THE USE OF *LACTOBACILLUS PARACASEI* AS A STARTER CULTURE WITH PROBIOTIC ACTIVITY

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Background

Food containing probiotic bacteria are well established on the market. Most are fermented dairy products but some fermented sausages had also been offered. We questioned the usefulness (Hammes and Haller, 1998) of application of probiotics in meat products, as virtually nothing is known about the survival of the probiotic bacteria along the way from the starter and the sausages to the large bowel. Especially, the adaptation to the ecological conditions prevailing in fermented sausages may cause problems with lactic acid bacteria that were originally isolated from the human intestinal tract. Poor survival of the bacteria or loss of metabolic fitness in the intestines may argue against their application connected with a defined health claim.

Objective

It was the objective of our studies to investigate the performance of a selected lactic starter culture in sausages and to describe the survival of the bacteria in human volunteers.

Methods

Lactobacillus paracasei LTH 2579 used in this study was previously isolated from a fermenting apple mash (Hammes et al., 1997). Fermented sausages were produced as described previously (Bunte et al., 2000). Starter organisms were employed to obtain final cell counts (CFU/g) in the meat mixture of 1.0×10^6 of *L. sakei* LTH 681, 8.0×10^6 of *Staphylococcus carnosus* LTH 3127, 1.5×10^6 of *Kocuria varians* LTH 2864, and 2.0×10^7 of *L. paracasei* LTH 2579. Control sausages were produced without employing strain LTH 2579. For microbial analysis 10 g of sausage were homogenised and the microbial counts were determined by surface plating.

The experimental group in this study consisted of 19 healthy volunteers. The study took place over a time of 83 days and was divided in three periods: (I) pre-period of 37 days, (II) administration period of 37 days, and (III) wash-out period of 7 days. During period I and II the volunteers consumed per day 50 g of the control sausages and sausages with strain LTH 2579, respectively. During the whole study the diet was of free choice except for the last 9 days of period I and II, in which the diet was individually assembled according to the volunteers eating habits during the first 4 weeks of the corresponding period. Faecal samples were taken at days 30 and 37 of periods I and II (period I, sample no. V3 and V9; period II, sample no. Z3 and Z9), and at days 2, 4, and 6 of period III (sample no. N2, N4, N6).

Faecal samples were taken and treated as described previously (Bunte et al., 2000). Species-specific PCR reactions for *L. paracasei* and strain-specific PCR reactions for *L. paracasei* LTH 2579 were carried out as described previously (Bunte et al., 2000) using crude nucleic acid extracts.

Results and discussion

The starter strain *L. paracasei* LTH 2579 had been isolated from strongly acidic fruit mash (pH ca. 2.0). This strain fulfils the precondition for survival of the intestinal passage tolerating the low pH prevailing in the stomach. The strain was also tolerant to bile (Hammes et al., 1997) and was performing well as a starter culture (Hammes et al., 1990), i.e. growing in the fermenting sausages to numbers $> 3 \times 10^8$ CFU/g and lowering efficiently the pH to technologically needed limits. The flavour was typical for fermented sausages with a characteristic pleasant note. In combination with established starter cultures the strain grew, maintained numbers of $> 10^8$ CFU/g and contributed to a characteristic flavour.

In a clinical study it was the aim to determine the survival of *L. paracasei* LTH 2579 in volunteers consuming fermented spreadable sausages. In addition, a correlation of that survival with probiotic effects derived from determining relevant biomarkers were studied. These studies were performed at the University of Jena (the co-operation with G. Jahreis was highly appreciated). These results will be published elsewhere. In our part we focused exclusively to the aspects of food technology and food microbiology.

The individual total cell counts of lactobacilli in the faeces of each volunteer were determined in period I. To determine the background of lactobacilli in the faeces of volunteers eating fermented sausages, 50 g of control sausages (without strain LTH 2579) were consumed per day. Lactobacilli were detected in faecal samples V3 and V9 of most of the volunteers. The cell counts of lactobacilli ranged from the detection level to 10^4 CFU/g faeces (2 volunteers), around 10^4 to 10^6 CFU/g faeces (9 volunteers), and 10^6 up to nearly 10^8 CFU/g faeces (8 volunteers). For 7 volunteers the cell counts of samples V3 and V9 varied in ranges of greater than one order of magnitude.

To prove the specificity of the PCR system for strain *L. paracasei* LTH 2579 within this study, the cells of 60 randomly picked colonies (3 of each volunteer) were subjected to the isolation of crude nucleic acid extracts. No PCR products were obtained using the nucleic acids as template. On the other hand, application of the species-specific PCR system revealed that 5 colonies consisted of cells of the species *L. paracasei*.

During period II the volunteers consumed per day 50 g of fermented sausages produced with strain *L. paracasei* LTH 2579. This consumption corresponds to an uptake of ca. 10^9 CFU of that test strain per day. After 4 weeks of consumption the cell counts of lactobacilli and strain *L. paracasei* LTH 2579 were determined by applying the culture technique in combination with the specific PCR system. Strain *L. paracasei* LTH 2579 was not detected in two volunteers and was present in 28 of 34 faecal samples of 17 volunteers. The cell counts ranged from 2.5×10^4 to 2.4×10^7 CFU/g faeces. The strain was detectable in faecal samples Z3 and Z9 of 11 volunteers and either in sample Z3 or Z9 of the remaining 6 volunteers. Taking into consideration the cell counts of background lactobacilli, *L. paracasei* LTH 2579 constituted a share of 13% to 100% of the total lactobacilli flora, with a share of > 90% in 14 faecal samples. In addition, no correlation was observed between the cell counts of strain LTH 2579 and those of the background lactobacilli.

To get insight into the persistence of *L. paracasei* LTH 2579 in the intestine, the volunteers did not consume any sausages either with or without *L. paracasei* LTH 2579 during period III. Investigation of the faecal samples taken after 2 (N2), 4 (N4), and 6 (N6) days revealed that the persistence of *L. paracasei* LTH 2579 did not correlate with the cell counts in the administration period. Two days after administration of the sausages (sample N2) the strain was detected in the faeces of 6 volunteers only at counts ranging from 8.2×10^3 to 2.2×10^7 CFU/g faeces. Remarkably, the strain was detectable throughout period III in the faecal samples of one volunteer (no. 9) only with decreasing levels from N2 to N6.

Conclusions

Our results show that lactobacilli isolated from food can be used as starter cultures and participate in and survive the fermentation process as well as the passage through the intestinal tract. The individual human volunteers exhibit a great variation in their quality as hosts for the lactobacilli which observation is consistent with results obtained in studies with probiotic bacteria of intestinal origin. Thus, non-human isolates exhibit survival and persistence characteristics in volunteers that are comparable with human isolates.

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