JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

IN VITRO MASS-SCREENING OF LACTIC ACID BACTERIA AS POTENTIAL BIOSORBENTS OF CESIUM AND STRONTIUM IONS

Hideki Kinoshita[*1], *Yuka Sato*[1], *Fumika Ohtake*[1], *Mitsuharu Ishida*[1], *Toshikazu Komoda*[1], *Haruki Kitazawa*[2], *Tadao Saito*[2] *and Kazuhiko Kimura*[1]

Address(es): Hideki Kinoshita,
[1]Miyagi University, Department of Food Management, School of Food, Agricultural and Environmental Sciences, 2-2-1 Hatatate, Sendai, Miyagi 982-0215, Japan. Phone: +81-22-245-1382.
[2] Tohoku University, Graduate School of Agricultural Science, 1-1 Tsutsumidori-Amamiyamachi, Sendai, Miyagi 981-8555, Japan.

*Corresponding author: kinoshih@myu.ac.jp

doi: 10.15414/jmbfs.2015.4.5.383-386

ARTICLE INFO

Received 19. 7. 2014
Revised 3. 9. 2014
Accepted 14. 1. 2015
Published 1. 4. 2015

Regular article

OPEN ACCESS

ABSTRACT

Many radionuclides were scattered by the explosion at the Fukushima Daiichi Nuclear Power Station. We examined whether lactic acid bacteria (LAB) can sorb cesium ions (Cs^+) and strontium ions (Sr^{2+}) for radioprotection. Many strains showed biosorption to Cs^+ and Sr^{2+} using an *in vitro* mass-screening although each strain showed different sorption. We selected MYU 111, MYU 758, and MYU 759 strains that showed especially high biosorption to Cs^+ and/or Sr^{2+}. MYU 111 was identified as *Lactobacillus plantarum*, and MYU 758 and 759 were *Pediococcus pentosaceus*. The selected strains tended to show higher biosorption when using the buffer method compared to the culture method. Further, they showed high biosorption at a low concentration of 1 ppb Cs^+ and Sr^{2+} (max 28.8% and 97.7% sorption, respectively). This is the first study where lactic acid bacteria are shown to have biosorption of Cs^+ and Sr^{2+}.

Keywords: Lactic acid bacteria, cesium, strontium, biosorption

INTRODUCTION

Many radionuclides such as iodine (I), cesium (Cs), and strontium (Sr) were scattered by the explosion at the Fukushima Daiichi Nuclear Power Station caused by the Great East Japan Earthquake on March 11, 2011. Steinhauser *et al.* **(2014)** estimated a total source term of 520 (340-780) peta-becquerels (PBq) or about one-tenth the radiation released during the 1986 Chernobyl disaster. The radiation has been released to soil, sea and river widely around Fukushima **(Kinoshita *et al.*, 2011, Hirose, 2012, Steinhauser *et al.*, 2013, Steinhauser, 2014)**, and has polluted water and food such as crops, animal products, fish and seafood **(Hamada *et al.*, 2012, Hamada & Ogino, 2012, Merz *et al.*, 2013, Yamamoto *et al.*, 2014)**. Especially, the exposure to radionuclides such as ^{134}Cs, ^{137}Cs, and ^{90}Sr may lead to long term ill health effects. Many reports show the effects of low level radiation. A comprehensive review of available biological and biophysical data supports a linear no-threshold (LNT) risk model where the risk of cancer occurs in a linear fashion at lower doses without a threshold (**NAS/NRC, 2006, Royal, 2008**). Romanenko *et al.* **(2012)** reported that exposure to low doses and to low dose-rates of radiation from post-Chornobyl cleanup work was associated with a significant increase of risk in leukemia. Nzabarushimana *et al.* **(2014)** report exposure to low doses of ^{56}Fe causes an increased in chemokine *Ccl3*, and interleukin *Il-4* expression; and interloikine and global DNA hypermethylation in the mouse lung. Taira *et al.* **(2014)** reported forewing size reduction, growth retardation, high mortality rates, and high abnormality rates in pale grass blue butterflies, *Zizeeria maha*, captured from the Fukushima area.

Lactic acid bacteria (LAB) have biosorption ability for heavy metals. Recently, we reported that LAB showed high biosorption of cadmium ion (Cd^{2+}) and mercury ion (Hg^{2+}) **(Kinoshita *et al.*, 2013)**. *Weissella viridescens* MYU 205 showed high biosorption, and it's cell surface proteins bound Hg^{2+}. Further reports show LABs have biosorption ability to various heavy metals such as Cd^{2+}, Pb^{2+}, and Cu^{2+} **(Ibrahim *et al.*, 2006, Schut *et al.*, 2011, Bhakta *et al.*, 2012)**. Recently, Sasaki *et al.* **(2012a)** reported about 90% of the radioactive Cs in the sediment mud of a school's swimming pool in Fukushima was removed using the alginate-immobilized photosynthetic bacterium *Rhodobacter sphaeroides* SSI. Therefore, we speculated LABs might be able to absorb and/or adsorb Cs^+ and Sr^{2+}. One of the primary features of LABs is high safety. People are eating many LAB strains with food, especially fermented food, and therefore LABs are GRAS organisms **(Stiles & Holzapfel, 1997, Feord, 2002)**. Oral ingestion of LAB showing high biosorption of Cs^+ and Sr^{2+} may prevent the absorption of Cs^+ and Sr^{2+} into the body, and may efficiently discharge them from the body via feces. Thus, our aims were to examine the Cs^+ and Sr^{2+} biosorption ability of LAB derived from food using an *in vitro* mass-screening.

MATERIALS AND METHODS

Seventy-seven strains of lactic acid bacteria were isolated from various foods, e.g., rice, bovine and porcine intestines (called Horumon in Japan), Japanese pickles, Japanese Amazake, kimchee, and yogurt. Bacterial strains were propagated twice at 37°C for 24 h in MRS broth with 2% (v/v) inoculum before the experiments.

Non-radioactive Cs^+ and Sr^{2+} were used in this study. The mass-screening of LAB having either Cs^+ or Sr^{2+} binding ability was performed using the culture method. MRS broth (Difco Laboratories, Detroit, MI) containing 10 ppm Cs^+ or Sr^{2+} was prepared using a 1,000 ppm Cs standard ($CsNO_3$ in 2-3% HNO_3) (Merck, Darmstadt, Germany) or 1,000 ppm Sr standard ($Sr(NO_3)_2$ in 2-3% HNO_3) (Merck, Darmstadt, Germany), respectively. Tests with ions were performed separately. Seventy-seven LAB strains isolated from food were propagated at 37°C for 24 h in 10 ml of MRS broth containing either 10 ppm Cs^+ or 10 ppm Sr^{2+}. Bacterial cells after culture were washed three times with sterile distilled water and the wet weight of the cells was measured. The pellets were suspended in 10% HNO_3 and heated at 105°C for 2 h. A few drops of hydrogen peroxide (H_2O_2) were added where the liquid was completely vaporized to lyse and degrade the cells. The remaining Cs^+ or Sr^{2+} was diluted with 10 ml of 5% HNO_3 and 5,000 ppm (final concentration) potassium nitrate solution (Merck, Darmstadt, Germany) was added; and the concentrations were measured using an atomic absorption spectrophotometer (AAS) SpectrAA-55 (Agilent Technologies, Santa Clara, CA).

The Cs^+ or Sr^{2+} biosorption by selected strains was tested in buffer (the buffer method). Bacterial cells cultured for 24 h were washed three times with distilled water, and the pellet was measured with the same weight as the culture method. The pellet was suspended in 10 ml of 10 mmol/L sodium citrate buffer (pH 6.0) containing 10 ppm Cs^+ or Sr^{2+}, and was incubated at 37°C for 1 or 24 h. After incubation, the suspension was centrifuged (8,000 rpm, 10 min, 4°C) and the Cs^+ or Sr^{2+} concentration of the supernatant with 5,000 ppm (final concentration) potassium nitrate solution added was measured using AAS. Buffer containing no

bacteria was used as a negative control (NC). The amount of biosorption (μg) was calculated by subtracting the sample value from the control value.
The Cs^+ or Sr^{2+} biosorption assay at low concentrations was performed using MilliQ water containing 1 ppb Cs^+ or Sr^{2+} following the same procedure as the buffer method. The bacterial cells cultured for 24 h were washed three times with MilliQ water, and 0.05 g of the pellet was suspended with 5 ml of MilliQ water containing 1 ppb Cs^+ or Sr^{2+}; and was incubated at 37°C for 1 h. After incubation, the bacterial cells were centrifuged (5,000 rpm, 10 min, 4°C); and the Cs^+ or Sr^{2+} concentration of the supernatant was measured using an inductively coupled plasma mass spectrometer (ICP-MS) ELAN DRC-e (Perkin Elmer SCIEX, Boston, MA). Cs^+ or Sr^{2+} solution (1 ppb) containing no bacteria was used as the control. The rate of biosorption (%) was calculated by subtracting the sample value from the control value.
The assays were performed in triplicate and all data were reported as the mean ± SD. The data was assessed using analysis of variance (ANOVA). When ANOVA was significant, the significance of differences was determined using the two-tailed t-test with Bonferroni adjustments.

RESULTS AND DISCUSSION

The mass-screening of LAB show Cs^+ and/or Sr^{2+} biosorption

Many strains showed biosorption of either Cs^+ or Sr^{2+} using the culture mass-screening method although each strain showed different sorptions. The average Cs^+ biosorption in 77 strains was 0.89 ± 0.81 μg showing 0.9% of total Cs^+. MYU 758 showed the highest at 2.4 ± 0.082 μg; and the second highest was MYU 759 at 2.4 ± 0.082 μg. The average Sr^{2+} biosorption was 0.76 ± 0.43 μg showing 0.8% of the total Sr^{2+}. MYU 759 showing the second highest in Cs^+ biosorption showed the highest Sr^{2+} sorption at 2.1 ± 0.082 μg. MYU 111 showed 1.9 ± 0.00 μg. This suggests LABs may be potential Cs^+ and Sr^{2+} sorbents. Because the chemical properties of stable isotopes and radioisotopes are the same, our data suggests LAB can also sorb ^{134}Cs, ^{137}Cs, and ^{90}Sr radioisotopes. The three strains were identified using the Gram stain, catalase test and 16S rDNA sequencing analysis. MYU 111 isolated from pickled Japanese radish was identified as *Lactobacillus plantarum*, and MYU 758 and 759 isolated from rice were *Pediococcus pentosaceus*. We selected these three strains showing high biosorption for further experiments.

Comparison of the culture method and the buffer method

Figure 1 shows the data for Cs^+ or Sr^{2+} biosorption using the culture and the buffer methods. Cs^+ biosorption using the buffer method incubating for 1 h was significantly higher than the culture method for *L. plantarum* MYU 111 and *P. pentosaceus* MYU 758 ($p<0.01$) although no significant difference was observed in *P. pentosaceus* MYU 759 comparing the two methods. The Cs^+ biosorption using the buffer method incubating for 24 h was lower than the buffer method incubating for 1 h; and had a significant difference at 24 h compared to the culture method (none for MYU 758). Cs^+ may be incorporated into the cells because Cs^+ behaves like K^+. A portion of the sorbed Cs^+ may be quenched by 24 h.
Sr^{2+} biosorption using the buffer method incubating for 1 h and 24 h was significantly higher than the culture method in all selected strains ($p<0.01$). Especially, *P. pentosaceus* MYU 759 showed the highest biosorption at 13.67 ± 0.05 μg, about 14% of the total Sr^{2+}. Sr^{2+} may be bound to cell surface proteins because Sr^{2+} behaves like Ca^{2+} whose concentration is lower inside a cell causing a gradient. The difference in the amount of biosorption using the buffer method and the culture method may be related to competitive ions such as Na^+, K^+, Ca^{2+}, and Mg^{2+}. And/or, the pH may influence sorption because pH becomes lower using the culture method after 24 h. The competitive ions and/or the low pH may have more strongly inhibited biosorption because the Sr^{2+} biosorption was higher than the Cs^+ biosorption using the buffer method. Halttunen *et al.* **(2008)** reported Cd^{2+} biosorption significantly decreased when using competitive ions such as Mg^{2+}, Ca^{2+}, Fe^{2+}, Zn^{2+}, and Pb^{2+} in *L. fermentum* ME3 and *Bifidobacterium longum* 46. When we measured the concentrations of metal ions in MRS broth, MRS broth contained 1,900 ppm Na^+, 1,800 ppm K^+, 50 ppm Mg^{2+}, and 70 ppm Ca^{2+}. The buffer contains about 566 ppm Na^+. In our previous study using Cd^{2+}, the amount of biosorption tended to be lower when the pH was low **(Kinoshita *et al.*, 2013)**. Comparable data are reported by others. Volesky *et al.* **(1993)** reported Cd^{2+} biosorption by *Saccharomyces cerevisiae* was inhibited by protons under acidic conditions. He and Tebo **(1998)** reported that pH-dependent copper biosorption with spores of a marine *Bacillus* sp. The amount of biosorption decreased by as much as 90% at pH 4.0 as compared to pH 8.0. The cell surface proteins of LAB are positively charged below the isoelectric point and may have difficulty adsorbing Sr^{2+} at low pH.

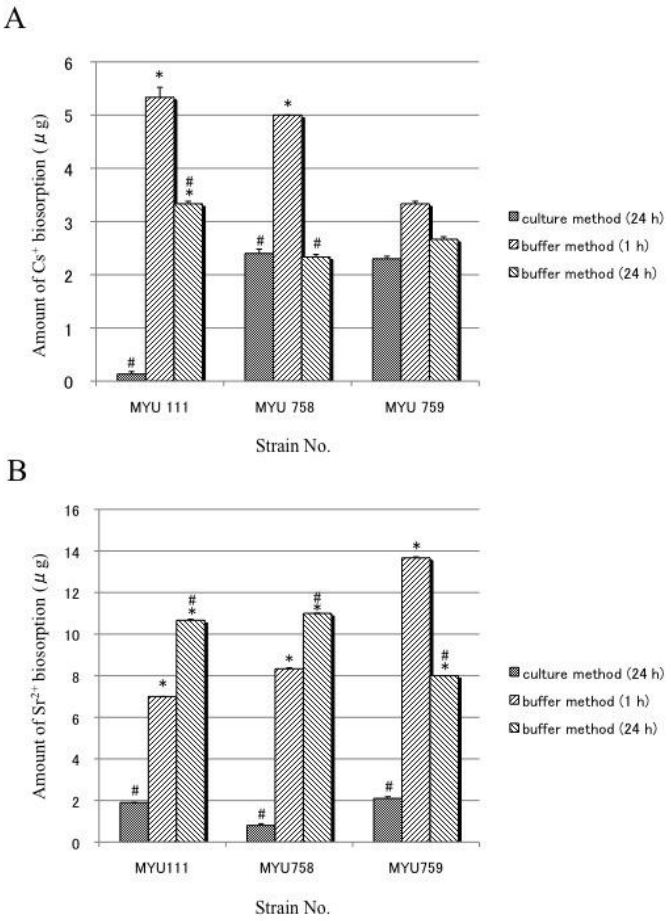

Figure 1 Comparison of Cs^+ (A) and Sr^{2+} (B) biosorption using the culture and the buffer method.

Using the culture method, the bacterial cells were propagated at 37°C for 24 h in MRS broth containing 10 ppm Cs^+ or Sr^{2+}. After washing with sterile distilled water, the Cs^+ or Sr^{2+} concentration of the pellets was measured (n=3).
Using the buffer method, the bacterial cells cultured for 24 h were washed three times with distilled water, the pellet was suspended with 10 mmol/L sodium citrate buffer (pH 6.0) containing 10 ppm Cs^+ or Sr^{2+}, and was incubated at 37°C for 1 or 24 h. After incubation, the suspension was centrifuged and the Cs^+ or Sr^{2+} concentration of the supernatant was measured (n=3). The amount of biosorption (μg) was calculated by subtracting the sample value from the control value.
The same weight of bacteria was used in both methods.
*: Significant difference compared to the culture method ($p<0.01$).
#: Significant difference compared at 1 h to the buffer method ($p<0.01$).

Biosorption assay at low concentrations of Cs^+ or Sr^{2+}

LABs are required to show biosorption at low concentrations because people may eat food containing very low concentrations of radioactive Cs^+ and Sr^{2+}. Figure 2 shows the data for the biosorption at 1 ppb Cs^+ or Sr^{2+}. The percent Cs^+ biosorption was 0% in MYU 111, 25.7 ± 1.6% in MYU 758, and 28.8 ± 2.9% in MYU759. The percent Sr^{2+} biosorption was 78.0 ± 0.3% in MYU 111, 97.7 ± 0.4% in MYU 758, and 95.6 ± 0.2% in MYU759. These selected strains showed high biosorption at low concentration of Cs^+ and Sr^{2+}. Further work is needed to show biosorption of radioactive Cs^+ and Sr^{2+} in the presence of a large amount of competitive ions such as Na^+, K^+, Ca^{2+}, and Mg^{2+}, and non-radioactive Cs^+ and Sr^{2+} naturally contained in food; and further if LABs can exclude radioactive Cs^+ and Sr^{2+} in human intestinal feces. The LAB showing high biosorption of Hg^{2+} can prevent uptake of Hg^{2+} into Caco-2 cells in our studies (unpublished data), although, further investigation is needed.

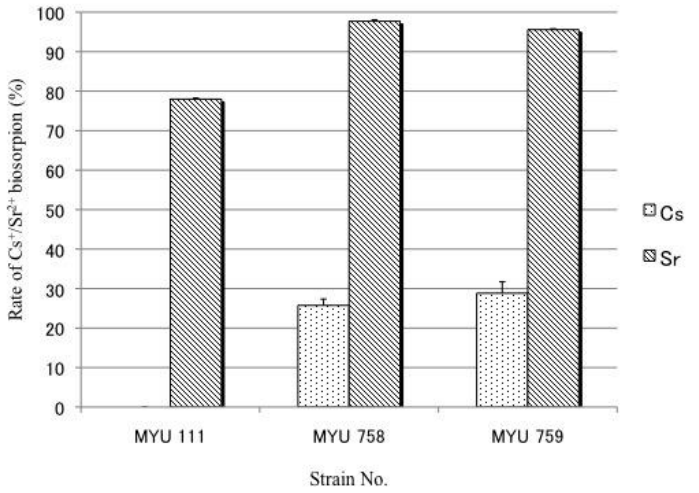

Figure 2 Rate of a 1 ppb solution Cs^+ or Sr^{2+} biosorption by lactic acid bacteria.

The bacterial cells cultured for 24 h were washed three times with MilliQ water; 0.05 g of the pellet was suspended with 5 ml of MilliQ water containing 1 ppb Cs^+ or Sr^{2+}; and was incubated at 37°C for 1 h. After incubation, the bacterial cells were centrifuged (5,000 rpm, 10 min, 4°C); and the Cs^+ and Sr^{2+} concentration of the supernatant was measured using ICP-MS (n=3), respectively. The rate of biosorption (%) was calculated by subtracting the sample value from the control value.

Possibility of radio protection using LAB

Some reports show the radio protective effects of LAB and yeast. Nomoto *et al.* **(1991)** report a single subcutaneous dose of heat-killed *L. casei* YIT 9018 (LC 9018) was highly radio protective when given shortly after irradiation in mice. Tsuneoka *et al.* **(1994)** reported a single dose of LC 9018 was radio protective, even when administered as late as 30 h after or as early as 7 days before irradiation. The extent of the radio protective effect was similar when LC 9018 was administered during the period from 2 days before irradiation to 9 h after irradiation, although the pre-irradiation treatment was slightly more effective than the post-irradiation treatment. Ciorba *et al.* **(2012)** reported orally administered *L. rhamnosus* GG (LGG) and its conditioned medium protected the murine small intestinal epithelium from radiation injury. LGG-mediated radio protection is dependent on MyD88, TLR-2, and COX-2, and occurs without significantly altering the bacterial family composition of the small intestine. Administering LGG did not change the COX-2 levels; however it results in a repositioning of COX-2-expressing mesenchymal stem cells of the lamina propria from the villi to the crypt region. Anzai *et al.* **(2008)** reported radio protection of mice using intraperitoneal administration of Zn-, Mn-, Cu-, or Se-containing heat-treated *S. serevisiae*. When mineral-yeasts were administered immediately after irradiation, the survival rate was higher where Zn- or Cu-yeast showed the highest rate (more than 90%). These facts show the possibility of lowering the internal exposure using LABs; however further study in long-term irradiation at low doses is needed because these previous studies were performed at lethal doses. Further, alginate-immobilized photosynthetic bacterium, *Rhodobacter sphaeroides* SSI, removed about 90% of the radioactive Cs in the sediment mud of a school's swimming pool in Fukushima, Japan **(Sasaki *et al.*, 2012a)** and the anaerobic digestion and lactic acid fermentation by lactic acid bacteria supported removal of Cs from soil **(Sasaki *et al.*, 2012b)**. Here, we showed the Cs^+ and Sr^{2+} biosorption ability of LAB. In addition, LABs properties, such as activation of an immune function **(Perdigón *et al.*, 2002)**, prevention of cancer **(Lim *et al.*, 2002)**., and anti-oxidation **(Yamamoto *et al.*, 2002)** may be helpful for radiation protection.

CONCLUSION

It is important to prevent the absorption of Cs^+ and Sr^{2+} into the body because they accumulate in muscle **(Fukuda *et al.*, 2013)** and bone **(Dahl *et al.*, 2001)**, respectively, during long-term radiation exposure. In this study, we tested the biosorption ability of 77 LABs and found LABs showed biosorption of Cs^+ and Sr^{2+}. This is the first study where LABs were shown to biosorp Cs^+ and Sr^{2+}. We believe oral ingestion of LAB showing high biosorption of Cs^+ and Sr^{2+} can prevent the absorption of Cs^+ and Sr^{2+} into the body, and may be discharged via feces from the body efficiently. This study is the first step to attempt lowering the internal radiation exposure using using LABs.

Acknowledgements: This study was financially supported by a grant from Miyagi University (2011-2012).

REFERENCES

Anzai K, Ikota N, Ueno M, Nyui M, Kagiya TV. 2008. Heat-treated mineral-yeast as a potent post-irradiation radioprotector. *Journal of Radiation Research* 49, 425-430. http://dx.doi.org/10.1269/jrr.07127.

Bhakta JN, Ohnishi K, Munekage Y, Iwasaki K, Wei MQ. 2012. Characterization of lactic acid bacteria-based probiotics as potential heavy metal sorbents. *Journal of Applied Microbiology* 112, 1193-1206. http://dx.doi.org/10.1111/j.1365-2672.2012.05284.x.

Ciorba MA, Riehl TE, Rao MS, Moon C, Ee X, Nava GM, Walker MR, Marinshaw JM, Stappenbeck TS, Stenson WF. 2012. *Lactobacillus* probiotic protects intestinal epithelium from radiation injury in a TLR-2/cyclo-oxygenase-2-dependent manner. *Gut* 61, 829-838. http://dx.doi.org/10.1136/gutjnl-2011-300367.

Dahl SG, Allain P, Marie PJ, Mauras Y, Boivin G, Ammann P, Tsouderos Y, Delmas PD, Christiansen C. 2001. Incorporation and distribution of strontium in bone. *Bone* 28, 446-453. http://dx.doi.org/10.1016/S8756-3282(01)00419-7.

Feord J. 2002. Lactic acid bacteria in a changing legislative environment. *Antonie Van Leeuwenhoek* 82, 353-360. http://dx.doi.org/10.1023/A:1020648018998.

Fukuda T, Kino Y, Abe Y, Yamashiro H, Kuwahara Y, Nihei H, Sano Y, Irisawa A, Shimura T, Fukumoto M, Shinoda H, Obata Y, Saigusa S, Sekine T, Isogai E. 2013. Distribution of artificial radionuclides in abandoned cattle in the evacuation zone of the Fukushima Daiichi nuclear power plant. *PLoS One* 8, e54312. http://dx.doi.org/10.1371/journal.pone.0054312.

Halttunen T, Salminen S, Meriluoto J, Tahvonen R, Lertola K. 2008. Reversible surface binding of cadmium and lead by lactic acid and bifidobacteria. *International Journal of Food Microbiology* 125, 170-175. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.03.041.

Hamada N, Ogino H, Fujimichi Y. 2012. Safety regulations of food and water implemented in the first year following the Fukushima nuclear accident. *Journal of Radiation Research* 53, 641-671. http://dx.doi.org/10.1093/jrr/rrs032.

Hamada N, Ogino H. 2012. Food safety regulations: what we learned from the Fukushima nuclear accident. *Journal of Environmental Radioactivity* 111, 83–99. http://dx.doi.org/10.1016/j.jenvrad.2011.08.008.

He LM, Tebo BM. 1998. Surface charge properties of and Cu (II) adsorption by spores of the marine *Bacillus* sp. strain SG-1. *Applied and Environmental Microbiology* 64, 1123-1129.

Hirose K. 2012. 2011 Fukushima Dai-ichi nuclear power plant accident: summary of regional radioactive deposition monitoring results. *Journal of Environmental Radioactivity* 111, 13-17. http://dx.doi.org/10.1016/j.jenvrad.2011.09.003.

Ibrahim F, Halttunen T, Tahvonen R, Salminen S. 2006. Probiotic bacteria as potential detoxification tools: assessing their heavy metal binding isotherms. *Canadian Journal of Microbiology* 52, 877-885. http://dx.doi.org/10.1139/w06-043.

Kinoshita H, Sohma Y, Ohtake F, Ishida M, Kawai Y, Kitazawa H, Saito T, Kimura K. 2013. Biosorption of heavy metals by lactic acid bacteria and identification of mercury binding protein. *Research in Microbiology* 164, 701-709. http://dx.doi.org/10.1016/j.resmic.2013.04.004.

Kinoshita N, Sueki K, Sasa K, Kitagawa J, Ikarashi S, Nishimura T, Wong Y, Satou Y, Handa K, Takahashi T, Sato M, Yamagata T. 2011. Assessment of individual radionuclide distributions from the Fukushima nuclear accident covering central-east Japan. *Proceedings of the National Academy of Sciences* 8, 19526–19529. http://dx.doi.org/10.1073/pnas.1111724108.

Lim BK, Mahendran R, Lee YK, Bay BH. 2002. Chemopreventive effect of *Lactobacillus rhamnosus* on growth of a subcutaneously implanted bladder cancer cell line in the mouse. *Japanese Journal of Cancer Research* 93, 36-41. http://dx.doi.org/10.1111/j.1349-7006.2002.tb01198.x.

Merz S, Steinhauser G, Hamada N. 2013. Anthropogenic radionuclides in Japanese food: environmental and legal implications. *Environmental Science & Technology* 47, 1248–1256. http://dx.doi.org/10.1021/es3037498.

NAS/NRC (National Academy of Sciences/National Research Council). 2006. Health risks from exposure to low levels of ionizing radiation, BEIR VII, phase 2. National Academy Press 424 p. ISBN 0-309-53040-7.

Nomoto K, Yokokura T, Tsuneoka K, Shikita M. 1991. Radioprotection of mice by a single subcutaneous injection of heat-killed *Lactobacillus casei* after irradiation. *Radiation Research* 125, 293-297.

Nzabarushimana E, Miousse IR, Shao L, Chang J, Allen AR, Turner J, Stewart B, Raber J, Koturbash I. 2014. Long-term epigenetic effects of exposure to low doses of 56Fe in the mouse lung. *Journal of Radiation Research* 55, 823-828. http://dx.doi.org/10.1093/jrr/rru010.

Perdigón G, Maldonado Galdeano C, Valdez JC, Medici M. 2002. Interaction of lactic acid bacteria with the gut immune system. *European Journal of Clinical Nutrition* 56 Suppl 4, S21-26. http://dx.doi.org/10.1038/sj.ejcn.1601658.

Romanenko AM, Ruiz-Saurí A, Morell-Quadreny L, Valencia G, Vozianov AF, Llombart-Bosch A. 2012. Microvessel density is high in clear-cell renal cell carcinomas of Ukrainian patients exposed to chronic persistent low-dose ionizing radiation after the Chernobyl accident. *Virchows Archiv* 460, 611-619. http://dx.doi.org/10.1007/s00428-012-1243-x.

Royal HD. 2008. Effects of low level radiation-what's new? *Seminars in Nuclear Medicine* 38, 392-402. http://dx.doi.org/10.1053/j.semnuclmed.2008.05.006.
Sasaki K, Morikawa H, Kishibe T, Mikami A, Harada T, Ohta M. 2012a. Practical removal of radioactivity from sediment mud in a swimming pool in Fukushima, Japan by immobilized photosynthetic bacteria. *Bioscience, Biotechnology, and Biochemistry* 76, 859-862. http://dx.doi.org/10.1271/bbb.110853.
Sasaki K, Morikawa H, Kishibe T, Takeno K, Mikami A, Harada T, Ohta M. 2012b. Practical removal of radioactivity from soil in Fukushima using immobilized photosynthetic bacteria combined with anaerobic digestion and lactic acid fermentation as pre-treatment. *Bioscience, Biotechnology, and Biochemistry* 76, 1809-1814. http://dx.doi.org/10.1271/bbb.120440.
Schut S, Zauner S, Hampel G, Konig H, Claus H. 2011. Biosorption of copper by wine-relevant lactobacilli. *International Journal of Food Microbiology* 145, 126-131. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.11.039.
Steinhauser G. 2014. Fukushima's forgotten radionuclides: A review of the understudied radioactive emissions. *Environmental Science & Technology*. 48, 4649-4663. http://dx.doi.org/10.1021/es405654c.
Steinhauser G, Brandl A, Johnson TE. 2014. Comparison of the Chernobyl and Fukushima nuclear accidents: A review of the environmental impacts. *Science of The Total Environment* 470-471, 800-817. http://dx.doi.org/10.1016/j.scitotenv.2013.10.029.
Steinhauser G, Schauer V, Shozugawa K. 2013. Concentration of strontium-90 at selected hot spots in Japan. *PLoS One* 8, e57760. http://dx.doi.org/10.1371/journal.pone.0057760.
Stiles ME, Holzapfel WH. 1997. Lactic acid bacteria of foods and their current taxonomy. *International Journal of Food Microbiology* 36, 1-29. http://dx.doi.org/10.1016/S0168-1605(96)01233-0.
Taira W, Nohara C, Hiyama A, Otaki JM. 2014. Fukushima's biological impacts: The case of the pale grass blue butterfly. *Journal of Heredity* 105, 710-722. http://dx.doi.org/10.1093/jhered/esu013.
Tokyo Electric Power Company. 2012. Estimation of the released amount of radioactive materials into the atmosphere as a result of the accident in the Fukushima Daiichi Nuclear Power Station. *TEPCO press release* May 24.
Tsuneoka K, Ishihara H, Dimchev AB, Nomoto K, Yokokura T, Shikita M. 1994. Timing in administration of a heat-killed *Lactobacillus casei* preparation for radioprotection in mice. *Journal of Radiation Research* 35, 147-156. http://dx.doi.org/10.1269/jrr.35.147.
Volesky B, May H, Holan ZR. 1993. Cadmium biosorption by *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering* 41, 826-829. http://dx.doi.org/10.1002/bit.260410809.
Yamamoto S, Yokoduka T, Fujimoto K, Takagi K, Ono T. 2014. Radiocaesium concentrations in the muscle and eggs of salmonids from Lake Chuzenji, Japan, after the Fukushima fallout. *Journal of Fish Biology* 84, 1607-1613. http://dx.doi.org/10.1111/jfb.12368.
Yamamoto Y, Poole LB, Hantgan RR, Kamio Y. 2002. An iron-binding protein, Dpr, from *Streptococcus mutans* prevents iron-dependent hydroxyl radical formation *in vitro*. *Journal of Bacteriology* 184, 2931-2939. http://dx.doi.org/10.1128/JB.184.11.2931-2939.2002.