Antiviral Effects of *Lactococcus lactis* on Feline Calicivirus, A Human Norovirus Surrogate

Hamada A. Aboubakr · Amr A. El-Banna · Mohammed M. Youssef · Sobhy A. A. Al-Sohaimy · Sagar M. Goyal

Received: 20 May 2014 / Accepted: 8 August 2014 / Published online: 17 August 2014
© Springer Science+Business Media New York 2014

**Abstract**  Foodborne viruses, particularly human norovirus (NV) and hepatitis virus type A, are a cause of concern for public health making it necessary to explore novel and effective techniques for prevention of foodborne viral contamination, especially in minimally processed and ready-to-eat foods. This study aimed to determine the antiviral activity of a probiotic lactic acid bacterium (LAB) against feline calicivirus (FCV), a surrogate of human NV. Bacterial growth medium filtrate (BGMF) of *Lactococcus lactis* subsp. *lactis* LM0230 and its bacterial cell suspension (BCS) were evaluated separately for their antiviral activity against FCV grown in Crandell–Reese feline kidney (CRFK) cells. No significant antiviral effect was seen when CRFK cells were pre-treated with either BGMF (raw or pH 7-adjusted BGMF) or BCS. However, pre-treatment of FCV with BGMF and BCS resulted in a reduction in virus titers of $1.3 \log_{10}$ tissue culture infectious dose (TCID)$_{50}$ and $1.8 \log_{10}$ TCID$_{50}$, respectively. The highest reductions in FCV infectivity were obtained when CRFK cells were co-treated with FCV and pH 7-adjusted BGMF or with FCV and BCS ($7.5 \log_{10}$ TCID$_{50}$ and $6.0 \log_{10}$ TCID$_{50}$, respectively). These preliminary results are encouraging and indicate the need for continued studies on the role of probiotics and LAB on inactivation of viruses in various types of foods.

**Keywords**  Norovirus · Feline calicivirus · Lactic acid bacteria · Probiotics · Antiviral activity · *Lactococcus lactis* · Foodborne viruses

**Introduction**

Foodborne illnesses associated with contaminated food continue to plague public health as well as world economies. The economic cost of foodborne illnesses is approximately $152 billion in the US alone (Scharff 2010). Enteric viruses, particularly human norovirus (NV) and hepatitis virus type A, are the leading causes of viral foodborne illnesses (Anonymous 2012; Koopmans and Duizer 2004). Human NV, one of the top five highest-ranking pathogens with respect to the total cost of foodborne illness in the US, belongs to family *Caliciviridae* and is a well-known cause of “winter-vomiting disease” or “stomach-flu” (ECDC 2013; Scharff 2012). The U.S. Centers for Disease Control and Prevention (2013) reported that NV causes 19–21 million cases of acute gastroenteritis annually in the US and leads to 1.7–1.9 million outpatient visits, 400,000 emergency room visits, 56,000–71,000 hospitalizations, and 570–800 deaths, mostly among young children. More than half of all foodborne disease outbreaks...
due to a known cause reported to CDC from 2006 to 2010 was attributed to NV. In the European Union, caliciviruses (primarily NV) were responsible for 507 of 675 foodborne viral outbreaks (European Food Safety Authority 2009).

The minimal effect of most food processing methods on the inactivation of foodborne viruses has been reviewed (Baert et al. 2009; FAO/WHO 2008; Hirneisen et al. 2010). In addition, recent experiments with NV in a variety of foods revealed that freezing, cooling, and mild heat treatment (minimal food processing) were not effective in significantly reducing virus titers (Mormann et al. 2010). Thus, development of novel, efficient and safe strategies for controlling viral contamination of foods is of great interest to food scientists and food producers. In this regard, biopreservation (control of one organism by another) has received much attention in the last decade (Dalié et al. 2010).

Among natural biological antagonists, lactic acid bacteria (LAB), a part of the intestinal microflora, have been widely used for the production of fermented foods. These bacteria have a long history of use in foods and are known to have beneficial health effects in humans. Many compounds are produced during LAB fermentation some of which have an antimicrobial activity. These compounds include: hydrogen peroxide, organic acids, diacetyl, hydroxyl fatty acids, proteinaceous compounds, and bacteriocins (Dalié et al. 2010). The antagonistic effects of LAB against pathogenic bacteria e.g., Listeria monocytogenes, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus sanguinis, Proteus mirabilis, and Yersinia spp. have been reported (Al Askari et al. 2012; Cizeikiene et al. 2013; Dalié et al. 2010; Koo et al. 2012; Schwenninger et al. 2011).

Recently, there has been an increased interest in using LAB and other probiotic bacteria as viral inhibitors against coronavirus (Maragkoudakis et al. 2010), herpes simplex virus (Khani et al. 2012), human immunodeficiency virus (Martin et al. 2010), influenza virus (Kobayashi et al. 2011; Lee et al. 2013; Youn et al. 2012), rotavirus (RV; Maragkoudakis et al. 2010), and vesicular stomatitis virus (VSV; Botić et al. 2007). Lactococcus lactis (formerly, Streptococcus lactis) is one of the most important LABs. It is a Gram-positive bacterium used extensively in the production of butter milk and cheese (Madigan et al. 2012). Other uses include the production of pickled vegetables, beer or wine, bread, and other fermented foodstuff, such as soymilk kefir. This organism has a homofermentative metabolism and produces l-(-)-lactic acid (Samaržija et al. 2001). It can also produce d-(-)-lactic acid when cultured at low pH (Åkerberg et al. 1998). The capability to produce lactic acid is one of the reasons why L. lactis is one of the most important microorganisms in the dairy and food industries and has achieved the GRAS (generally regarded as safe) status (FDA 2012). The present study was undertaken to determine the antiviral activity of L. lactis subsp. lactis LM0230 against feline calicivirus (FCV), a surrogate of NV.

Materials and Methods

Bacterial Strain

Lactococcus lactis subsp. lactis LM0230 was kindly provided by Dr. Dan O’Sullivan, Professor of Food Microbiology, Department of Food Science and Nutrition, University of Minnesota. The strain was maintained at –20 °C in De Man, Ragosa, and Sharp (MRS) broth (Oxoid, Basingstoke, England) supplemented with 20 % (v/v) glycerol as a cryoprotective agent.

Preparation of Bacterial Growth Medium Cell-Free Filtrate (BGMF) and Bacterial Cell Suspension (BCS)

The bacterium was grown in 30 mL MRS broth for 24 h at 30 ± 02 °C, under anaerobic conditions. The culture was centrifuged at 2,000×g for 15 min. The supernatant was collected and divided into two portions. One portion (its measured pH was 3.7) was filter-sterilized using 0.22 μm PVDF membrane filters (Millex®GV, Millipore, Bedford, MA) and was labeled as ‘raw BGMF’. The second portion was adjusted to pH 7.0 ± 0.05 using 1 M sodium hydroxide solution, filter-sterilized, and labeled as ‘pH-7 adjusted BGMF’. The BCS was prepared by washing the pellet of bacteria obtained above twice with sterile peptone phosphate water broth (PPWB, Fluka, Switzerland) to remove excess MRS followed by centrifugation at 2,000×g for 15 min. The washed pellet was re-suspended in 10 mL of PPWB. The viable bacterial cell count was determined spectrophotometrically by measuring the optical density (OD) at 620 nm against cell-free PPWB as a blank. A standard curve was created by plotting ODs of 10-fold serial dilutions of a standard BCS versus mathematically calculated colony forming units (CFUs)/mL of each dilution. The CFU/mL of the standard BCS was measured initially using the plate count technique on MRS agar plates.

Cell Line and Growth

A Crandell–Reese feline kidney (CRFK) cell line was obtained from Veterinary Diagnostic Laboratory, University of Minnesota, USA. Cells were grown in Corning cellgro minimum essential medium (MEM) with Earle’s salts and l-glutamine (Mediatech, Inc., USA) supplemented with 8 % fetal bovine serum (FBS) and standard...
antibiotics at 37 °C in 5 % CO₂ in tissue culture flasks until confluent monolayer of cells is formed. The cell culture was regularly passaged. To perform biological assays, the cells were seeded in 96 well plates (5 × 10³ cells/well) and incubated for 48 h at 37 °C under 5 % CO₂ to reach the monolayer.

Virus Propagation and Titration

FCV, strain 255, was used in the experiments. The virus was propagated in CRFK monolayers. Flasks containing CRFK cell monolayers were infected with FCV. When cytopathic effect (CPE) was observed by inverted microscope (24–48 h after infection and incubation at 37 °C) the supernatant containing the virus was collected after freezing and thawing three times followed by centrifugation at 3,000×g for 15 min. Virus was stored at −80 °C until used. For virus titration, the 50 % tissue culture infectious dose (TCID₅₀) method was used. In which, serial 10-fold dilutions of samples were prepared in MEM containing 4 % FBS and inoculated in confluent CRFK monolayers prepared in 96-well microtiter plates using three wells per dilution. The cells were examined for the development of CPE daily up to 5 days. The endpoint was taken as the highest dilution of the virus which produced CPE in 50 % of the inoculated cells. Viral titers were calculated by the Karber formula (Karber 1931) and were expressed as TCID₅₀/0.1 mL.

Cytotoxicity Assay

The minimum non-toxic dilutions (MNTDs) of each type of BGMF were determined based on cellular morphological alteration method described by Orhan et al. (2010). Briefly, several dilutions of each BGMF prepared in MEM were inoculated in monolayers of CRFK cells contained in 96-well microplates at 100 µL/well followed by incubation for 48 h at 37 °C under 5 % CO₂. Dilutions that were not toxic to viable cells were labeled as non-toxic and were also compared with non-treated cells (negative control) for confirmation. The lowest non-toxic dilutions were chosen as (MNTDs).

Antiviral Assays

The anti-FCV activity of L. lactis LM0230 and its metabolites was assayed by three different methods. In which, FCV titers of treated and non-treated virus or cells (control) were calculated.

(i) **Pre-treatment of cells with BGMF** after discarding its growth medium, the CRFK cell monolayers were covered with 100 µL of non-toxic dilutions (MNTDs) of the two different types of BGMF (1:10 diluted and undiluted) from raw and pH 7-adjusted BGMF, respectively. After incubation at 37 °C in 5 % CO₂ incubator for various incubation times (30 min, 90 min, and 24 h), the monolayers were washed with MEM. Immediately, the washed monolayers were infected with 100 µL of FCV 10-fold serial dilutions.

(ii) **Pre-treatment of cells with BCS**, the CRFK monolayers were incubated with 20, 50, and 100 µL of BCS (5.1 × 10⁸ CFU/mL) for 30, 60, and 90 min at 37 °C in a 5 % CO₂ incubator. After incubation the non-bound bacteria were removed by washing two times with MEM 100 µL each. The monolayers were then infected with FCV dilutions.

(iii) **Pre-treatment of virus with BGMF**, aliquots (250 µL) of FCV suspension were mixed separately with equal volumes of raw BGMF and pH 7-adjusted BGMF (both undiluted) in 1.5 mL sterile Eppendorf tubes. After incubation at 37 °C in 5 % CO₂ incubator for different times (30 min, 90 min, and 24 h), 10-fold serial dilutions were prepared from each mixture followed by infection of CRFK monolayers.

(iv) **Pre-treatment of virus with BCS (virus adsorption to bacterial cells)**, aliquots (250 µL) of FCV suspension were separately mixed with equal volumes of BCS containing different bacterial cell counts (1 × 10⁸, 2 × 10⁸, 3 × 10⁸ CFU/250 µL) in 1.5 mL sterile Eppendorf tubes. After incubation at 37 °C in 5 % CO₂ incubator for different times (30 min, 90 min, and 24 h), the mixtures were centrifuged at 12,000×g for 3 min. 10-Fold serial dilutions of the supernatant were prepared in MEM and 100 µL of each dilution was used to infect the CRFK monolayers for titration.

(v) **Co-treatment of cells and virus with BGMF**, 10-fold serial dilutions of FCV were prepared in different solutions of raw BGMF and pH 7-adjusted BGMF as diluents followed by infection of CRFK monolayers. Three different dilutions of raw BGMF and pH 7-adjusted BGMF were used: 1:10, 1:20, 1:30, and undiluted, 1:5, 1:10 v/v in MEM medium were used, respectively.

(vi) **Co-treatment of CRFK cells with BCS and virus**, the CRFK monolayers were inoculated with 20, 50, and 100 µL of BCS (5.1 × 10⁸ CFU/mL). Immediately, the monolayers were infected with serial 10-fold dilutions of FCV prepared in MEM. After the fifth day of incubation, the wells were washed two times with MEM 100 µL each, to
remove the bacterial cells overlaying layers which prevent observation of CPEs under microscope. Cell control and bacterial-treated cell control wells were done for discrimination between CPE versus intact cells, and normal CPE versus bacterial-contaminated cells, respectively.

Statistical Analysis

Each titration was carried out in triplicate and each experiment was triplicated. The results are the mean ± standard deviation. The analysis of variance (ANOVA) was generated by F test. The statistical analysis was carried out using STATISTICA software, v. 10 (Statsoft, Inc., USA).

Results

Cytotoxicity of BGMF–CRFK Cells

Raw BGMF exhibited toxicity to CRFK cells at 0 and 1:5 dilutions while higher dilutions exhibited no toxicity. On the other hand, pH 7-adjusted BGMF did not show any toxicity in diluted or undiluted forms.

Antiviral Activity of *L. lactis* LM0230

(i) **Pre-treatment of cells with BGMF**, the CRFK cells were pre-treated with raw and pH 7-adjusted BGMFs at their MNTDs (1:10 and 0 dilution, respectively) for 30 min, 90 min or 24 h. As shown in Fig. 1, there is no significant decrease (*P ≥ 0.01*) in FCV titer after pre-treatment of CRFK cells either with raw or pH 7-adjusted BGMF. The time of pre-treatment also had no significant effect (*P ≥ 0.01*).

(ii) **Pre-treatment of cells with BCS**, the CRFK cells were pre-treated with various BCS volumes (20, 50, and 100 µL) to examine the effect of number of bacterial cells on the capability of CRFK cells to support FCV replication. The cells were pre-treated for 30, 60, or 90 min for each BCS volume. Except for a little decrease in FCV titer (0.5 log₁₀ TCID₅₀/0.1 mL) with CRFK treated with 100 µL of BCS for 90 min, neither bacterial cell count nor the treatment time had any significant effect on FCV titer (*P < 0.01*; data not shown).

(iii) **Pre-treatment of FCV with BGMF**, the pre-treatment of FCV with raw BGMF for 30 min, 90 min, and 24 h resulted in significant reductions (*P < 0.01*) in FCV titer by approximately 0.7, 1.0, and 1.3 log₁₀ TCID₅₀/0.1 mL, respectively, whereas pre-treatment with pH 7-adjusted BGMF led to non-significant decreases (*P ≥ 0.01*) with all pre-treatment times (Fig. 2).

(iv) **Pre-treatment of FCV with BCS (virus adsorption to bacterial cells)**, the pre-treatment of FCV with BCS containing 1 × 10⁸, 2 × 10⁸ and 3 × 10⁸ CFU of *L. lactis* LM0230 resulted in non-significant decreases (*P ≥ 0.01*) in FCV titers at either 30 or 90 min (Fig. 3). However, virus titers were significantly reduced (*P < 0.01*) when the virus was treated for 24 h with BCS containing 1 × 10⁸, 2 × 10⁸, and 3 × 10⁸ CFU (approximately 1.2, 1.3, and 1.8 log₁₀ TCID₅₀/0.1 mL reductions, respectively).
Co-treatment of cells and virus with BGMF, co-treatment of CRFK simultaneously with BGMFs and FCV as virus infection led to significant decreases in FCV titers \( (P < 0.01; \text{Fig. 4}) \). The highest decrease in virus titer \( (7.5 \log_{10} \text{TCID}_{50}/0.1 \text{mL}) \) was obtained by co-treatment with pH 7-adjusted BGMF at MNTD (0 dilution). There were lower decreases in FCV titers with higher dilutions of pH 7-adjusted BGMF (1.3 and 1.0 \( \log_{10} \text{TCID}_{50}/0.1 \text{mL} \) with 1:5 and 1:10 dilutions). Similar trend was seen with co-treatment with raw BGMF. The highest decrease in FCV \( (P < 0.01) \) was attained with MNTD (1:10 dilution) of raw BGMF followed by 1:20 dilutions to be 1.5, 1.3 \( \log_{10} \text{TCID}_{50}/0.1 \text{mL} \) reduction, respectively. The highest dilution (1:30) showed non-significant decrease in the virus titer \( (0.3 \log_{10} \text{TCID}_{50}/0.1 \text{mL}) \).

Co-treatment of cells with BCS and virus, the co-treatment of CRFK monolayers with different volumes (20, 50, and 100 µL) of BCS \( (5.1 \times 10^8 \text{CFU/mL}) \) during simultaneous FCV infection resulted in significant decreases \( (P < 0.01) \) in FCV titer versus its titer with control monolayers (without BCS). The highest decrease in FCV titer \( (6.0 \log_{10} \text{TCID}_{50}/0.1 \text{mL}) \) was attained by treatment with 100 µL followed by approximately 5.7 and 5.0 \( \log_{10} \text{TCID}_{50}/0.1 \text{mL} \) when CRFK monolayer was treated by 50 and 20 µL of BCS, respectively (Fig. 5). There were no statistically significant differences \( (P \geq 0.01) \) between the decreasing values attributed to the three BCS volumes used.

**Discussion**

To study the antiviral activity of LAB and probiotics, *L. lactis ssp. lactis* LM0230 was chosen as a model because it is a common LAB with probiotic properties (Heoa et al. 2013). The FCV was chosen as a surrogate of NV because the former does not grow in vitro although several attempts have been made to accomplish this task (Guix et al. 2007; Malik et al. 2005; Straub et al. 2007). In addition, the FCV has been used as a surrogate to evaluate the efficacy of
common preservation processes used in the food industry (Baert et al. 2009; Butot et al. 2008). The FCV belongs to the same Caliciviridae family as does human NV (Bidawid et al. 2000; D’Souza et al. 2006).

To avoid interference of BGFM toxicity with viral CPEs, preliminary cytotoxicity assays were done to determine the MNTD of each type of BGFM. The cytotoxicity of raw BGFM at 0 and 1:5 dilutions may have been due to their low pH (pH 3.7 and 5.0, respectively) since 0 dilution of pH 7-adjusted BGFM did not show any toxicity.

Pre-treatment of CRFK cells with BGFM or BCS had non-significant decreases in FCV titer ranged from 0 to 68 % (less than one log_{10} TCID_{50}/0.1 mL). This result is in agreement with an earlier report in which 68 and 60 % of VSV infectivity was diminished when IPEC-J2 cells were pre-treated with BGFM or BCS of certain LAB strains (Botić et al. 2007).

Significant time-dependent decrease in FCV titer was obtained by pre-treatment of FCV with raw BGFM but not with pH 7-adjusted BGFM. The main difference between the two types of BGFM is the status of lactic acid excreted in the growth medium by L. lactis. Lactic acid was neutralized by sodium hydroxide in pH 7-adjusted BGFM. Therefore, its effect was eliminated by transforming lactic acid into its sodium salt (sodium lactate) at pH 7.0. This explanation is supported by a similar study in which pre-treatment of FCV with 0.3 % D,L-lactic acid solution (pH 3.4–3.5) at 20 °C led to 1.3 log_{10} reduction in FCV titer (Straube et al. 2011). The pH of undiluted raw BGFM in our study was 3.7. We hypothesize that the viral capsid proteins are denatured due to the effects of acid pH on non-enveloped viruses (Rodger et al. 1977; Straube et al. 2011), thus preventing viral attachment to its host cells.

Pre-treatment of FCV with BCS resulted in a decreased virus titer after 24 h but not after 30 or 90 min. Similarly, Botić et al. (2007) reported 70 % reduction in infectivity of VSV after 24 h incubation with different LAB strains. They attributed this reduction to the adsorption or binding of the virus on the surface of LAB strains probably because peptidoglycans in the cell walls of LAB trapped the virus (Botić et al. 2007). The cell wall of L. lactis is also known to have a peptidoglycan structure consisting of A4\alpha-type peptidoglycan, with a monomer primary structure (Glc\nac-Mur\nac-\alpha-D-Glu-L-Lys-D-Ala) and a D-Asp in the interpeptide bridge, attached to the \alpha-amino group of Lys (Courtin et al. 2006). Some Lactobacillus strains have been shown to trap HIV virions by binding the mannose sugar rich “dome” of their attachment glycoprotein gp 120 (Carlson et al. 2004; Chang et al. 2009). A similar mechanism may also have worked in the bacterium–virus interaction system of the present study.

In co-treatment experiments, the FCV titers were reduced by both types of BGMFs, but complete inhibition of FCV infectivity was only attained when undiluted pH 7-adjusted BGFM was used (Fig. 4). We hypothesize that the extracellular metabolites of L. lactis excreted in BGFM might prevent the attachment of FCV to the cells affecting its entrance into the cells. The observed antiviral activities of pH 7-adjusted BGFM indicate that lactic acid may not be the key factor in this action where it was transformed to sodium lactate during pH adjustment of BGFM. It has been reported previously that metabolites of L. lactis such as bacteriocins (Akkoç et al. 2011; Choi et al. 2000; Samaržija et al. 2001) and hydrogen peroxide (Grufferty and Condon 1983; Samaržija et al. 2001; Van Niel et al. 2002) may be responsible for such action. Antiviral activity of bacteriocins and bacteriocin-like substances produced by LAB, probiotics, and certain other bacteria has been reported (Ermolenko et al. 2010; Saeed et al. 2007; Todorov et al. 2005; Torres et al. 2013; Wachsmann et al. 2003). Hydrogen peroxide is also a well-known antiviral substance (Roberts and Antonoplos 1998). Antiviral activity of probiotic bacteria against VSV has been attributed to their metabolites (Botić et al. 2007).

Co-infection of CRFK with FCV and BCS showed about 7.5 log_{10} TCID_{50}/0.1 mL (~100 %) reduction in FCV infectivity (Fig. 4). In similar work, the infectivity of VSV was decreased by 60 % when IPEC-J2 cells co-infected with VSV and different LAB strains (Lactobacilli and Bifidobacteria) and VSV (Botić et al. 2007). Our results are also in agreement with Maragkoudakis et al. (2010) who observed significant decreases in infectivity of transmissible gastroenteritis coronavirus (TGEV) and RV when hosting cells co-infected with the viruses in presence of Lactobacillus sp. It was hypothesized that LAB cells induced release of reactive oxygen species such as NO– and H_{2}O_{2}, which may be responsible for killing the studied viruses (TGEV and RV; Maragkoudakis et al. 2010).

Competition between bacterial cells and FCV for attaching to the functional receptors on the cells may also help elucidate these results. It is also possible that LAB may establish a “cross talk” (some sort of signaling) or alter the state of the epithelial cells and macrophages, which leads to an antiviral response as suggested by Botić et al. (2007).

Finally, four possible mechanisms of the anti-FCV effect of L. lactis subsp. lactis LM0230 can be proposed. First, the lower p\text{H} related to the excretion of lactic acid by LAB may be responsible for denaturation of capsid proteins of the virus preventing its attachment to host cells. Second, the peptidoglycan structure of LAB may trap viral particles. Third, production of different metabolites (such as bacteriocins and hydrogen peroxide) can prevent the entrance of the virus into host cells thereby inhibiting its replication. Finally, the competition between the bacterial cells and the virus for attachment on host cells may be occurred. In addition, the induction effect of the bacterium
for the host cells to produce reactive oxygen substances might kill the virus.

In conclusion, this study reported for the first time, an antiviral effect of *L. lactis* subsp. *lactis* LM0230 (as a dual model of LAB and probiotics) against FCV as a human NV surrogate. This indicates that LAB and probiotics-based fermented food may hold a promise in preventing foodborne viruses and that these bacteria hold promise as bio-preservative agents in controlling the contamination of foods with viruses. Although preliminary, the results presented here are of particular importance and merit further investigation to understand deeply the mechanisms of LAB and probiotics antiviral effect and to study its activity in food models.

Acknowledgments Funding provided by the Cultural Affairs and Mission Sector, Ministry of Higher Education and Scientific Research, Egypt is gratefully acknowledged.

References

Äkerberg, C., Hofvendahl, K., Zacchi, G., & Hahn-Hägerdal, B. (1998). Modelling the influence of pH, temperature, glucose and lactic acid concentrations on the kinetics of lactic acid production by *Lactococcus lactis* ssp. *lactis* ATCC 19435 in whole-wheat flour. *Applied Microbiology and Biotechnology*, 49, 682–690.

Akköç, N., Ghamat, A., & Akçelik, M. (2011). Optimization of bacteriocin production of *Lactococcus lactis* subsp. *lactis* MA23, a strain isolated from Boza. *International Journal of Dairy Technology*, 64, 425–432.

Al Askari, G., Kahouadji, A., Khedid, K., Charof, R., & Mennane, Z. (2012). Screenings of lactic acid bacteria isolated from dried fruits and study of their antibacterial activity. *Middle-East Journal of Scientific Research*, 11, 209–215.

Anonymous. (2012). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2010. *EFSA Journal*, 10(3):2597. doi:10.2903/j.efsa.2012.2597.

Baert, L., Debevere, J., & Uyttendaele, M. (2009). The efficacy of preservation methods to inactivate foodborne viruses. *International Journal of Food Microbiology*, 131, 83–94.

Bidawid, S., Farber, J. M., & Sattar, S. A. (2000). Contamination of foods by food handlers: Experiments on hepatitis A virus production to food and its interruption. *Applied and Environmental Microbiology*, 66, 2759–2763.

Botić, T., Klingberg, T. D., Weingartl, H., & Cencić, A. (2007). A novel eukaryotic cell culture model to study antiviral activity of potential probiotic bacteria. *International Journal of Food Microbiology*, 115, 227–234.

Butot, S., Putallaz, T., & Sanchez, G. (2008). Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. *International Journal of Food Microbiology*, 126, 30–35.

Carlson, S. J., Pavlova, S. I., Spear, G. T., Anzinger, J. J., & Tao, L. (2004). *Lactobacillus* lectin as a natural HIV trap. [abstract 2280]. In IADR/AADR/CADR 82nd General Session, Honolulu, Hawaii. https://iadr.confex.com/iadr/2004Hawaii/techprogram/abstract_47196.htm.

Chang, R., Pavlova, S., Caffrey, M., Spear, G., Tanzer, J., Thompson, A., & Tao, L. (2009). Blocking milk-borne HIV transmission by virus-capturing *Lactobacilli*. In *The Mouth and AIDS: The Global Challenge 6th World Workshop on Oral Health and Disease*, Beijing. http://www.hivotdent.org/6thWWOHDA/6WWOHDA_BMBHTV.htm.

Choi, H.-J., Cheigh, C.-I., Kim, S.-B., & Pyun, Y.-R. (2000). Production of a nisin-like bacteriocin by *Lactococcus lactis* subsp. *lactis* A164 isolated from Kimchi. *Journal of Applied Microbiology*, 88, 563–571.

Cizelkienė, D., Juodeikiene, G., Paskevičius, A., & Bartkiene, A. (2013). Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganisms isolated from food and their control in wheat bread. *Food Control*, 31, 539–545.

Courtin, P., Miranda, G., Guillet, A., Wessner, F., Mérange, C., Domakova, E., et al. (2006). Peptidoglycan structure analysis of *Lactococcus lactis* reveals the presence of an L,D-carboxypeptidase involved in peptidoglycan maturation. *Journal of Bacteriology*, 188, 5293–5298.

D’Souza, D. H., Sair, A., Williams, K., Papafragkou, E., Jean, J., Moore, C., et al. (2006). Persistence of caliciviruses on environmental surfaces and their transfer to food. *International Journal of Food Microbiology*, 108, 84–91.

Dalié, D. K. D., Deschamps, V., & Richard-Forget, F. (2010). Lactic acid bacteria—Potential for control of mould growth and mycotoxins: A review. *Food Control*, 21, 370–380.

ECDC. (2013). http://www.ecdc.europa.eu/en/healthtopics/norovirus_infection/basic_facts/Pages/basic_facts.aspx.

Ermolenko, E. I., Furaeva, V. A., Isakov, V. A., Ermolenko, D. K., & Suworov, A. N. (2010). Inhibition of herpes simplex virus type 1 reproduction by probiotic bacteria in vitro. *Voprosy Virologii*, 55, 25–28.

European Food Safety Authority. (2009). The community summary report for foodborne outbreaks in the European Union in 2007. *EFSA Journal*, 271. http://www.efsa.europa.eu/EFSA/efsa_ locale1178620753812_121902515341.htm.

FAO/WHO. (2008). *Microbiological hazards in fresh leafy vegetables and herbs: Meeting Report*. *Microbiological Risk Assessment Series No. 14*. Rome. http://www.who.int/foodsafety/publications/micro/Viruses_in_food_MRA.pdf.

FDA. (2012). *GRAS notification for the use of lactic acid bacteria to control pathogenic bacteria in meat and poultry products*. http://www.accessdata.fda.gov/scripts/fcn/gras_notices/GRN000463.pdf.

Grufferty, R. C., & Condon, S. (1983). Effect of fermentation sugar on hydrogen peroxide accumulation by *Streptococcus lactis* C10. *Journal of Dairy Research*, 50, 481–489.

Guix, S., Asanaka, M., Katayama, K., Crawford, S. E., Neill, F. H., Atmar, R. L., et al. (2007). Norwalk virus RNA is infectious in mammalian cells. *Journal of Virology*, 81, 12238–12248.

Heoa, W. S., Kima, Y. R., Kima, E. Y., Baib, S. C., & Kong, I. S. (2013). Effects of dietary probiotic, *Lactococcus lactis* subsp. *lactis* I2, supplementation on the growth and immune response of olive flounder (*Paralichthys olivaceus*). *Aquaculture*, 376–379, 20–24.

Hirmeisen, K. A., Black, E. P., Cascarino, J. L., Fino, V. R., Hoover, D. G., & Kniel, K. E. (2010). Viral inactivation in foods: A review of traditional and novel food-processing technologies. *Comprehensive Reviews in Food Science and Food Safety*, 9, 3–20.

Karber, G. (1931). 50% End point calculation. *Archiv fur Experimentelle Pathologie und Pharmakologie*, 162, 480–483.

Khani, S., Motamedifar, M., Golmoghaddamb, H., Hosseini, H. M., & Hashemizadeh, Z. (2012). In vitro study of the effect of a probiotic bacterium *Lactobacillus rhamnosus* against herpes
simplex virus type 1. *Brazilian Journal of Infectious Diseases*, 16, 129–135.

Kobayashi, N., Saito, T., Uematsu, T., Kishi, K., Toba, M., Kohda, N., et al. (2011). Oral administration of heat-killed *Lactobacillus pentosus* strain b240 augments protection against influenza virus infection in mice. *International Immunopharmacology*, 11, 199–203.

Koo, O. K., Eggleton, M., O’Bryan, C. A., Crandall, P. G., & Ricke, S. C. (2012). Antimicrobial activity of lactic acid bacteria against *Listeria monocytogenes* on frankfurters formulated with and without lactate/diacetate. *Meat Science*, 92, 533–537.

Koopmans, M., & Duizer, E. (2004). Foodborne viruses: An emerging problem. *International Journal of Food Microbiology*, 90, 23–41.

Lee, Y., Youn, H., Kwon, J., Lee, D., Park, J., Yuk, S., et al. (2013). Sublingual administration of *Lactobacillus rhamnosus* affects respiratory immune responses and facilitates protection against influenza virus infection in mice. *Antiviral Research*, 98, 284–290.

Madigan, M., Martinko, J., Stahl, D., & Clark, D. (2012). Chapter 12. In *Brock biology of microorganisms* (13th ed.). San Francisco: Benjamin Cummings.

Malik, Y. S., Maherchandani, S., Allwood, P. B., & Goyal, S. M. (2005). Evaluation of animal origin cell cultures for in vitro cultivation of Noroviruses. *The Journal of Applied Research in Clinical and Experimental Therapeutics*, 5, 312–317.

Maragoudakis, P. A., Chingwaru, W., Gradinsnik, L., Tsakalidou, E., & Cenic, A. (2010). Lactic acid bacteria efficiently protect human and animal intestinal epithelial and immune cells from enteric virus infection. *International Journal of Food Microbiology*, 141, S91–S97.

Martin, V., Maldonado, A., Fernandez, L., Rodriguez, J., & Connor, R. (2010). Inhibition of human immunodefiiciency virus type 1 by lactic acid bacteria from human breastmilk. *Breastfeeding Medicine*, 5, 153–158.

Mormann, S., Dabisch-Ruthe, M., & Becker, B. (2010). Inactivation of norovirus in foods. Inoculation study using human norovirus. *Fleischwirtschaft*, 90, 116–121.

Orhan, D. D., Ozcelik, B., Ozgen, S., & Ergun, F. (2010). Antibacterial, antifungal, and antiviral activities of some flavonoids. *Microbiological Research*, 165, 496–504.

Roberts, C., & Antonoplos, P. (1998). Inactivation of human immunodeficiency virus type 1. *Virology*, 175, 175–178.

Saeed, S., Rasool, S. A., Ahmed, S., Zaidi, A. Z., & Rehmani, S. (2007). Antiviral activity of staphylococcin 188: A purified bacteriocin like inhibitory substance isolated from *Staphylococcus aureus* AB188. *Research Journal of Microbiology*, 2, 796–806.

Samarˇzija, D., Antunac, N., & Havranek, J. L. (2001). Taxonomy, physiology and growth of *Lactococcus lactis*: A review. *Mijekarstvo*, 51, 35–48.

Scharf, R. L. (2010). Health-related costs from foodborne illness in the United States. [http://www.pewhealth.org/uploadedFiles/PHGI_Content_Level_Pages/Reports/PSP-Scharff%20v9.pdf.](http://www.pewhealth.org/uploadedFiles/PHGI_Content_Level_Pages/Reports/PSP-Scharff%20v9.pdf)

Scharf, R. L. (2012). Economic burden from health losses due to foodborne illness in the United States. *Journal of Food Protection*, 75, 123–131.

Schwenninger, S. M., Meile, L., & Lacroix, C. (2011). Antifungal lactic acid bacteria and propionibacteria for food biopreservation. In C. Lacroix (Ed.), *Protective cultures, antimicrobial metabolites and bacteriophages for food and beverage biopreservation* (pp. 27–62). Philadelphia: Woodhead Publishing Limited.

Straub, T. M., Honerzu, B. K., Orosz-Coghlan, P., Dohnalkova, A., Mayer, B. K., Bartholomew, R. A., et al. (2007). In vitro cell culture infectivity assay for human noroviruses. *Emerging Infectious Diseases Journal*, 13, 396–403.

Torres, N. I., Noll, K. S., Xu, S., Li, J., Huang, Q., Sinko, P. J., et al. (2013). Safety, Formulation and in vitro antiviral activity of the antiviral peptide subtilosin against Herpes Simplex Virus Type 1. *Probiotics and Antimicrobial Proteins*, 5, 26–35.

Van Niel, E. W. J., Hofvendahl, K., & Hahn-Hägerdal, B. (2002). Formation and conversion of oxygen metabolites by *Lactococcus lactis* subsp. *lactis* ATCC 19435 under different growth conditions. *Applied and Environmental Microbiology*, 68, 4350–4356.

Wachsman, M. B., Farias, M. E., Takeda, E., Sesma, F., Holgado, A. P., Torres, R. A., et al. (2003). Enterocin CRL35 inhibits late stages of HSV-1 and HSV-2 replication in vitro. *Antiviral Research*, 58, 17–24.

Youn, H., Lee, D., Lee, Y., Park, J., Yuk, S., Yang, S., et al. (2012). Intranasal administration of live *Lactobacillus* species facilitates protection against influenza virus infection in mice. *Antiviral Research*, 93, 138–143.