Letters in Applied Microbiology

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

DNA-protection and antioxidant properties of fermentates from *Bacillus amyloliquefaciens* B-1895 and *Bacillus subtilis* KATMIRA1933

E.V. Prazdnova[1], V.A. Chistyakov[1], M.N. Churilov[1], M.S. Mazanko[1], A.B. Bren[1], A. Volski[2] and M.L. Chikindas[3,4,5]

1 Academy of Biology and Biotechnologies, Southern FederalUniversity, Rostov-on-Don, Russia
2 School of Arts and Science, Rutgers State University, New Brunswick, NJ, USA
3 Astrabiol, LLC, Highland Park, NJ, USA
4 Health Promoting Naturals Laboratory, School of Environmental and Biological Sciences, Rutgers State University, New Brunswick, NJ, USA
5 Center for Digestive Health, New Jersey Institute for Food, Nutrition and Health, New Brunswick, NJ, USA

Significance and Impact of the Study: In humans, oxidative stress is a cause or an important component of many serious diseases, as well as being one of the age influencing factors. Environmental stresses lead to the increase in levels of reactive oxygen species (ROS). Oxidative DNA damage is a side effect of nonspecific inflammation. These human health challenging factors trigger the search for health-promoting bacteria capable of production of antioxidants and DNA-protectors. In this study, two *Bacillus* strains of interest were shown to produce noticeable DNA protective and antioxidant activities.

Keywords
antioxidant activity, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, DNA damage, genotoxicity, *Lux* biosensors, probiotic.

Correspondence
Evgeniya V. Prazdnova, Research Institute of Biology, Southern Federal University, Prospect Stachki, 194/1, Rostov-on-Don, Russia.
E-mail: prazdnova@sfedu.ru

2015/0352: received 20 February 2015, revised 25 August 2015 and accepted 31 August 2015

doi:10.1111/lam.12491

Abstract

DNA protective and antioxidant activity of *Bacillus amyloliquefaciens* B-1895 and *Bacillus subtilis* KATMIRA1933 were evaluated by *Escherichia coli*-based *Lux* biosensors. Two biosensor strains of *E. coli*, MG1655 (pColD-lux) and MG1655 (pSoxS-lux), which react on DNA damage and superoxide-anion radical activity, were used. SOS-response and *Sox*-response were stimulated by addition of dioxidine (2,3-Quinoxalinedimethanol,1,4-dioxide) and paraquat (*N*,*N*′-dimethyl-4,4′-bipyridinium dichloride) respectively. Preparations of both *Bacillus* fermentates demonstrated DNA protective and antioxidant (superoxide scavenging) activity (up to 60·19%). The strain K1933 is, in general, characterized by higher DNA protective activity (28·85%), with parameters of antioxidant activity of both bacilli strains being statistically not significantly different. Sporogenous potential probiotic micro-organisms with antioxidant and DNA protective activities can become an effective tool for compensation of various negative oxidative stress processes in humans.

Introduction

According to the WHO definition, probiotics are live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO 2001; FAO/WHO 2002). While immunomodulation and protection against various infections are the most commonly known and desired functions of probiotics (Floch 2014), there are other features such as stimulation of muscular tissue growth in animals, control of obesity (Kadooka *et al.* 2010), and even impact on behaviour (Putignani *et al.* 2014; Stilling *et al.* 2014; Cox and Dalloul 2014). Probiotics are effective in addressing health conditions which are not directly associated with infections, in particular allergies, toxicoses of different aetiology, etc. (Priebe *et al.* 2002; Vanderhoof and Mitmesser 2010). A wide range of adaptogenic activities can be related to release of metabolites, which protect eukaryotic host cells from most harmful consequences of stress: reactive oxygen species production and DNA damage (Marnett 2000; Masood *et al.* 2014; Skulachev 2005).

Oxidative stress underlies action of many health-threatening factors (Fridovich 1999; Wells *et al.* 2005; Sedelnikova *et al.* 2010). Among these are hypoxia/ischaemia, inflammatory and autoimmune disorders, and poisonous effect of the sitotoxins, in particular mycotoxins (Atroshi *et al.* 2002).

Various environmental stresses lead to the increase in levels of reactive oxygen species (ROS) and damage of the eukaryotic organism's DNA (Ames 1999; Ames and Gold 2000; Halliwell 2005). These human health challenging factors justify the emerging search for probiotic strains capable of production of antioxidants and DNA protectors (Kodali and Sen 2008).

The majority of micro-organisms used as probiotics belong to the lactic acid bacteria (Burgain *et al.* 2014). However, sporeforming health-promoting bacteria are broadly used in agricultural practice (Quigley *et al.* 2013) and are on the rise in various human applications, with the *Clostridium* and *Bacillus* genera being among them (Cutting 2011; Bader *et al.* 2012).

Bacillus amyloliquefaciens B-1895 (soil isolate) is a nonpathogenic micro-organism with reported high level proteolytic activity. Probiotic properties of B-1895 manifest in stimulation of growth and intolerance to fish and bird pathogens (Chistyakov *et al.* 2015; Karlyshev *et al.* 2014). *Bacillus subtilis* KATMIRA1933 was found in a fermented dairy product and was isolated from several independent batches of this product over the period of 3 years (Sutyak *et al.* 2008a). The strain KATMIRA1933 produces the ribosomally synthesized antimicrobial protein (bacteriocin) subtilosin (Zheng and Slavik 1999), previously isolated from *B. subtilis* 168, a laboratory derivative of a Marburg strain ATCC 6051 of unidentifiable origin (Lamanna 1954). The subtilosin preparation obtained from the strain KATMIRA1933, was confirmed as being safe for human tissues, having spermicidal activity (Sutyak *et al.* 2008b), and activity against foodborne (Amrouche *et al.* 2010) and vaginal (Noll *et al.* 2011) pathogens.

Here, we report on DNA protective and antioxidant activity of supernatants of two bacilli strains, *B. amyloliquefaciens* B-1895 and *B. subtilis* KATMIRA1933 evaluated with *Escherichia coli*-based *Lux* biosensors.

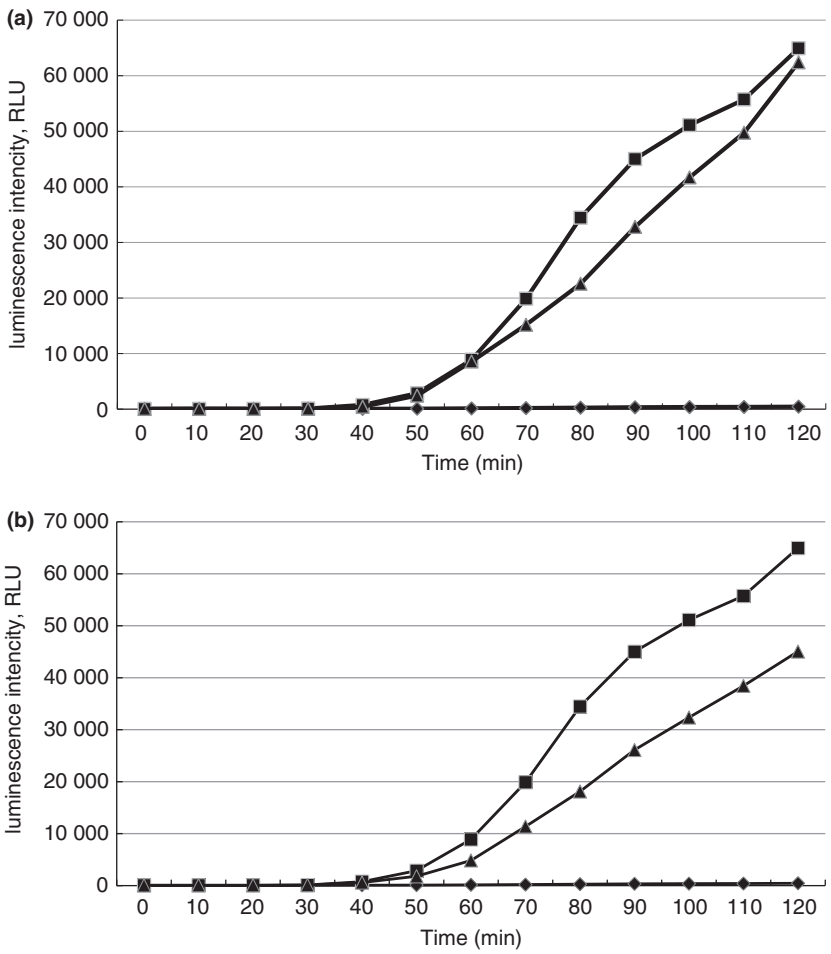

Figure 1 Response of *Escherichia coli* MG1655 pColD-lux to dioxidine with and without B 1895 strain metabolites (Part a), and K 1933 strain metabolites (Part b), where —◆— is the usual level of luminescence, —■— is the response to dioxidine, and —▲— is the response to dioxidine in the presence of the studied bacilli fermentates.

Results and discussion

Data on the protective activity of the supernatants of two probiotic strains' fermentates (cell-free supernatants) as determined based on biosensors tests, are shown in Figs 1 and 2 and Table 1. Preparations of both fermentates demonstrate DNA protective and antioxidant activity. The strain K1933 is characterized by higher DNA protective activity, with parameters of antioxidant activity of both probiotic strains being statistically not significantly different. Hundred-fold dilution does not result in considerable loss of activity. This may be indicative of the protector's concentration being much higher than saturation level. The observed protective effect became significantly lower only at a thousand-fold dilution of the supernatants. Data on dose–response are presented in Fig. 3.

In addition, a series of experiments to examine thermostability of active components was performed. It was shown that heating of supernatant for 30 min decreases protective (antioxidant) effect of the strain B-1895 by 31·47%, and heating for 10 min decreases it by 7·57%. The strain's K-1933 supernatant heating for 10 min produces a decrease of 7·79%, whereas heating for 30 min completely removes the antioxidant activity.

Dioxidin (2,3-Quinoxalinedimethanol,1,4-dioxide) is a mutagenic substance widely used for treatment of infectious diseases (Piopov *et al.* 2013). Its mutagenicity has been demonstrated for a broad range of prokaryotes and eukaryotes. It was shown to act by the reactive oxygen species (ROS) production mechanism (Ordzhonikidze *et al.* 2011). Paraquat (*N*,*N*′-dimethyl-4,4′-bipyridinium dichloride) is a commonly used reference chemical inducer of ROS generation. It triggers oxidative stress, switch-

Table 1 DNA protective and superoxide-scavenging activity of *Bacillus* strains

	Protective activity (*P*), %	
Bacilli strain/ *Escherichia coli* strain	*E. coli* MG 1655 pColD-lux (DNA protection)	*E. coli* MG1655 pSoxS-lux (Antioxidant)
B1895	19·53 ± 4·48	42·79 ± 13·45
K1933	28·85 ± 3·82	60·19 ± 3·69

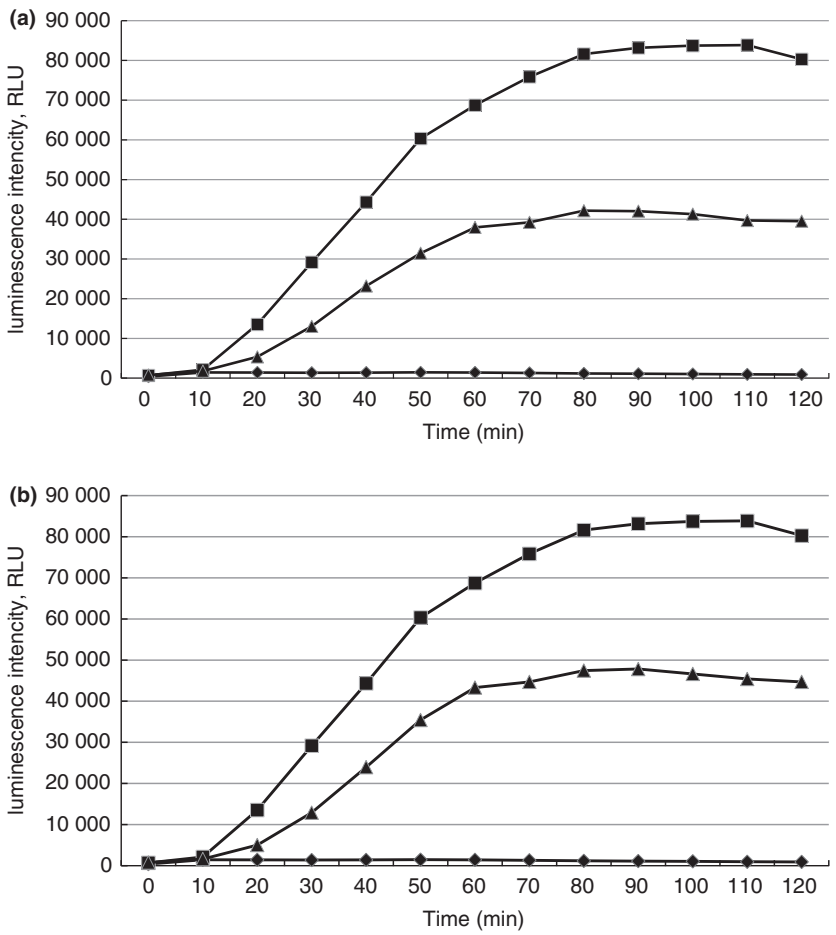

Figure 2 Response of *Escherichia coli* MG1655 pSoxS-lux to paraquat with and without fermentates of the studied strains B 1895 (Part a) and K 1933 (Part b), where —◆— is the usual level of luminescence (control), —■— is the level of luminescence in the presence of paraquat, and —▲— is the level of luminescence in the presence of paraquat and fermentate.

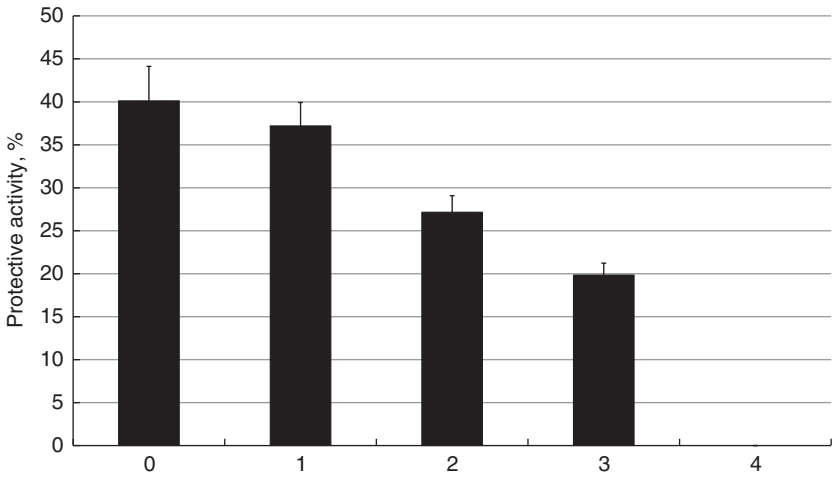

Figure 3 Dose-effect relationship of antioxidant activity of *Bacillus subtilis* KATMIRA1933 cell-free supernatant, where 0 is nondiluted supernatant, 1 is the ten-fold dilution, 2 is the hundred-fold dilution, 3 is thousand-fold dilution, 4 is ten-thousand-fold dilution.

ing cell's bioenergetics to the superoxide-anion generation instead of ATP synthesis (Liochev *et al.* 1994; Miller *et al.* 2007).

Ability of health-promoting lactobacilli and bifidobacteria to produce DNA protective agents has been known for more than two decades (Renner and Münzner1991). These bacteria generate agents which lower genotoxicity of substances such as 4-nitroquinoline-1-oxide, nitrosoguanidine, 2-amino-3,4-dimethyl, imidaso[4,5-f]quinoline, polyaromatic hydrocarbons, aflatoxins etc. (McBain and MacFarlane 2001; Lo *et al.* 2004). Also, high antimutagenic activity of sporogenous bacilli was recently described (Cenci *et al.* 2008; Piopov *et al.* 2013).

The reports on the connection between antimutagenic activity of probiotic bacteria and their production of antioxidants are scattered (Gotteland *et al.* 2006; Shen *et al.* 2011; Achuthan *et al.* 2012), and so far it has not been reported for sporogenous probiotics. Probiotic micro-organisms that generate substances with antioxidant and DNA protective activity can become an effective tool for compensation of the aforementioned negative processes.

We assume the bacterial model is acceptable for this study because antioxidant mechanisms are quite general for all living organisms. Nevertheless, it should be taken into account that there is a considerable gap between the *in vitro* assays performed on *E. coli* here and the antimutagenic activity in humans.

Materials and methods

Two bacilli strains were grown in Luria–Bertani (LB) broth (Difco, Detroit, MI) at 37°C on the circular shaker Biosan (Riga, Latvia) during 14 h until early stationary growth phase had been achieved (OD = 0·7, approx. $3{\cdot}5 \times 10^9$ CFU ml^{-1}).

The strains' cell-free supernatants were collected by centrifugation (Minispin-plus; Eppendorf, Leipzig, Germany) at 6000 ***g*** for 7 min. The DNA protective and antioxidant activities of these supernatants were evaluated with bacterial *Lux* biosensors. Supernatants were not filter sterilized, assuming that for a rapid biosensor test it is not necessary.

Escherichia coli strains MG1655 (pColD-lux) and MG1655 (pSoxS-lux) (obtained from Manukhov, State Scientific Center Genetika, Moscow, Russia) were used as *Lux* biosensors, identifying induction of *ColD* gene and *Sox* operon, which are involved in SOS-reparation and serve as a part of the cellular antioxidant defence system respectively (Zavilgelsky *et al.* 2007).

DNA protective and antioxidant activity was evaluated by ability of the secreted bacterial metabolites to reduce SOS-response and *Sox*-response, stimulated by addition of dioxidine (2,3-Quinoxalinedimethanol,1,4-dioxide, Biosintez, Penza, Russia) up to $2{\cdot}25 \times 10^{-5}$ mol l^{-1} and paraquat (*N*,*N*′-dimethyl-4,4′-bipyridinium dichloride, Sigma-Aldrich, Saint-Louis, MO) up to 10^{-3} mol l^{-1} concentrations respectively (Chistyakov *et al.* 2012). The methodology for *Lux* biosensors bioluminescence detection was thoroughly described by Manukhov *et al.* (1999) and Zavilgelsky *et al.* (2007). Thirty-minute pre-incubation of supernatant with culture was performed. For luminescence measurements, an LM-01A automatic microplate luminometer (Immunotech, Praha, Czech Republic) was used. Measurements were carried out every 10 min for 120 min. For evaluation of the influence of the studied factors on *Sox* operon expression, the induction factor (I_s) was calculated according to the formula:

$$I_s = (L_e/L_k) - 1, \quad (1)$$

where L_k and L_e are luminescence intensities of control and experimental samples respectively. A statistically sig-

nificant excess of L_e over L_k estimated with the *t*-criterion was considered as a sign of significant influence on the induction effect.

Protective activity (P, %) was calculated taking induction into account in the presence of corresponding protector concentrations:

$$P = (1 - (Ia/Ip)) \times 100\%, \quad (2)$$

where Ia and Ip are induction factors of the SOS-response with the investigated influence in the presence of protector and in the control sample respectively.

To characterize the protective activity of the studied concentration of the substance, the mean value of P during the whole duration of measurements was used.

Each experiment was conducted at least three times in triplicate and the statistical analysis was performed using Student's *t*-test. Confidence intervals were calculated by program MICROSOFT EXCEL (Microsoft Corporation, Redmond, WA) for $P = 0{\cdot}05$.

To study the thermostability of cell-free supernatants, preparations were heated in a water bath to 85°C.

Acknowledgements

This research was supported by project of the Ministry of Education and Science of Russia № 6.1202.2014/K. Analytical work was carried out on the equipment of Centers for collective Use of Southern Federal University 'High Technology', grant RFMEFI59414X0002.

MLC is thankful is grateful to Jiangsu Sinoyoung Biopharmaceutical Co., Ltd., for their support. The publication was prepared in the framework of 'Providing of scientific research' in Southern Federal University.

Conflict of Interest

The authors declare no conflict of interests.

References

Achuthan, A.A., Duary, R.K., Madathil, A., Panwar, H., Kumar, H., Batish, V.K. and Grover, S. (2012) Antioxidative potential of lactobacilli isolated from the gut of Indian people. *Mol Biol Rep* **39**, 7887–7897.

Ames, B.N. (1999) Micronutrient deficiencies: a major cause of DNA damage. *Ann N Y Acad Sci* **889**, 87–106.

Ames, B.N. and Gold, L.S. (2000) Paracelsus to parascience: the environmental cancer distraction. *Mutat Res* **447**, 3–13.

Amrouche, T., Noll, K.S., Wang, Y., Huang, Q. and Chikindas, M.L. (2010) Antibacterial activity of subtilosin alone and combined with curcumin, poly-lysine and zinc lactate against Listeria monocytogenes strains. *Probiotics Antimicrob Proteins* **2**, 250–257.

Atroshi, F., Rizzo, A., Westermarck, T. and Ali-Vehmas, T. (2002) Antioxidant nutrients and mycotoxins. *Toxicology* **15**, 151–167.

Bader, J., Albin, A. and Stahl, U. (2012) Spore-forming bacteria and their utilisation as probiotics. *Benef Microbes* **3**, 67–75.

Burgain, J., Scher, J., Francius, G., Borges, F., Corgneau, M., Revol-Junelles, A.M., Cailliez-Grimal, C. and Gaiani, C. (2014) Lactic Acid Bacteria in dairy food: surface characterization and interactions with food matrix components. *Adv Colloid Interface Sci* **213**, 21–35.

Cenci, G., Caldini, G., Trotta, F. and Bosi, P. (2008) In vitro inhibitory activity of probiotic spore-forming bacilli against genotoxins. *Lett Appl Microbiol* **46**, 331–337.

Chistyakov, V.A., Prazdnova, E.V., Gutnikova, L.V., Sazykina, M.A. and Sazykin, I.S. (2012) Superoxide scavenging activity of plastoquinone derivative 10-(6'-plastoquinonyl) decyltriphenylphosphonium (SkQ1). *Biochemistry (Mosc)* **77**, 776–778.

Chistyakov, V.A., Melnikov, V.G., Chikindas, M.L., Khutsishvili, M., Chagelishvili, A., Bren, A., Kostina, N., Cavera, V. *et al.* (2015) Poultry-beneficial solid-state *Bacillus amyloliquefaciens* B-1895 fermented soybean formulation. *Biosci Microbiota Food Health* **34**, 25–38.

Cox, C.M. and Dalloul, R.A. (2014) Immunomodulatory role of probiotics in poultry and potential in ovo application. *Benef Microbes* **6**, 45–52.

Cutting, S.M. (2011) Bacillus probiotics. *Food Microbiol* **28**, 214–220.

FAO/WHO (2001) Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. Cordoba, Argentina. Viewed on-line on January 25, 2015: ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf

FAO/WHO (2002) Guidelines for the evaluation of probiotics in food. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food; London, Ontario, Canada. Viewed on-line on January 25, 2015: ftp://ftp.fao.org/es/esn/food/wgreport2.pdf

Floch, M.H. (2014) Recommendations for probiotic use in humans- a 2014 update. *Pharmaceuticals (Basel)* **7**, 999–1007.

Fridovich, I. (1999) Fundamental aspects of reactive oxygen species, or what's the matter with oxygen? *Ann N Y Acad Sci* **893**, 13–18.

Gotteland, M., Brunser, O. and Cruchet, S. (2006) Systematic review: are probiotics useful in controlling gastric colonization by *Helicobacter pylori*? *Aliment Pharmacol Ther* **23**, 1077–1086.

Halliwell, B. (2005) Free radicals and other reactive species in disease. *eLS*, 1–9. doi: 10.1038/npg.els.0003913.

Kadooka, Y., Sato, M., Imaizumi, K., Ogawa, A., Ikuyama, K., Akai, Y., Okana, M., Kagoshima, M. *et al.* (2010)

Regulation of abdominal adiposity by probiotics (Lactobacillus gasseri SBT2055) in adults with obese tendencies in a randomized controlled trial. *Eur J Clin Nutr* **64**, 636–643.

Karlyshev, A.V., Melnikov, V.G. and Chistyakov, V.A. (2014) Draft genome sequence of *Bacillus amyloliquefaciens* B-1895. *Genome Announc* **2**, e00633-14.

Kodali, V.P. and Sen, R. (2008) Antioxidant and free radical scavenging activities of an exopolysaccharide from a probiotic bacterium. *Biotechnol J* **3**, 245–251.

Lamanna, C. (1954) The problem of the nomenclature of *Bacillus subtilis* and *Bacillus vulgatus*. *Int Bull Bacteriol Nomencl Taxon* **4**, 133–139.

Liochev, S.I., Hausladen, A., Beyer, W.F. Jr and Fridovich, I. (1994) NADPH: ferredoxin oxidoreductase acts as a paraquat diaphorase and is a member of the soxRS regulon. *Proc Natl Acad Sci USA* **91**, 1328–1331.

Lo, P.R., Yu, R.C., Chou, C.C. and Huang, E.C. (2004) Determinations of the antimutagenic activities of several probiotic bifidobacteria under acidic and bile conditions against benzo[a]pyrene by a modified Ames test. *Int J Food Microbiol* **93**, 249–257.

Manukhov, I.V., Eroshnikov, G.E., Vissokikh, M.Y. and Zavilgelsky, G.B. (1999) Folding and refolding of thermolabile and thermostable bacterial luciferases: the role of DnaKJ heat-shock proteins. *FEBS Lett* **448**, 265–268.

Marnett, L.J. (2000) Oxyradicals and DNA damage. *Carcinogenesis* **21**, 361–370.

Masood, N., Fatima, K. and Luqman, S. (2014) A modified method for studying behavioral paradox of antioxidants and their disproportionate competitive kinetic effect to scavenge the peroxyl radical formation. *ScientificWorldJournal* **2014**, 931581.

McBain, A.J. and MacFarlane, G.T. (2001) Modulation of genotoxic enzyme activities by non-digestible oligosaccharide metabolism in in-vitro human gut bacterial ecosystems. *J Med Microbiol* **50**, 833–842.

Miller, R.L., Sun, G.Y. and Sun, A.Y. (2007) Cytotoxicity of paraquat in microglial cells: involvement of PKCdelta- and ERK1/2-dependent NADPH oxidase. *Brain Res* **1167**, 129–139.

Noll, K.S., Sinko, P.J. and Chikindas, M.L. (2011) Elucidation of the molecular mechanisms of action of the natural antimicrobial peptide subtilosin against the bacterial vaginosis-associated pathogen *Gardnerella vaginalis*. *Probiotics Antimicrob Proteins* **3**, 41–47.

Ordzhonikidze, K.G., Zanadvorova, A.M. and Abilev, S.K. (2011) Organ specificity of the genotoxic effects of cyclophosphane and dioxidine: an alkaline comet assay study. *Genetika* **47**, 853–855.

Piopov, D.A., Anuchina, N.M., Terent'ev, A.A., Kostiuk, G.V., Blatun, L.A., Rusanova, E.V., Aleksandrova, I.A., Pkhakadze, T.I.A. *et al.* (2013) Dioxidin: antimicrobial activity and prospects of its clinical use at present. *Antibiot Khimioter* **58**, 37–42.

Priebe, M.G., Vonk, R.J., Sun, X., He, T., Harmsen, H.J. and Welling, G.W. (2002) The physiology of colonic metabolism. Possibilities for interventions with pre-and probiotics. *Eur J Nutr* **41**, 2–10.

Putignani, L., Del Chierico, F., Petrucca, A., Vernocchi, P. and Dallapiccola, B. (2014) The human gut microbiota: a dynamic interplay with the host from birth to senescence settled during childhood. *Pediatr Res* **76**, 2–10.

Quigley, L., O'Sullivan, O., Stanton, C., Beresford, T.P., Ross, R.P., Fitzgerald, G.F. and Cotter, P.D. (2013) The complex microbiota of raw milk. *FEMS Microbiol Rev* **37**, 664–698.

Renner, H.W. and Münzner, R. (1991) The possible role of probiotics as dietary antimutagen. *Mutat Res* **262**, 239–245.

Sedelnikova, O.A., Redon, C.E., Dickey, J.S., Nakamura, A.J., Georgakilas, A.G. and Bonner, W.M. (2010) Role of oxidativelyinduced DNA lesions in human pathogenesis. *Mutat Res* **704**, 152–159.

Shen, Q., Shang, N. and Li, P. (2011) In vitro and in vivo antioxidant activity of *Bifidobacterium animalis* 01 isolated from centenarians. *Curr Microbiol* **62**, 1097–1103.

Skulachev, V.P. (2005) How to clean the dirtiest place in the cell: cationic antioxidants as intramitochondrial ROS scavengers. *IUBMB Life* **57**, 305–310.

Stilling, R.M., Dinan, T.G. and Cryan, J.F. (2014) Microbial genes, brain and behaviour – epigenetic regulation of the gut-brain axis. *Genes Brain Behav* **1**, 69–86.

Sutyak, K.E., Anderson, R.A., Dover, S.E., Feathergill, K.A., Aroutcheva, A.A., Faro, S. and Chikindas, M.L. (2008a) Spermicidal activity of the safe natural antimicrobial peptide subtilosin. *Infect Dis Obstet Gynecol* **2008**, 540758.

Sutyak, K.E., Wirawan, R.E., Aroutcheva, A.A. and Chikindas, M.L. (2008b) Isolation of the *Bacillus subtilis* antimicrobial peptide subtilosin from the dairy product-derived *Bacillus amyloliquefaciens*. *J Appl Microbiol* **104**, 1067–1074.

Vanderhoof, J.A. and Mitmesser, S.H. (2010) Probiotics in the management of children with allergy and other disorders of intestinal inflammation. *Benef Microbes* **4**, 351–356.

Wells, P.G., Bhuller, Y., Chen, C.S., Jeng, W., Kasapinovic, S., Kennedy, J.C., Kim, P.M., Laposa, R.R. *et al.* (2005) Molecular and biochemical mechanisms in teratogenesis involving reactive oxygen species. *Toxicol Appl Pharmacol* **207**, 354–366.

Zavilgelsky, G.B., Kotova, V. and Manukhov, I.V. (2007) Action of 1,1-dimethylhydrazine on bacterial cell sis determined by hydrogen peroxide. *Mutat Res* **634**, 172–176.

Zheng, G. and Slavik, M.F. (1999) Isolation, partial purification and characterization of a bacteriocin produced by a newly isolated *Bacillus subtilis* strain. *Lett Appl Microbiol* **28**, 363–367.