŽEVIĆ-VUKČEVIĆ[2]

[1] *Faculty of Biology, Chair of Comparative Physiology and Ecophysiology, University of Belgrade,* 11000 Belgrade, Serbia
[2] *Faculty of Biology, Chair of Microbiology, University of Belgrade,* 11000 Belgrade, Serbia

*Abstract* - The protective effect of probiotic bacteria against cadmium (Cd)-induced genotoxicity was studied in rat hepatocytes *in vivo* and *in vitro*. Male Wistar rats, *Rattus norvegicus*, were treated for five weeks with (i) $CdCl_2$ (70 ppm in the drinking water), (ii) a mixture of lyophilized probiotic bacteria *Lactobacillus rhamnosus*, *L. acidophilus* and *Bifidobacterium longum* ($5\times10^8$ cfu/g of food), or (iii) $CdCl_2$ and probiotic bacteria. In addition, single cells obtained from the untreated rat liver were exposed to $CdCl_2$ (70 ppm), probiotic bacteria (1.28 mg/ml), or $CdCl_2$ and probiotic bacteria, for 15 min at 22°C in the dark. The level of Cd-induced DNA damage in hepatocytes was determined by the comet assay. The obtained results show that probiotic bacteria significantly reduced Cd-induced genotoxicity, both *in vivo* and *in vitro* (20% and 48%, respectively). Moreover, the toxicity of Cd to lactobacilli in the gastrointestinal tracts of rats was significantly decreased in the probiotic-treated animals. The binding of $Cd^{2+}$ to probiotic bacteria was proposed as the most probable protection mechanism.

*Key words*: Cadmium, toxicity, lactic acid bacteria, genotoxicity, rat hepatocytes

## INTRODUCTION

Cadmium (Cd) is a non-essential heavy element that represents a serious environmental hazard (Satarug et al., 2003). Human exposure to Cd occurs through food, air, water, tobacco smoke, industrial products and by occupational exposure (Fowler, 2009). Cd is extremely harmful for human beings and animals (Jarup, 2003; Waalkes, 2003). It has been found to be toxic to almost every organ in the body, causing hypertension, renal tubular dysfunction, bone fracture and cancer (Jin et al., 2003; Satarug et al., 2003). Cd has been classified as a group I carcinogen by the International Agency for Research on Cancer (Boffetta, 1993), and as a group B1 (probable human carcinogen) by the Environmental Protection Agency (Fotakis and Timbrell, 2006).

The mechanisms by which Cd induces a wide variety of adverse health effects and cancer are poorly understood, but induction of reactive oxygen species and inhibition of DNA repair seem to have a predominant role at the molecular level (Filipic et al., 2006; Rao, 2009). Prolonged exposure to low concentrations of Cd can increase glutathione levels, whereas high Cd concentrations lead to glutathione depletion *in vitro*. *In vivo* and *in vitro* depletion of glutathione results in increased toxicity following the administration of Cd (Fotakis and Timbrell, 2006). In addition to oxidative stress, inflammation has also been implicated in Cd-induced tissue injury (Oteiza

et al., 1999). Several antioxidants and anti-inflammatory agents were found to be effective in minimizing Cd-induced organ damage (Sen Gupta et al., 2004; Kara et al., 2007; Yadav and Khandelwal, 2008; Abdel-Aziem et al., 2011).

Cd has long been considered a non-genotoxic carcinogen, and the evidence that $Cd^{2+}$ causes gene mutations in bacteria or mammalian cells was weak. However, alterations in testing protocols have recently led to better evidence of its mutagenicity, especially in bacteria. The fact that bacterial and mammalian cells appear to sustain some type of repairable DNA damage after exposure to Cd suggests that the damage is caused in an indirect manner. Cd-metallothionein complex is able to cause DNA strand breaks and to induce a "pro-oxidant state" by causing a depletion of cellular glutathione. It has also been suggested that the mechanism responsible for the genotoxicity of Cd may involve the direct interaction with DNA through the binding of $Cd^{2+}$ at G, A and T bases (Valverde et al., 2001; Hossain and Huq, 2002). Cd can also inhibit DNA repair, and can therefore act synergistically with certain mutagens and carcinogens (Rossman et al., 1992). The inhibition of an essential DNA mismatch repair by Cd compounds results in a high level of genetic instability (Jin et al., 2003).

Numerous investigations indicate that probiotic bacteria can play an important role in the body's natural processes of detoxification and elimination of Cd (Haskard et al., 2001; Rao, 2009). Their beneficial properties are related to their capacity to adhere to or bind different targets. Probiotic bacteria such as *Lactobacillus rhamnosus* GG strain (ATCC 53103) and *L. rhamnosus* LC-705 strain (DSM 7061) can bind toxic compounds, such as aflatoxin B1 (Haskard et al., 2001), mutagens from food (Turbic et al., 2002), or microcystin-LR (Halttunen et al., 2007).

In this work, we have assessed the effect of commercial preparations of probiotic bacteria, *Lactobacillus rhamnosus*, *L. acidophilus* and *Bifidobacterium longum* on Cd-induced genotoxicity in rat hepatocytes. Male Wistar rats were treated with $CdCl_2$, a lyophilized mixture of probiotic bacteria, or $CdCl_2$ and probiotic bacteria for five weeks. In addition, single cells obtained from the livers of untreated rats were exposed to $CdCl_2$, probiotic bacteria, or $CdCl_2$ and probiotic bacteria *in vitro*. The level of Cd-induced DNA damage in the hepatocytes was determined by comet assay. In addition, we evaluated the number of lactobacilli and bifidobacteria in the feces of treated animals in order to assess the effect of the probiotic on Cd-induced toxicity to bacterial microflora in rat gastrointestinal tract.

## MATERIALS AND METHODS

### *Animals*

Male Wistar rats (*Rattus norvegicus*), weighing 130±10 g, were acclimatized to 22±2°C in metabolic cages (3 rats/cage) and maintained under a 12 h light/dark cycle. The rats were fed with commercial rat food and drank tap water ad *libitum*.

### *Probiotic bacteria*

The probiotic used in this study was the commercial preparation PROBIOTIC®, Ivančić i sinovi d.o.o., Belgrade, Serbia. The capsules are declared to contain $5x10^9$ lyophilized cells of *Lactobacillus rhamnosus Rosell-11*, *Lactobacillus acidophilus Rosell-52* and *Bifidobacterium longum Rosell-175* strains.

### *Chemicals, media, and culture conditions*

Cadmium chloride ($CdCl_2$, analytical grade) was from Fisher Scientific UK. Hank's BBS (HBSS, Lot No. H00911-0648) and Dulbecco's PBS (Lot No. H00911-1191) were from PAA Laboratories GmbH, Austria. Lactobacilli were cultured on MRS agar (ScharlauChemie S.A., Spain) for 48 h at 37°C. Bifidobacteria were plated on *Bifidobacterium* agar (HiMedia, India) in anaerobic conditions for 24 h at 37°C. Peptone water "Torlak", Belgrade (Lot. No. 364201) contained 10 g pepton-4 "Torlak", 5 g NaCl, 0.01 g Fuchsin S per liter of distilled water. *L. rhamnosus*, *L. acidophilus* and *B. longum* were identified

using an API 50 CH identification kit (bioMerieux, Lot. No. 842019201).

*In vivo exposure*

The rats were randomly divided into four groups of 6 males each. All groups were treated for five weeks as follows: Group 1 (control group) was given food and tap water *ad libitum*; Group 2 was given the probiotic mixed with food at a dose of $5\times10^8$ colony forming units (cfu)/g of food; Group 3 received $CdCl_2$ at a dose of 70 ppm in the drinking water, and Group 4 received both $CdCl_2$ and probiotic. After sacrificing the animals, the livers were processed to obtain single-cell suspensions and the comet test was performed to assess DNA damage.

*Preparation of single-cell suspensions*

Single-cell suspensions of liver tissue were prepared using a method adapted from Wilson et al. (1998). The liver was excised and chopped separately 10 times in 0.2 ml of HBSS using two fresh scalpel blades in a scissor-like movement on a Petri dish, washed off gently into a 15 ml centrifuge tube with a further 2.8 ml HBSS and 0.03 ml of 0.5% trypsin. The suspension was gently rocked for 10 min at room temperature, after which 10 ml of HBSS was added and the suspension passed through a 40 μm sieve to remove any large fragments that remained. After centrifugation (800 g for 5 min), the supernatant was discarded and the pellet carefully re-suspended in 1 ml of HBSS. Cell viability was measured by trypan blue dye exclusion method (Altman et al., 1993) and cell density was adjusted to $3 \times 10^5$ cell/ml.

*In vitro exposure*

The single-cell suspension obtained from the untreated rat liver (control group) was used to prepare comet slides embedded in agarose as described below. The slides were exposed to $CdCl_2$ (70 ppm), probiotic (1.28 mg/ml) and a combination of $CdCl_2$ and probiotic for 15 min at 22°C in the dark. Slides exposed to PBS only served as controls. The concentrations of Cd and probiotic bacteria corresponded to the concentrations for *in vivo* treatment. After exposure, the slides were rinsed with PBS to remove residues and submitted to comet assay procedure as described below.

*Comet assay*

The assay was performed as described by Tice et al. (2000). The cell suspension (30 μl) was mixed with 70 μl of 1% LMP (low melting point) agarose and added to slides, previously precoated in 1% NMP (normal melting point) agarose. The slides were incubated in lysis buffer (2.5 M NaOH, 0.1 M EDTA, 0.01 M Tris and TritonX-100, pH 10) for 1 h at 4 °C, transferred into an electrophoresis solution (300 mM NaOH, 1 mM EDTA, pH 13) for 20 min to allow DNA unwinding, and subjected to electrophoresis for 20 min at 25 V and 300 mA. Finally, the slides were neutralized with 0.4 M Tris buffer (pH 7.5), stained with 20 μl ethidium bromide (5 μg/ml), and analyzed using a fluorescence microscope (Leica) and image analysis software (Comet IV, Perceptive Instruments). Fifty nuclei were analyzed per experimental point (in triplicate), and the percentage of the fluorescence in the comet tail was scored as a reflection of DNA damage.

*Identification of probiotic bacteria*

To obtain pure cultures of probiotic bacteria, MRS broth was inoculated with the content of one capsule of PROBIOTIC® and incubated at 30°C. After 48 h the culture was streak-inoculated on MRS agar and *Bifidobacterium* agar and incubated for 48 h at 30°C aerobically and for 24 h at 37°C anaerobically, respectively. Using Gram staining and an API 50 CH identification kit we identified small grey colonies on the MRS agar as *Lactobacillus rhamnosus*, big white colonies on the same medium as *Lactobacillus acidophilus* and colonies on the Bifidobacterium agar as *Bifidobacterium longum*.

*Microbiological analysis of feces*

Feces samples from the four treatment groups were

collected and immediately transported to the laboratory. A feces specimen (4 g from each group), was suspended in 36 ml of peptone water and homogenized. Samples were vigorously vortexed for 1 min and then centrifuged at 65 g for 5 min to deposit any remaining solid matter. The number of lactobacilli and bifidobacteria was determined by spreading appropriate dilutions onto the MRS agar and Bifidobacterium agar plates, respectively. Small grey colonies on the MRS were considered to be *Lactobacillus rhamnosus*, while big white colonies on the same medium were considered to be *Lactobacillus acidophilus*. Experiments were performed twice, each with duplicate samples.

### *Statistical analysis*

Data for bacterial enumeration were statistically evaluated by one-way analysis of variance (ANOVA), using SigmaStat (Version 3.1) software. Holm-Sidak comparisons were performed when ANOVA was significant. The level of significance was set at $p<0.05$. For the results of the comet assay, one-way analysis of variance (non-parametric ANOVA, Kruskal-Wallis test) was used to analyze differences between the treatments within each experiment. The Duncan Post Hoc test was used to compare median values of the percent of fluorescence in comet tails for all treatments; $p<0.05$ was considered as statistically significant.

## RESULTS

### *The effect of probiotic on Cd-induced genotoxicity in rat hepatocytes*

Two experimental procedures, *in vivo* and *in vitro,* were used to study and compare the effect of a probiotic on Cd-induced genotoxicity in rat hepatocytes. As shown in Fig. 1, the percent of DNA damage in control and probiotic-treated cells was similar in both experimental conditions. The percent of DNA in the comet tail was 1.71% in the controls, 1.53% *in vivo* and 1.64% *in vitro* after the treatment with probiotic. The treatment with Cd resulted in a significant increase in DNA damage: the percent of DNA in the comet tail increased from 1.71% to 8.46% *in vivo* and from 1.71% to 3.43% *in vitro*. When the animals or cells were simultaneously treated with Cd and probiotic there was a significant decrease of Cd-induced genotoxicity. The percent of DNA in the comet tail declined from 8.45% to 6.78% *in vivo* (a 20% decrease) and from 3.43% to 1.82% *in vitro* (a 48% decrease).

### *The number of lactic acid bacteria in the feces of rats exposed to Cd and probiotic*

The number of bifidobacteria in the feces of rats from all groups is shown in Fig. 2. While there was no significant difference between the control and probiotic-treated groups ($1.0 \times 10^7$ and $1.1 \times 10^7$ cells/g of feces), exposure to Cd significantly decreased the abundance of bifidobacteria compared with control group (by 64%). Adding probiotic to Cd resulted in an increase of bifidobacteria counts, from $3.6 \times 10^6$ to $4.6 \times 10^6$cells/g, but this difference was not statistically significant.

The number of *L. acidophilus* and *L. rhamnosus* in the feces of differently treated rats is shown in Figs. 3 and 4. In the probiotic-treated animals, the number of *L. acidophilus* was significantly higher ($1.2 \times 10^8$ cells/g) compared with the control ($9.3 \times 10^7$cells/g). Exposure to Cd significantly reduced *L. acidophilus* counts compared with the control (by about 95%). In animals simultaneously treated with Cd and probiotic the number of *L. acidophilus* was significantly increased, from $4.5 \times 10^6$ to $5.5 \times 10^7$cells/g of feces.

As in the case of *L. acidophilus*, the number of *L. rhamnosus* in probiotic-treated animals was significantly higher than in the control ($9.2 \times 10^5$ and $5.5 \times 10^5$ cells/g, respectively). Exposure to Cd resulted in the significant reduction of *L. rhamnosus* counts compared with the control (by 40%). Interestingly, treatment with the probiotic completely diminished Cd-induced toxicity. The number of *L. rhamnosus* increased from $3.3 \times 10^5$ to $1 \times 10^6$ cells/g of feces, which is insignificantly different from the number in the probiotic-treated animals ($9.2 \times 10^5$ and, respectively).

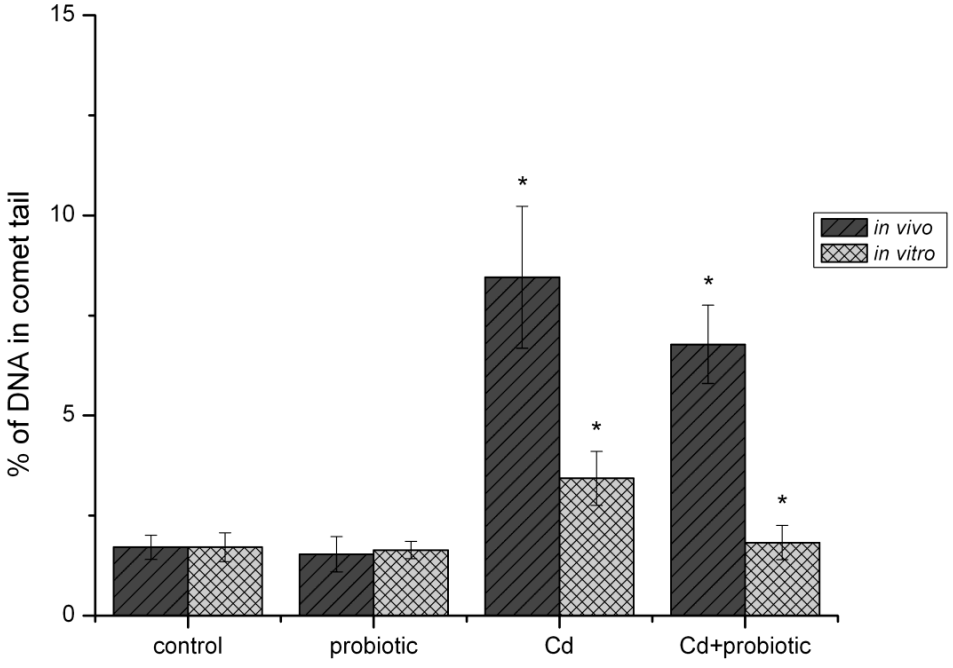


**Fig. 1.** The effect of Cd and probiotic on DNA damage in rat hepatocytes exposed *in vivo* and *in vitro*. The level of DNA strand breaks is expressed as the percentage of DNA in the comet tails. Fifty cells were analyzed per experimental point. *significantly different from the control group ($p<0.05$)

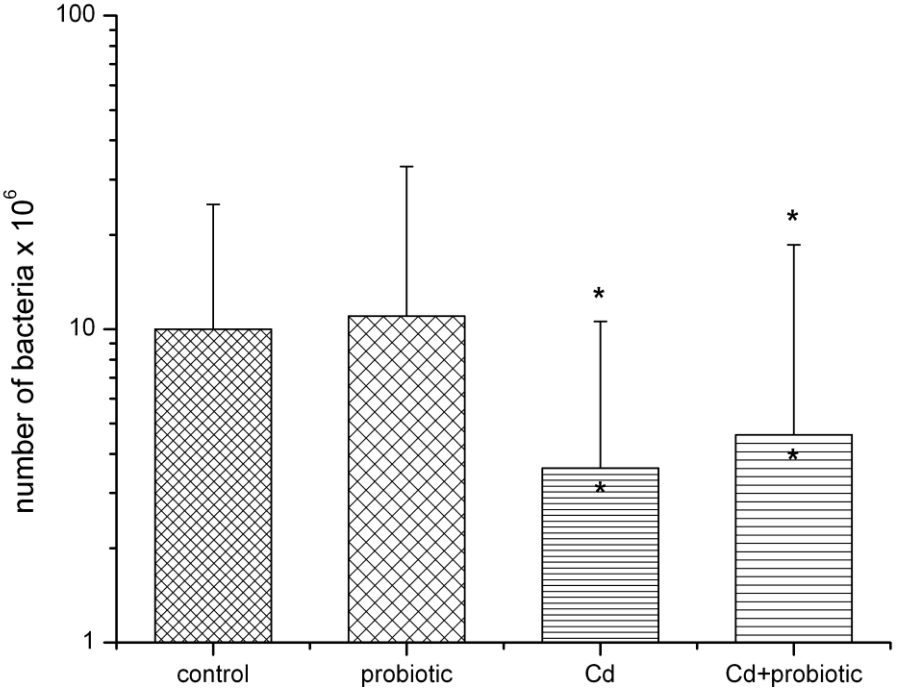


**Fig. 2.** The number of fecal bifidobacteria in Cd-exposed rats, with or without probiotic. Presented values are the means of two independent experiments ± SD. *above the bar indicates significant difference from control group; *inside the bar indicates significant difference from probiotic group ($p<0.05$)

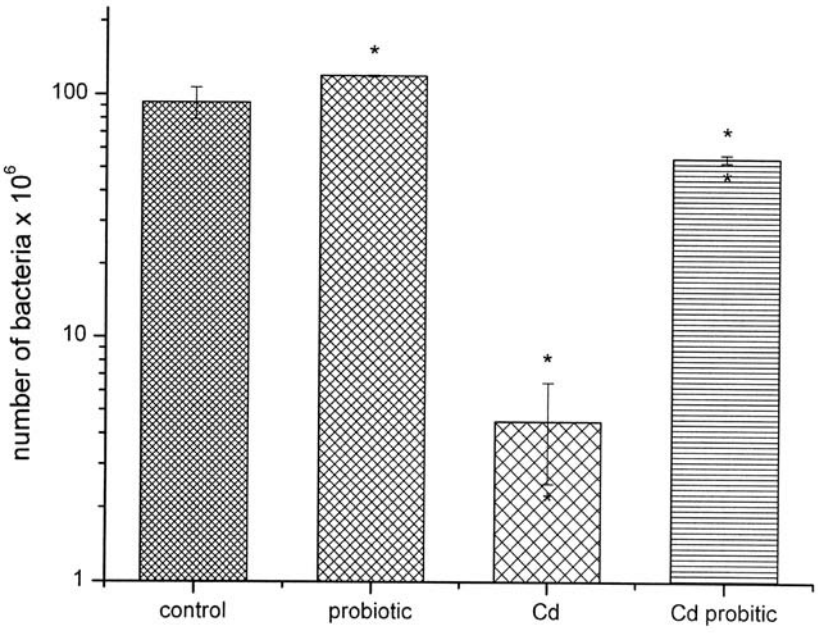


**Fig. 3.** The number of fecal *L. acidophilus* in Cd-exposed rats with or without probiotic. Presented values are the means of two independent experiments ± SD. *above the bar indicates significant difference from control group; *inside the bar indicates significant difference from probiotic group ($p<0.05$)

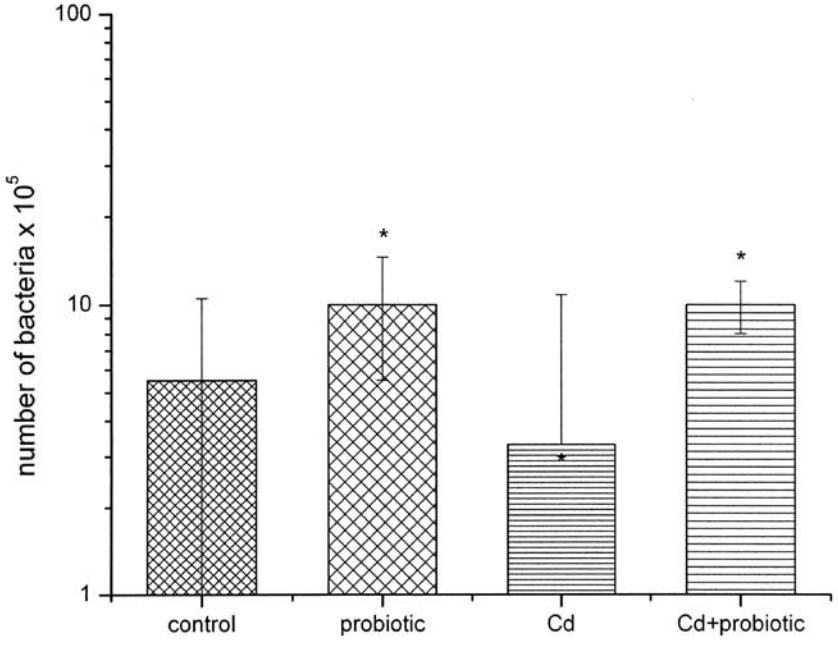


**Fig. 4.** The number of fecal *L. rhamnosus* of in Cd-exposed rats with or without probiotic. Presented values are the means of two independent experiments ± SD. * above the bar indicates significant difference from control group; *inside the bar indicates significant difference from probiotic group ($p<0.05$)

## DISCUSSION

A number of reports have convincingly demonstrated the genotoxic potential of Cd in animals and mammalian cells (Waisberg et al., 2003; Bertin and Averbeck, 2006). In the present work, we investigated the protective effect of probiotic bacteria against liver genotoxicity induced by Cd, using the comet assay. The experiments were performed *in vivo* and *in vitro*, and the genotoxic effect of Cd was significant in both cases. From our data it is obvious that after chronic *in vivo* exposure to Cd (5 weeks) the percent of DNA damage was higher compared to short *in vitro* exposure (15 min). It is known that the genotoxicity of Cd involves the induction of oxidative stress and inhibition of different DNA repair mechanisms (Rossman et al., 1992; Jin et al., 2003; Rao, 2009; Jomova and Valko, 2011). It is to be expected that both processess occured during the chronic *in vivo* exposure, leading to substantial DNA damage. In the case of *in vitro* exposure, the time was probably too short for saturation of the antioxidative defense of the hepatocytes, and the amount of DNA lesions was consequently lower. In addition, the low concentration of vitamin C present in the PROBIOTIC® preparation could have acted as an antioxidant and reduced oxidative stress (Sen Gupta et al., 2004).

Nowdays there is an increasing interest in the use of probiotic bacteria to mitigate the toxic/genotoxic potential of heavy metals. On the basis of our results it is clear that the protective effect of probiotic bacteria aginst Cd-induced genotoxicity exists. The protection was more pronounced in *in vitro* conditions (48% compared to 20% *in vivo*), indicating that the most probable mechanism was the direct binding of $Cd^{2+}$ to the probiotic bacteria. The surface of lactic acid bacteria is composed of a thick layer of peptidoglycan, teichoic acid, proteins and polysaccharides; moreover, some *Bifidobacterium* strains and *L. rhamnosus* GG are known to produce exopolysaccharides. These structures contain different kinds of negatively charged groups such as carboxyl, hydroxyl and phosphate groups. Therefore, probiotic bacteria have a great number of different possible ligands able to bind cations such as $Cd^{2+}$ (Teemu et al., 2008). It is possible that in the mixture of liver cells, probiotic bacteria and Cd *in vitro*, bacterial cells bind $Cd^{2+}$ more efficiently than in the rat intestines, preventing more effectively the induction of DNA damage. The ability of probiotic strains to perform auto-aggregation, as well as co-aggregation with other bacteria in the intestines, might reduce the binding of heavy metals (Kos et al., 2003; Salim Abdelgader et al., 2011), which could be one of the reasons for the weaker protection observed *in vivo*.

Heavy metals have inhibitory effects on the growth of a number of bacteria (Forsberg, 1978; Ravikumar et al., 2007). In this study, we noticed significant reduction in the counts of all the studied bacteria in the feces of rats treated with Cd compared with the control group. The toxicity increased, in order *L. rhamnosus* (40%), bifidobacteria (64%), and *L. acidophilus* (95%), indicating the highest resistance of *L. rhamnosus* to Cd. Our results are similar to those of Fazeli et al. (2011), who reported that Cd has a toxic effect on gastrointestinal bacteria in mice. The cytotoxicity of Cd is well documented, and it appears that it is mainly due to the oxidative deterioration of biomolecules, including DNA, proteins and lipids (Bertin and Averbeck, 2006).

The natural inhabitants of the intestine play a pivotal role through different mechanisms in the physiological functions of the organism and pathological processes. Knowledge is accumulating that lactic acid bacteria modulate gut physiology, immunological functions, and may produce many beneficial effects (Erickson and Hubbard, 2000). This has led scientists to investigate the efficacy of probiotics, prebiotics and synbiotics in the prevention and treatment of diseases and toxicities. Administration of probiotics enhances the intestine immune system, decreases bacteria translocation and prevents the overgrowth of enteric pathogens; it also has beneficial effects on acute hepatocellular disease (Fernandes et al., 1987; Mao et al., 1996; Adawi et al., 2001; Salminen et al., 2010). The mechanisms by which probiotics exert biological effects are still poorly understood. The adsorption of heavy metals to the bacterial surface appears to be the main mechanism for their removal by

probiotics. Halttunen et al. (2008) found that different lactic acid bacteria were effective in the removal of heavy metals, microcystin-LR and aflatoxin B1. Moreover, they found clear differences in metal and toxin removal efficiency between the strains. The assessment of $Cd^{2+}$ removal also indicated a strongly pH-dependent process, with the highest binding at a neutral pH (Halttunen et al., 2007). Knowing that the pH values in different parts of the gastrointestinal tract of rats vary from 5.3-7.4 (McConnell et al., 2008), we can propose that the highest binding of $Cd^{2+}$ occurs in the large intestine where pH values are close to neutral.

Rowland et al. (1998) reported that feeding rats with *Bifidobacterium longum* decreased the levels of ammonia in the caecum and reduced the incidence of preneoplastic lesions induced by azoxymethane. On the contrary, our results do not indicate a protective effect of bifidobacteria against Cd-induced toxicity. In the group of rats treated with probiotic, the number of *L. acidophilus* and *L. rhamnosus* in the feces significantly increased in comparison with the control, while the number of bifidobacteria remained unchanged. Moreover, in the group treated with Cd plus probiotic, the number of *L. acidophilus* and *L. rhamnosus* significantly increased compared to the Cd-treated group, while the increase of bifidobacteria counts was insignificant. Obtained results point at the different behavior of bifidobacteria and lactobacilli in relation to binding $Cd^{2+}$. It seems that lactobacilli possess some capacity to bind $Cd^{2+}$, which enables their better survival. However, the probiotic potential of different bacterial strains, even within the same species, differs. Different strains of the same species may have different areas of adherence (site-specific), specific immunological effects, and the actions on healthy/inflamed mucosa may be distinct from each other (Soccol et al., 2010).

## CONCLUSIONS

This study reports on the efficiency of probiotic bacteria against Cd-induced toxicity to gut microflora and genotoxicity to liver cells. The health-promoting properties of probiotic bacteria and the implications from the present study indicate that the application of probiotics may be a good approach for counteracting the negative effect of Cd and possibly other heavy metals. Further research is needed to define a specific probiotic combination that would be the most effective in binding $Cd^{2+}$ and other heavy metals, as well as different toxins and carcinogens.

*Acknowledgments* – This research was financially supported by the Ministry of Education and Science of the Government of Serbia, projects No 172058 and 173023. Adel Jama benefited from the doctoral fellowship of The Libyan Republic.

## REFERENCES

*Abdel-Aziem, S.H., El-Nekeety, A.A., Barakat, I.A., Mohamed, M.I.* and *M.A. Abdel-Wahhab* (2011). Aquilegia vulgaris extract protects against the oxidative stress and the mutagenic effects of cadmium in Balb/c mice. *Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie*. **63**, 337-344.

*Adawi, D., Ahrne, S.* and *G. Molin* (2001). Effects of different probiotic strains of Lactobacillus and Bifidobacterium on bacterial translocation and liver injury in an acute liver injury model. *International journal of food microbiology*. **70**:213-220.

*Altman, S.A., Randers, L.* and *G. Rao* (1993). Comparison of trypan blue dye exclusion and fluorometric assays for mammalian cell viability determinations. *Biotechnology progress*. **9**, 671-674.

*Bertin, G.* and *D. Averbeck* (2006). Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). *Biochimie*. **88**, 1549-1559.

*Boffetta, P.* (1993). Carcinogenicity of trace elements with reference to evaluations made by the International Agency for Research on Cancer. *Scandinavian journal of work, environment & health*. **19 Suppl 1**, 67-70.

*Erickson, K.L.* and *N.E. Hubbard* (2000). Probiotic immunomodulation in health and disease. *J Nutr*. **130**:403S-409S.

*Fazeli, M., Hassanzadeh, P.* and *S. Alaei* (2011). Cadmium chloride exhibits a profound toxic effect on bacterial microflora of the mice gastrointestinal tract. *Human & experimental toxicology*. **30**, 152-159.

*Fernandes, C.F., Shahani, K.M.* and *M.A. Amer* (1987). Therapeutic role of dietary lactobacilli and lactobacillic fermented dairy products. *FEMS microbiology letters*. **46**, 343-356.

*Filipic, M., Fatur, T.* and *M. Vudrag* (2006). Molecular mechanisms of cadmium induced mutagenicity. *Human & experimental toxicology*. **25**, 67-77.

*Forsberg, C.W.* (1978). Effects of heavy metals and other trace elements on the fermentative activity of the rumen microflora and growth of functionally important rumen bacteria. *Canadian journal of microbiology*. **24**, 298-306.

*Fotakis, G.* and *J.A. Timbrell* (2006). Sulfur amino acid deprivation in cadmium chloride toxicity in hepatoma cells. *Environmental toxicology and pharmacology*. **22**, 334-337.

*Fowler, B.A.* (2009). Monitoring of human populations for early markers of cadmium toxicity: a review. *Toxicol Appl Pharmacol*. **238**, 294-300.

*Halttunen, T., Collado, M.C., El-Nezami, H., Meriluoto, J.* and *S. Salminen* (2008). Combining strains of lactic acid bacteria may reduce their toxin and heavy metal removal efficiency from aqueous solution. *Letters in applied microbiology*. **46**, 160-165.

*Halttunen, T., Salminen, S.* and *R. Tahvonen* (2007). Rapid removal of lead and cadmium from water by specific lactic acid bacteria. *International journal of food microbiology*. **114**, 30-35.

*Haskard, C.A., El-Nezami, H.S., Kankaanpaa, P.E., Salminen, S.* and *J.T. Ahokas* (2001). Surface binding of aflatoxin B(1) by lactic acid bacteria. *Appl Environ Microbiol*. **67**, 3086-3091.

*Hossain, Z.* and *F. Huq* (2002). Studies on the interaction between Cd(2+) ions and nucleobases and nucleotides. *J Inorg Biochem*. **90**, 97-105.

*Jarup, L.* (2003). Hazards of heavy metal contamination. *Br Med Bull*. **68**, 167-182.

*Jin, Y.H., Clark, A.B., Slebos, R.J., Al-Refai, H., Taylor, J.A., Kunkel, T.A., Resnick, M.A.* and *D.A. Gordenin* (2003). Cadmium is a mutagen that acts by inhibiting mismatch repair. *Nat Genet*. **34**, 326-329.

*Jomova, K.* and *M. Valko* (2011). Advances in metal-induced oxidative stress and human disease. *Toxicology*. **283**, 65-87.

*Kara, H., Cevik, A., Konar, V., Dayangac, A.* and *M. Yilmaz* (2007). Protective effects of antioxidants against cadmium-induced oxidative damage in rat testes. *Biological trace element research*. **120**, 205-211.

*Kos, B., Suskovic, J., Vukovic, S., Simpraga, M., Frece, J.* and *S. Matosic* (2003). Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. *Journal of applied microbiology*. **94**, 981-987.

*Mao, Y., Nobaek, S., Kasravi, B., Adawi, D., Stenram, U., Molin, G.* and *B. Jeppsson* (1996). The effects of Lactobacillus strains and oat fiber on methotrexate-induced enterocolitis in rats. *Gastroenterology*. **111**, 334-344.

*McConnell, E.L., Basit, A.W.* and *S. Murdan* (2008). Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. *J Pharm Pharmacol*. **60**, 63-70.

*Oteiza, P.I., Adonaylo, V.N.* and *C.L. Keen* (1999). Cadmium-induced testes oxidative damage in rats can be influenced by dietary zinc intake. *Toxicology*. **137**, 13-22.

*Rao, K.S.* (2009). Free radical induced oxidative damage to DNA: relation to brain aging and neurological disorders. *Indian journal of biochemistry & biophysics*. **46**, 9-15.

*Ravikumar, S., Williams, G.P., Shanthy, S., Gracelin, N.A., Babu, S.* and *P.S. Parimala* (2007). Effect of heavy metals (Hg and Zn) on the growth and phosphate solubilising activity in halophilic phosphobacteria isolated from Manakudi mangrove. *Journal of environmental biology / Academy of Environmental Biology, India*. **28**, 109-114.

*Rossman, T.G., Roy, N.K.* and *W.C. Lin* (1992). Is cadmium genotoxic? *IARC scientific publications*.367-375.

*Rowland, I.R., Rumney, C.J., Coutts, J.T.* and *L.C. Lievense* (1998). Effect of Bifidobacterium longum and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. *Carcinogenesis*. **19**, 281-285.

*Salim Abdelgader, B., Badawy, H.L.* and *S.S. Kasem* (2011). Effect of lactic acid bacteria against heavy metals toxicity in rats. *Journal of American Science*. **7**, 264-274.

*Salminen, S., Nybom, S., Meriluoto, J., Collado, M.C., Vesterlund, S.* and *H. El-Nezami* (2010). Interaction of probiotics and pathogens--benefits to human health? *Current opinion in biotechnology*. **21**, 157-167.

*Satarug, S., Baker, J.R., Urbenjapol, S., Haswell-Elkins, M., Reilly, P.E., Williams, D.J.* and *M.R. Moore* (2003). A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. *Toxicol Lett*. **137**, 65-83.

*Sen Gupta, R., Sen Gupta, E., Dhakal, B.K., Thakur, A.R.* and *J. Ahnn* (2004). Vitamin C and vitamin E protect the rat testes from cadmium-induced reactive oxygen species. *Mol Cells*. **17**, 132-139.

*Soccol, C.R., Vandenberghe, L.P., Spier, M.R., Medeiros, A.B., Yamaguishi, C.T., Linder, J.D.* and *V. Thomaz-Socol* (2010). The Potential of Probiotics: A Review. *Food Technology and Biotechnology*. **48**, 413-434.

*Teemu, H., Seppo, S., Jussi, M., Raija, T.* and *L. Kalle* (2008). Reversible surface binding of cadmium and lead by lactic acid and bifidobacteria. *International journal of food microbiology*. **125**, 170-175.

*Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.C.* and *Y.F. Sasaki* (2000). Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environmental and molecular mutagenesis*. **35**, 206-221.

*Turbic, A., Ahokas, J.T.* and *C.A. Haskard* (2002). Selective in vitro binding of dietary mutagens, individually or in combination, by lactic acid bacteria. *Food additives and contaminants*. **19**, 144-152.

*Valverde, M., Trejo, C.* and *E. Rojas* (2001). Is the capacity of lead acetate and cadmium chloride to induce genotoxic damage due to direct DNA-metal interaction? *Mutagenesis*. **16**, 265-270.

*Waalkes, M.P.* (2003). Cadmium carcinogenesis. *Mutat Res*. **533**, 107-120.

*Waisberg, M., Joseph, P., Hale, B.* and *D. Beyersmann* (2003). Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology*. **192**, 95-117.

*Wilson, J.T., Pascoe, P.L., Parry, J.M.* and *D.R. Dixon* (1998). Evaluation of the comet assay as a method for the detection of DNA damage in the cells of a marine invertebrate, Mytilus edulis L. (Mollusca: Pelecypoda). *Mutat Res*. **399**, 87-95.

*Yadav, N.* and *S. Khandelwal* (2008). Effect of Picroliv on cadmium induced testicular damage in rat. *Food Chem Toxicol*. **46**, 494-501.