EXPRESSION OF THE CATALASE GENE OF *LACTOBACILLUS SAKEI* IN *L. CURVATUS* IMPROVES THE SENSORY QUALITY OF FERMENTED SAUSAGES

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Background

Malfermentation may occur in fermented sausages when ecological factors and technological conditions are unfavourable during the fermentation process. For example, in presence of oxygen hydrogen peroxide (H_2O_2) may be formed by lactic acid bacteria (LAB). This strongly oxidizing compound may accumulate and exert undesired effects in the products such as colour defects and rancidity (Rozier, 1971). Numerous species of LAB possess peroxidase and/or catalase to prevent these deleterious effects (Engesser and Hammes, 1994). Another group, the true catalases, are active upon addition of hematin. The second group are so called non-heme, pseudo-, or manganese catalases which are found in only a few species, e.g. *Lactobacillus plantarum* (Igarashi et al., 1996).

Lactobacillus curvatus and *L. sakei* are the most prevalent organisms in meat fermentations. In contrast to *L. curvatus*, *L. sakei* possesses a heme dependent catalase, which can function in meat products as these substrates contain abundant heme sources. The gene *katA* of *Lactobacillus sakei* LTH 677 was already cloned and characterized (Knauf et al., 1992). It is a potential candidate to improve meat starter strains, such as *L. curvatus*, by endowing the organisms with the property to produce catalase. This manipulation has the potential to simplify starter preparations as there is no longer a need to combine *L. curvatus* with catalase positive species such as *Staphylococcus carnosus* or *Kocuria varians* (Hammes and Hertel, 1998).

Objective

It was the objective of our studies to investigate the potential of the catalase KatA to prevent the deleterious effects of H_2O_2 on the sensory quality of fermented sausages. For this purpose, the gene *katA* of *L. sakei* LTH 677 was expressed in the catalase-deficient strain *L. curvatus* LTH1432 and the resulting recombinant strain LTH 5292 was used as starter organism in sausage fermentation.

Methods

Lactobacillus curvatus LTH 1432 is a plasmid-cured derivative of the catalase-deficient strain LTH 683, a component of a starter preparation used for sausage fermentation, and was used as host. For expression of the catalase gene *katA* of *L. sakei* LTH 677 (Knauf et al., 1992), *katA* without promoter was amplified and the generated PCR fragment was introduced into the shuttle-vector pLPV111 (Axelsson and Holck, 1995). The promoter of the *ptsHI* operon of *L. sakei* (Stentz et al., 1997) was transcriptionally fused to the promoterless *katA* resulting in plasmid pCB100. This plasmid was introduced into *L. curvatus* LTH 1432 resulting in strain *L. curvatus* LTH 5292. The construction was confirmed by sequencing. A quantitative analysis of the catalase activities in intact cells of lactobacilli was performed as described previously (Hertel et al., 1998).

Fermented sausages were produced as described previously (Cavadini et al., 1998) with the following modification: After mixing of the frozen meat and back fat glucose (3 g), curing salt (28 g), and pepper (2.0 g) were added. Starter organisms were employed to obtain final cell counts (CFU/g) in the meat mixture of 7.5×10^6 of *L. sakei* LTH 947 (catalase-positive), 1.1×10^7 *L. sakei* LTH 677 ($KatA^+$), 3.7×10^6 of *L. curvatus* LTH 673 ($KatA^-$), 1.2×10^7 of *L. curvatus* LTH 5292 ($KatA^+$), or *L. curvatus* LTH 4002 ($KatA^+$). Neither staphylococci nor micrococci (*Kocuria varians*) were added. For microbial analysis 10 g of sausage were homogenised and the microbial counts were determined by surface plating. The brightness (L* value), the red value (a* value), and the yellow value (b* value) of the fermented sausages were determined using the chroma meter CR-200 (Minolta).

Results and discussion

Recently, it was shown that the expression of the catalase gene *katA* of *L. sakei* LTH 677 is regulated by oxygen and H_2O_2, respectively (Hertel et al., 1998). This regulation took also place in *L. curvatus* LTH 1432 as demonstrated with the recombinant strain *L. curvatus* LTH 4002 containing *katA* under the control of its own promoter. To ensure constant expression of *katA* in *L. curvatus* LTH 1432 during sausage fermentation, the strong and constitutive promoter of the *ptsHI* operon of *L. sakei* was fused to promoterless *katA*. The resulting recombinant strain LTH 5292 exhibited a catalase activity of ca. 72 µg of O_2 per min per 10^8 CFU, a activity which was comparable to that of the strain LTH4002 under inducing (aerobic) conditions (ca. 67 µg of O_2 per min per 10^8 CFU). However in contrast to LTH4002, KatA of strain LTH 5292 was also expressed under non-inducing (anaerobic) conditions.

High catalase activity was found for the gene donor strain *L. sakei* LTH 677 (ca. 100 µg of O_2 per min per 10^8 CFU) as well as *L. sakei* LTH 947 (ca. 183 µg of O_2 per min per 10^8 CFU) which is a component of an industrial used starter preparation.

Fermented sausages were produced employing the catalase-positive wild-type strains *L. sakei* LTH 947 and LTH 677, the $KatA^+$ recombinant strains *L. curvatus* LTH 5292 and LTH4002, and the $KatA^-$ host *L. curvatus* LTH 673 as a starter. After 4 days of fermentation the lactobacilli reached a maximum in cell counts of > 5 x 10^8 CFU/g and caused a drop in pH to 5.2. During the fermentation the development of the colour on the surface and in the inside of the sausages was monitored. With regard to the inside of the sausages no difference in the values was obtained. However, differences were found for the L* and a* values on the surface of the sausages. The a* value of the sausages reached a maximum after 3 days of fermentation and thereafter decreased continuously. At the end of the fermentation the a* values of the sausages produced with the recombinant strains LTH 5292 and LTH 4002 (7.8 ± 0.5 and 7.3 ± 0.5, respectively) were significantly increased compared to that of the control sausage with strain LTH673 (5.5 ± 0.8). However, sausages produced with *L. sakei* LTH 947 and LTH 677 showed the highest values (11.7 ± 0.5 and 9.9 ± 0.6, respectively).

Conclusions

The experiments unambiguously reveal the indispensable role of catalase activity in starter organisms. The catalase gene *katA* of *L. sakei* was successfully expressed in the catalase-deficient meat starter organism *L. curvatus*. The use of the recombinant starter strain LTH 5292 permitted to prevent discoloration during sausage fermentation. Thus, this gene is a potential candidate to improve catalase-deficient meat starter strains by endowing the organisms with the property to produce catalase.

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