Food Environ Virol (2014) 6:42–47
DOI 10.1007/s12560-013-9135-z

ORIGINAL PAPER

Antiviral Potential of Selected Starter Cultures, Bacteriocins and D,L-Lactic Acid

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Received: 24 June 2013 / Accepted: 25 November 2013 / Published online: 3 December 2013
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Abstract The antiviral potential of selected bacteria species [lactic acid bacteria (LAB) and *micrococcaceae*] was examined. By this, the effect of their cell-free supernatants as well as of certain species-related metabolites (sakacin A, nisin, and lactic acid) was investigated on different viruses after exposure at 24 °C for 3 days. Viruses were incubated with supernatants and metabolites in a dilution ratio of 1:10. Data for antiviral effects towards murine norovirus S99 (MNV), influenza A virus A/WSN/33 (H1N1), Newcastle disease virus Montana (NDV) and feline herpesvirus KS 285 (FHV) were generated in vitro simulating pH and temperature conditions according to raw sausage fermentations. Investigations showed no antiviral effect of sakacin A and nisin on MNV, H1N1, FHV and NDV. Furthermore, the antiviral potential of D,L-lactic acid was determined for MNV and H1N1. At raw sausage-related pH values (5.0–6.2) it could be shown that the virus titre for MNV and H1N1 was reduced by a maximum of 3.25 log and 2.5 log units, respectively. In addition, 29 culture supernatants of different bacteria species, mainly LAB and staphylococci, were tested for their antiviral activity against MNV. Only the cell-free supernatant of a *Lb. curvatus* strain showed a higher virus titre reduction of MNV by 1.25 log units compared to the control. Further studies on the characterisation of this cell-free supernatant were carried out, however, the antiviral substance could not be identified so far.

Keywords Murine norovirus · Influenza virus · Virus inactivation · Starter culture · Raw sausage

Introduction

Virus transmission due to the consumption of contaminated food is an emerging public health issue. From an epidemiological point of view, mainly human noroviruses (NoV), rotaviruses and hepatitis A viruses are foodborne pathogens of significant health concerns. In Germany, human NoV have a great importance due to the high number of registered cases, for example 112.364 in 2012 (RKI 2013).

For others, like SARS coronavirus or influenza virus H5N1 or H1N1, the foodborne transmission route has been postulated even this is so far not convincingly shown.

Food can be contaminated during preparation by infected persons that can excrete large quantities of viruses (Carter 2005).

Possible risk products are raw-eaten food like shellfish, fruits or raw sausages (Wichmann et al. 2008; Sarvikivi et al. 2012; Smith et al. 2012).

In the context of potential risks associated with foodborne viruses, questions emerged about survival time and inactivation kinetics of several pathogens during food processing. As a consequence, studies were carried out to examine virus stability towards different food-preserving technologies. They were mainly focused on the impact of heat treatment, chilling, freezing, acidification or high hydrostatic pressure. Results were recently reviewed by Bertrand et al. (2012), Cliver (2010) and Baert et al.

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(2009). It seems that apart from heat treatment, measures like acidification, chilling or freezing are not sufficient enough to effectively inactivate most foodborne viruses.

In contrast, little is known about the antiviral effect of different microorganisms and their metabolites which are widely used for processing of raw meat and different dairy products. For example, use of starter cultures [e.g., lactic acid bacteria (LAB), staphylococci, and micrococci] for microbial fermentation is a crucial step to obtain microbiological safety of short fermented raw sausages as it can prevent growth of pathogenic microorganisms in these products. For that, starter cultures must be quickly increased at the initial ripening time (Luecke and Hechelmann 1985). They contribute to an improvement in hygienic safety, high and constant levels of quality, shelf-life and sensory attractiveness (Hammes and Knauf 1994). Besides the positive effect of improving organoleptic properties, different microorganisms are used as protective cultures (Abee et al. 1995). They compete with other organisms about nutrition and are able to produce antimicrobial substances like organic acid, hydrogen peroxide, diacetyl or bacteriocins (Jay 1982a, b; Kroeckel 1998).

An antiviral effect of bacteriocins as well as of bacterial supernatants, e.g., originated from LAB or staphylococci, was described in different studies, i.e., for herpes simplex virus (Todorov et al. 2010), influenza A virus (Serkedjieva et al. 2000) and Newcastle disease virus (NDV) (Saeed et al. 2007). Up to now mechanisms causing the antiviral effect of these bacteria are not known.

To broaden knowledge about the antiviral potential of food-relevant bacteria species, the impact of selected starter cultures used for raw sausage fermentation as well as of their relevant metabolites was examined in the present study. For this, the antiviral effect of the two bacteriocins sakacin A and nisin on the non-enveloped murine norovirus (MNV), widely used as a surrogate for human norovirus, as well as on influenza virus H1N1 (H1N1), feline herpesvirus (FHV) and NDV was screened. The latter ones are not food associated, but by using them an antiviral effect on the virus envelope should be assessed. In addition, the influence of lactic acid on the infectivity of MNV and H1N1 was examined. Furthermore, a screening for a possible antiviral effect of cell-free bacterial supernatants (LAB, *Staphylococcus* spp., *Kocuria varians*) was carried out for MNV. These bacteria are commonly used during raw sausage processing.

Materials and Methods

Virus and Infectivity Assay

The mouse leukaemic monocyte macrophage cell line RAW 264.7 (ATCC-TIB-71) was used for the propagation and detection of MNV S99 (Dr. Schreier, RKI, Berlin). For the propagation and detection of the viruses H1N1 (Strain A/WSN/33, Dr. Stech, FLI, Riems) and FHV (KS 285), Madin–Darby canine kidney cells (MDCK+, RHI 671, ZBV, FLI, Riems) and Crandell-Reese feline kidney cells (CRFK, RIE 769, ZBV, FLI, Riems) were used, respectively. NDV (Montana, Prof. Dr. Kaleta, Justus Liebig-University, Gießen) was grown in 10-days-old embryonated chicken eggs. The amniotic–allantoic fluid was harvested 72 h after inoculation and MDCK+ cells were used for the determination of the virus titre. All virus fluids were pooled for working stocks and stored at −80 °C. The titres of the harvested viruses were determined by the virus titration method of Spearman and Kaerber (Horzinek 1985). For all test series, the virus titres were determined initially and after 3 days of exposure. Moreover, cytotoxic controls with different cell lines were performed.

Antiviral Effect of Bacteriocins

The in vitro antiviral effect of the synthesized bacteriocins sakacin A (GenScript, USA; HPLC purity: 83.7 %) and nisin (Nisitrol, Schechen, Germany) was investigated for MNV, H1N1, FHV and NDV. The viruses were exposed in a dilution ratio of 1:10 to the bacteriocins solved in phosphate-buffered saline (PBS) for 3 days at 24 °C (pH 6.2). The experiment was performed twice. The biological activity of the used bacteriocins was tested towards the bacteriocin-sensitive strains *Listeria innocua* 1 using the listeria agar as described by Con et al. (2001) and *Pediococcus pentosaceus* 3 (both from Dr. Kroeckel, Max Rubner Institute, Kulmbach) by a well-diffusion assay according to Schillinger and Luecke (1989) (Fig. 1). The activity of the tested bacteriocins was expressed as

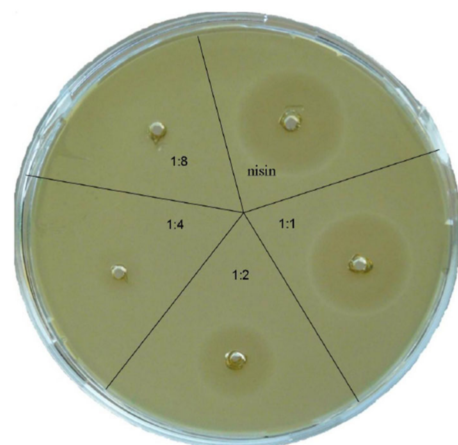

Fig. 1 The biological activity of nisin demonstrated by the inhibition zone of the bacteriocin-sensitive strain *Pediococcus pentosaceus* 3 by the well-diffusion assay (*nisin* pure, *numbers* serial twofold dilution of the bacteriocin)

arbitrary units (AU) per ml, defined as the reciprocal of the highest serial twofold dilution showing a clear zone of growth inhibition of the indicator strain (Ivanova et al. 1998). Virus-containing PBS was used as control.

Antiviral Effect of D,L-Lactic acid

For setting various pH values, D,L-lactic acid (90 %, synthetic, Carl Roth + Co KG, Germany, 3957.1) was used. Sterile-filtrated D,L-lactic acid, prediluted in PBS, was mixed with the virus suspension of MNV or H1N1 in a dilution ratio of 10:1. Typical raw sausage-related pH values between 5.0 and 6.2 were adjusted. The pH value was determined from an aliquot of the test batches immediately after mixing, because otherwise the pH value deviated from the desired one (data not published). After 3 days of exposure at 24 °C, the pH values were determined too. Mean virus titre reductions were compared to reductions obtained at pH 6.2. The experiment was performed five times.

Antiviral Effect of Bacterial Supernatants

For the study, different strains of *Lactobacillus* (*Lb.*) spp., *Staphylococcus* (*S.*) spp., *Pediococcus* (*P.*) spp. and *Kocuria* (*K.*) *varians*, commonly used for raw sausage fermentation, were selected (Table 1). Differentiation of the bacterial strains was performed biochemically by API 50 CH and API-ID32-STAPH test kit (both bioMerieux® sa, Marcy l'Etoile, France) and by MALDI-TOF mass spectrometry using the MALDI Biotyper microflex (Bruker Daltonics GmbH, Germany) with the method according to Albert et al. (2011).

LAB were grown on MRS agar (MERCK, Germany, TN 1201). After 3 days of incubation at 30 °C, one colony of each strain was transferred to MRS broth (MERCK, 1.10661) and incubated for 24 h at 30 °C. Thereafter, 4 ml of the culture was transferred to 36 ml fresh MRS broth, incubated for 24 h at 24 °C and was finally centrifuged (15 min, 10,000×*g*, 4 °C). The selected strains of *Staphylococcus* spp. and *Kocuria* spp. were grown on Plate-count Agar (SIFIN GmbH Berlin, Germany, TN 1189) for 24 h at 37 °C. A single colony was transferred to brain heart infusion broth (MERCK, 1.10493) and incubated for 24 h (37 °C). Thereafter, 4 ml of the culture was brought into 36 ml fresh brain heart broth, incubated for 24 h at 24 °C and finally centrifuged (15 min, 10,000×*g*, 4 °C).

In order to eliminate the effect of lactic acid on the test organisms, all cell-free supernatants were adjusted to pH 6.2 with NaOH (5 mol, ROTIPURAN®, >32 % p.a. Carl Roth, T196,) and filtrated through a 0.22 µm PES diaphragma (TPP Techno Plastic Products AG, Trasadingen, Switzerland, 99722). Altogether, the antiviral potential of 29 cell-free supernatants originated from species listed in Table 1 was tested on the infectivity of MNV. MNV was incubated in a dilution ratio of 1:10 with the supernatant for 3 days at 24 °C (pH 6.2). The virus titre reductions of all experiments were compared to the control (native MRS-/brain heart broth with MNV). The pH value (Symp Hony SB 70P, VWR International) was controlled in all assays before and after 3 days of incubation.

Table 1 Overview of bacteria used for deriving cell-free supernatants

Typed reference strains		Strains isolated from different meat products		Commercial starter cultures	Isolates derived from Prof. Vogel, TU Weihenstephan, Munich	
Lb. sakei 1	DSMZ 6333	*Lb. sakei* 4	Sheepmeat	*Lb. sakei* 3	*Lb. sakei* 2	TMW 1.454 (LTH 673)
Lb. curvatus 4	DSMZ 20019T	*Lb. sakei* 5	"Teewurst" (raw sausage)	*Lb. plantarum* 1	*Lb. curvatus* 3	TMW 1.17 (LTH 1174)
S. carnosus 1	DSMZ 20501	*Lb. sakei* 6	Salami	*P. pentosaceus* 1		
S. xylosus 1	DSMZ 20266	*Lb. curvatus* 1	Air-dried raw sausage	*P. acidilactici* 2		
K. varians 1	DSMZ 20033	*Lb. curvatus* 2	Wild boar meat	*S. carnosus* 2		
		Lb. paracasei 1	Salami	*S. carnosus* 5		
		Lb. paracasei 2	Salami	*S. xylosus* 2		
		Lb. plantarum 2	"Teewurst" (raw sausage)	*S. xylosus* 3		
		P. pentosaceus 2	Salami	*K. varians* 2		
		P. acidilactici 1	"Zwiebelettwurst" (raw sausage)			
		S. carnosus 3	Black Forest ham			
		S. carnosus 4	Turkey rolled filet of ham			
		S. xylosus 4	Salami			

Lb., *Lactobacillus*; *P.*, *Pediococcus*; *S.*, *Staphylococcus*; *K.*, *Kocuria*

Because of the results derived from the screening of bacterial supernatants, where an effect of *Lb. curvatus* 1 for MNV was detected, further studies were carried out using different methods of treatments to identify possible antiviral substances. The supernatant was frozen (−21 °C, 1 h), heated (100 °C, 10 min) and treated with bovine liver catalase to exclude an inhibition due to hydrogen peroxide production. These experiments were repeated once. In addition, catalase treatment of the supernatant was performed four times. In three tests, 2 µl catalase/ml supernatant (equivalent to 68 U/ml, Sigma-Aldrich Chemie GmbH, Steinheim, Germany, C1345-1G) was used and in a fourth experiment the catalase volume per ml was doubled. Furthermore, the supernatant was separated according to different molecular weights. For this purpose VIVACON®500 concentrators with membrane cut offs by 2, 10, 30, 50 and 100 kDa (VWR International GmbH, Darmstadt, Germany, 518-0010/0011) were used. This experiment was performed twice. The virus titre reductions of all experiments were controlled regarding their significant differences compared to the control (native MRS broth with MNV) and the untreated cell-free supernatant of *Lb. curvatus* 1 (native supernatant) with MNV. For each test, the mean reduction of the virus titre was determined from each of five sample approaches.

Statistical Analysis

Statistical analyses were performed with IBM SPSS Statistics 20. All virus titres were $\log_{10}$ transformed and analysed by the Mann–Whitney *U* test. Bonferroni–Holm test was used for determination of significance of treated *Lb. curvatus* 1 supernatants (Holm 1979). All graphs were prepared with SigmaPlot 11.0.

Results and Discussion

Contrary to the results of Serkedjieva et al. (2000), Saeed et al. (2007) and Todorov et al. (2010), who demonstrated that bacteriocins can have an antiviral activity against influenza A virus (H7N7, H7N1), NDV and Herpes Simplex Virus-1, no antiviral effect could be detected by the tested bacteriocins in the present study. The bacteriocins sakacin A and nisin did not reduce the infectivity of MNV, H1N1, FHV and NDV. It can be seen in Table 2 that the mean virus titre reductions did not greatly differ from their controls. Different high bacteriocin activities (400–25.600 AU/ml) in the test series had no additional effect on the virus titre (data not shown). In all trials, the adjusted pH value (6.2) was stable before and after incubation. Thus, a statement regarding an effect on the viral envelope could not be made. Moreover, a direct transfer of these results to food is not possible and further studies should be carried out. In addition, bacteriocin-producing strains should be investigated in fermented sausages, because only the use of those is legal in Germany, while the application of bacteriocins is not allowed.

Table 2 Influence of bacteriocins on the mean virus titre reduction of MNV, H1N1, NDV, and FHV

	n	Mean reduction of virus titre [$\log_{10}$ $TCID_{50}$]			
		MNV	H1N1	NDV	FHV
Sakacin A	2	0.00	1.38	0.31	0.19
Control sakacin A	2	0.00	1.13	0.44	0.44
Nisin	2	0.56	1.25	0.31	0.56
Control nisin	2	0.00	1.13	0.44	0.44

n number of trails, $TCID_{50}$ tissue culture infectious dose 50, *MNV* murine norovirus, *H1N1* influenza A virus H1N1, *FHV* feline herpesvirus, *NDV* Newcastle disease virus, *control* PBS and virus

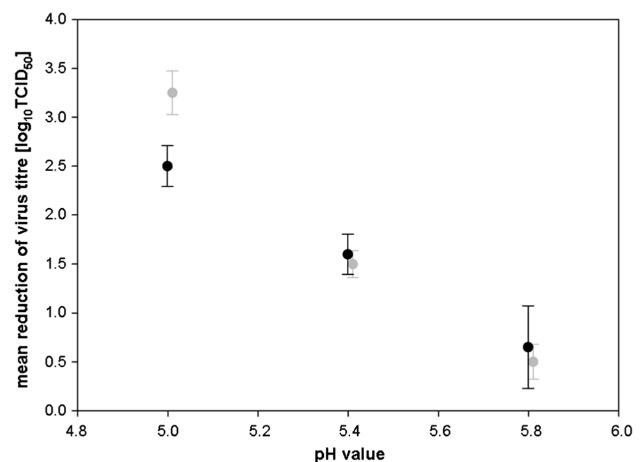

Fig. 2 Inactivation of MNV and H1N1 in acidified buffer (PBS/D,L-lactic acid), (The shown virus titre reductions were compared to the obtained values at pH 6.2. *Grey filled circle* MNV—mean reduction of virus titre ($n = 5$), *black filled circle* H1N1—mean reduction of virus titre ($n = 5$), *error bars* 2 × standard error of mean, $TCID_{50}$ tissue culture infectious dose 50)

Another important metabolite of LAB during the ripening of raw sausages is lactic acid (Kroeckel 1998), which can also be used as an additive. Therefore, the effect of D,L-lactic acid on MNV and H1N1 was examined. The mean reduction of virus titres in comparison to values obtained at pH 6.2 is shown in Fig. 2. The virus titre was reduced between pH values of 5.0 and 6.2 by a maximum of 3.25 log units (MNV) and 2.5 log units (H1N1), respectively. The pH values did not differ from each other immediately and after 3 days of the exposure. All samples without virus were assessed as not cytotoxic. Also Straube et al. (2010) could detect a virus inactivation of influenza A viruses (H3N8) with lactic acid. However, it is known that the inactivation of influenza by acid depends on the different subtypes (Scholtissek 1985). Therefore, a

direct transfer of the results to another subtype is limited. According to the results, it could be assumed that the pH decrease in fermented food like raw sausages could be an effective measure for risk reduction concerning viruses. An application of starter cultures with rapid and high acidification might improve the safety of raw sausages in relation to viral pathogens. However, investigations in fermented sausages with lactic acid should be carried out to confirm this.

However, the obtained results of our study concerning MNV S99 are contrary to those of Cannon et al. (2006). They determined virus titre reductions of less than 1 log unit at pH 2 only, by exposing MNV-1 in buffered solutions (citrate buffer, phosphate buffer and carbonate buffer) of certain pH values from 2 to 10 for up to 2 h. A reason for different results could be the use of different test systems for the detection of viral infectivity. While Cannon et al. (2006) used the plaque assay, in the present study the cytopathic effect was visually determined using the endpoint dilution assay. Another possible reason for contrary results could be the use of different virus strains. Therefore, a direct transfer of the results to another strain is not possible.

In addition to these individual factors, the antiviral effect of complex culture supernatants from starter and protective cultures (Table 1) was examined towards MNV. The screening did not indicate any antiviral activity by 28 out of 29 tested cell-free supernatants (single data not shown). Only the cell-free supernatant of *Lb. curvatus* 1 showed a higher virus titre reduction of the MNV by 1.25 log units in comparison to the control. This antiviral effect was confirmed by five trials. Furthermore, it was verified that the mean virus titre reduction of MNV differed significantly from the control ($p < 0.05$). This supposes a bacterial strain-specific production of antiviral metabolites (Botic et al. 2007). Further investigations were carried out for determination of the antiviral properties of the cell-free supernatant. No significant antiviral effect could be obtained anymore ($p > 0.05$) after heating, whereas freezing did not significantly influence the antiviral effect on MNV compared to the native cell-free supernatant ($p > 0.05$). Therefore it might be assumed that the antiviral substance is of proteinaceous nature. An effect by hydrogen peroxide, often produced by different LAB, can be excluded by catalase treatment, because it did not significantly influence the antiviral effect of the supernatant on MNV ($p > 0.05$) (Fig. 3). Moreover, the cell-free supernatant was separated according to different molecular weights. All collected fractions from 2 to 100 kDa exhibited the same significant antiviral effect ($p = 0.02$) like the entire supernatant. Virus titre reductions up to 2 log units were detected (single data not shown). It can be assumed that the potential antiviral substance of *Lb. curvatus* 1 has a molecular weight of less than or equal to 2 kDa.

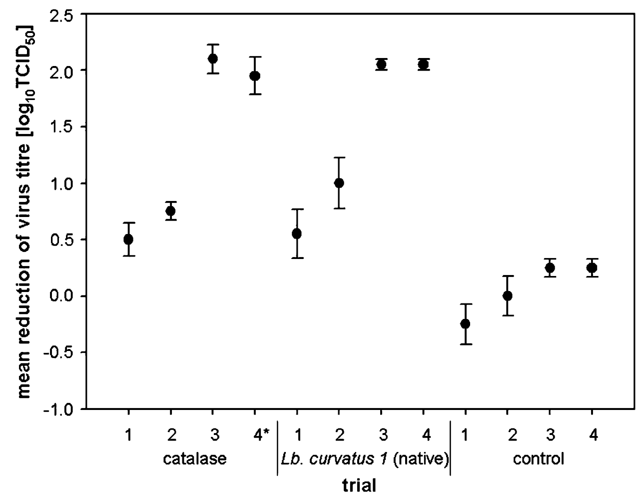

Fig. 3 Influence of catalase on the antiviral activity of the cell-free supernatant of *Lb. curvatus* 1 on MNV (*Lb.*: *Lactobacillus*, mean reduction of virus titre ($n = 5$); *error bars* 2 × standard error of mean; $TCID_{50}$: tissue culture infectious dose 50; catalase: *Lb. curvatus* 1 supernatant treated with catalase; asterisk: 4 µl catalase per ml of *Lb. curvatus* 1 supernatant (trials 1–3: 2 µl); *Lb. curvatus* 1 (native): untreated *Lb. curvatus* 1 supernatant; control: MRS broth)

Based on these results, no final conclusions on the antiviral substance can be drawn. The heat lability is indicative for a bacteriocin. However, in the literature heat-labile bacteriocins are described with a molecular weight >30 kDa (Klaenhammer 1993). Moreover, it is not clear if the antiviral effect is caused by a single component of this supernatant or by an interaction of different metabolites. Such synergistic effect of bacterial metabolites has already been proven several times for bacteria and fungi (Corsetti et al. 1998; Niku-Paavola et al. 1999). For example, the synergistic activity against moulds has been identified by a mixture of LAB-formed organic acids (Corsetti et al. 1998). Further studies should be carried out to identify the antiviral component of the cell-free culture supernatant, e.g., with HPLC and gas chromatography–mass spectrometry (GC–MS) methods. But despite of these methods, it seems to be very difficult to clean small molecules and to identify them (Niku-Paavola et al. 1999).

Moreover, the antiviral mode of action of *Lb. curvatus* 1 is unknown. Interactions of the supernatant with virus replication, cell adsorption or with the monolayer cell line are proposed. For example, Serkedjieva et al. (2000) assumed that the bacteriocin B1 from *Lb. delbrueckii* inhibits some of the intracellular specific steps in the viral reproduction.

Summing up, bacteriocins sakacin A and nisin are not a suitable alternative for virus inactivation. Moreover, lactic acid is important for virus inactivation and could improve the product safety regarding to viral pathogens. For this

purpose, further investigations have to be done. The MNV, which is described as stable in different studies, could be reduced in its infectivity by the cell-free supernatant of *Lb. curvatus* 1. The supernatant was characterized, however, the antiviral metabolite could not be identified. In further studies the bacterium or the supernatant should be also tested on human NoV in a variety of products or dosage forms. It might be possible that those results lead to a prevention of food-associated norovirus infections.

Acknowledgments This research project was supported by the FEI e.V. Bonn (Research Association of the German Food Industry), the German Federation of Industrial Research Associations (AiF), the Ministry of Economics and Technology and the Federal Association of the German Meat Industry (BVDF e.V.) AiF-Project No. 15189 BR. We thank Nadine Rossner and Mario Reinhardt for technical assistance as well as Dr. Kroeckel and Prof. Vogel for providing bacterial cultures.

References

Abee, T., Kroeckel, L., & Hill, C. (1995). Bacteriocins: Modes of action and potentials in food preservation and control of food poisoning. *International Journal of Food Microbiology, 28*(2), 169–185.

Albert, T., Große-Herrenthey, A., Lange-Starke, A., & Kroeckel, L. (2011). Identifizierung von Staphylokokken aus Rohschinkenaufschnitt. *Rundschau Fleischhygiene, Lebensmittelueberwachung., 63*, 387–390.

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

Bertrand, I., Schijven, J. F., Sánchez, G., Wyn-Jones, P., Ottoson, J., Morin, T., et al. (2012). The impact of temperature on the inactivation of enteric viruses in food and water: A review. *Journal of Applied Microbiology, 112*(6), 1059–1074.

Botic, T., Klingberg, T. D., Weingartl, H., & Cencic, A. (2007). A novel eukaryotic cell culture model to study antiviral activity of potential probiotic bacteria. *International Journal of Food Microbiology, 115*(2), 227–234.

Cannon, J. L., Papafragkou, E., Park, G. W., Osborne, J., Jaykus, L. A., & Vinje, J. (2006). Surrogates for the study of norovirus stability and inactivation in the environment: A comparison of murine norovirus and feline calicivirus. *Journal of Food Protection, 69*(11), 2761–2765.

Carter, M. J. (2005). Enterically infecting viruses: Pathogenicity, transmission and significance for food and waterborne infection. *Journal of Applied Microbiology, 98*(6), 1354–1380.

Cliver, D. O. (2010). Early days of food and environmental virology. *Food and Environmental Virology, 2*, 1–23.

Con, A. H., Gokalp, H. Y., & Kaya, M. (2001). Antagonistic effect on *Listeria monocytogenes* and *Listeria innocua* of a bacteriocin-like metabolite produced by lactic acid bacteria isolated from sucuk. *Meat Science, 59*(4), 437–441.

Corsetti, A., Gobbetti, M., Rossi, J., & Damiani, P. (1998). Antimould activity of sourdough lactic acid bacteria: Identification of a mixture of organic acids produced by *Lactobacillus sanfrancisco CB1*. *Applied Microbiology and Biotechnology, 50*(2), 253–256.

Hammes, W. P., & Knauf, H. J. (1994). Starters in the processing of meat-products. *Meat Science, 36*(1–2), 155–168.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics, 6*(2), 65–70.

Horzinek, M. C. (1985). Das Virus als Partikel. In M. C. Horzinek (Ed.), *Kompendium der allgemeinen Virologie* (pp. 13–75). Berlin: Paul Parey.

Ivanova, I., Miteva, V., Stefanova, T., Pantev, A., Budakov, I., & Danova, S. (1998). Characterization of a bacteriocin produced by *Streptococcus thermophilus 81*. *International Journal of Food Microbiology, 42*(3), 147–158.

Jay, J. M. (1982a). Antimicrobial properties of diacetyl. *Applied and Environmental Microbiology, 44*(3), 525–532.

Jay, J. M. (1982b). Effect of diacetyl on foodborne microorganisms. *Journal of Food Science, 47*(6), 1829–1831.

Klaenhammer, T. R. (1993). Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiology Reviews, 12*(1–3), 39–86.

Kroeckel, L. (1998). Biokonservierung von Fleischerzeugnissen. *Forschungsreport, 2*(1998), 26–29.

Luecke, F.-K., & Hechelmann, H. (1985). Starterkulturen fuer Rohwurst und Rohschinken- Zusammensetzung und Wirkung. In *Mikrobiologie und Qualitaet von Rohwurst und Rohschinken*. Institut fuer Mikrobiologie, Toxikologie und Histologie der Bundesanstalt fuer Fleischforschung (pp. 193–218). Kulmbach.

Niku-Paavola, M. L., Laitila, A., Mattila-Sandholm, T., & Haikara, A. (1999). New types of antimicrobial compounds produced by *Lactobacillus plantarum*. *Journal of Applied Microbiology, 86*(1), 29–35.

Robert Koch-Institut (RKI) (2013). Aktuelle Statistik meldepflichtiger Infektionskrankheiten, Deutschland, 52. Woche 2012 (Datenstand: 16.01.2013). *Epidemiologisches Bulletin*, 3/2013, 28–30.

Saeed, S., Rasool, S. A., Ahmad, S., Zaidi, S. 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*(11), 796–806.

Sarvikivi, E., Roivainen, M., Maunula, L., Niskanen, T., Korhonen, T., Lappalainen, M., et al. (2012). Multiple norovirus outbreaks linked to imported frozen raspberries. *Epidemiologie and Infection, 140*(2), 260–267.

Schillinger, U., & Luecke, F. K. (1989). Antibacterial activity of *Lactobacillus sake* isolated from meat. *Applied and Environmental Microbiology, 55*(8), 1901–1906.

Scholtissek, C. (1985). Stability of infectious influenza-A viruses to treatment at low pH and heating. *Archives of Virology, 85*(1–2), 1–11.

Serkedjieva, J., Danova, S., & Ivanova, I. (2000). Antiinfluenza virus activity of a bacteriocin produced by *Lactobacillus delbrueckii*. *Applied Biochemistry and Biotechnology, 88*(1–3), 285–298.

Smith, A. J., McCarthy, N., Saldana, L., Ihekweazu, C., McPhedran, K., Adak, G. K., et al. (2012). A large foodborne outbreak of norovirus in diners at a restaurant in England between January and February 2009. *Epidemiology and Infection, 140*(9), 1695–1701.

Straube, J., Manteufel, J., Heinze, J., Fehlhaber, K., Truyen, U., & Albert, T. (2010). Low pathogenic avian influenza viruses (H3N8, H5N6): In vitro influence of D, L-lactic acid and sodium chloride on infectivity and virus persistence in short fermented raw poultry sausage. *Food and Environmental Virology, 2*(2), 74–82.

Todorov, S. D., Wachsman, M., Tome, E., Dousset, X., Destro, M. T., Dicks, L. M. T., et al. (2010). Characterisation of an antiviral pediocin-like bacteriocin produced by *Enterococcus faecium*. *Food Microbiology, 27*(7), 869–879.

Wichmann, O., Schimanski, S., Koch, J., Kohler, M., Rothe, C., Plentz, A., et al. (2008). Phylogenetic and case–control study on hepatitis E virus infection in Germany. *Journal of Infectious Diseases, 198*(12), 1732–1741.