H_2S PRODUCTION BY STRAINS OF *LACTOBACILLUS SAKE*: GROWTH CONDITIONS AND PLASMID CONTENT

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Key words: hydrogen sulphide, lactic acid bacteria, plasmids

Background

Lactic acid bacteria are the dominating flora on vacuum- and gas-packaged meat, and their low spoilage potential explains the long shelf life attained under such conditions. Because of their ability to outcompete other bacteria there also is increasing interest in using them as protective cultures in meat and meat products. There are, however, reports of spoilage caused by lactic acid bacteria in the form of slime production, discolouration or objectionable odours (Egan et al., 1989; Borch and Agerhem 1992). It is, therefore, important to know more about the spoilage potential of those organisms and the conditions under which they are expressed. In 1981, Shay and Egan reported spoilage of vacuum-packed meat caused by a strain of *Lactobacillus sake* (L13) which produced hydrogen sulphide. Shay et al. (1988) showed H_2S production to be associated with the organisms ability to use cysteine for growth in a mineral/yeast extract medium . This ability was unstable, being lost either spontaneously or after applying curing procedures, probably due to the loss of a plasmid.

Objectives

In the present study we have investigated (1) H_2S production in media and on meat of *L. sake* strains isolated from meat and meat products (2) if the production was plasmid dependent.

Material and methods

***L. sake* strains tested:**, NFCB 2714, 210-230 from gas packed bologna, 120, 132, 134, 138, 140, 147, 151and 159 from gas packed pork (all isolated at MATFORSK), 706 and 790, from meat and meat products (obtained from Federal Centre for Meat Research in Kulmbach, Germany)

Medium: A mineral medium (M56) with the addition of yeast extract (0.3 %), Tween-80 (0.1 %), $MnSO_4$ (0.004 %) and cysteine hydrocloride (0.1 %) as described by Shay et al. (1988) was used for H_2S production.The medium was filled in Hungate tubes and inoculated with the bacterial strains.

Detection of H_2S: Samples (0.1 - 0.2 ml) from the headspace in the tubes were injected directly into 30 x 0.53 mm i.d. packed capillary column og GSQ (J&W Scientific), in the oven of a gas chromatograph fitted with a flame photometric detector.

Storage of vacuum packed pork: Sliced pork (1-2 cm) was vacuum packed (Super 7-E with O_2 transmission rate of 8 $cm^3/m^2/atm$), inoculated with *L. sake* strains and stored at 4 °C for 3 and 6 weeks. 10 ml. of N_2 was injected in the packages 30 min. before samples for headspace analysis were taken.

Results and discussion

Gas chromatography of headspace samples from fully grown cultures gave a peak that was identified as H_2S (Figure1c) for 29 of the 30 strains of *L. sake* grown under strictly anaerobic conditions. Leakage of air into poorly sealed tubes did not affect the growth of the bacteria, but it did stop or reduce the production of H_2S. Only strain 706 never produced any measurable amounts. Tubes giving an H_2S peak always smelled of the gas. In headspace samples containing high levels of H_2S an unidentified peak with a retention time of about 10 minutes was also detected (Figure 1b). The production of H_2S was dependent on the addition of cysteine, but addition of 2.5 mM glucose had no effect and was not included in the medium.

To find out if the strains tested in the cysteine medium also produced H_2S on meat, some of the strains were inoculated onto vacuum-packed pork. The meat had a normal pH (5.6-5.8) and was packaged in a film of low permeability. Figure 1 c and d show that both strain 2714 and 225 produced H_2S when growing on the meat while strain 706 only produced a negligible amount. In addition to H_2S, a peak with a retention time of about 8 minutes (probably dimethyl-sulphide, Figure 1a) and an unknown peak with the same retention time as the unknown compound produced in liquid medium. No sulphmyoglobin was detected in these packages, in accordance with findings of Egan et al. (1988) whith packaging film of low permeability. Two of the uninoculated meat samples also gave negligible sulfide production (Figure 1e), but the third (Figure 1f) gave large amounts and also sulphmyoglobin production. Microbial analysis showed that this piece of meat had been contaminated by *Hafnia alvei* which under these conditions had a higher spoilage potential than the lactic acid bacteria.

Shay et al. (1988) found in their *L. sake* strain (L13) a connection between the ability to produce H_2S and the possession of two plasmids of 8.3 and 2.7 kb. Of the strains isolated at MATFORSK numbers 120, 225, 217 and 159 each had 3-4 plasmids (Figure 2) but none were as small as the ones described by Shay et al. (1988). Curing failed to eliminate any of the plasmids in strains 120 and 225 and reduced plasmid numbers from 4 to 3 in strain 217 and from 3 to 1 in strain 159. Loss of the plasmid(s) in the two strains was not associated with loss of H_2S production. Parent and cured strains of 706 and 790 were also tested. Neither parent or cured strains of 706 produced H_2S while both parent and cured strains of 790 produced equal amounts of H_2S. Taken together these results would suggest that H_2S production in different strains of *L. sake* is not necessarily dependent on plasmid content.

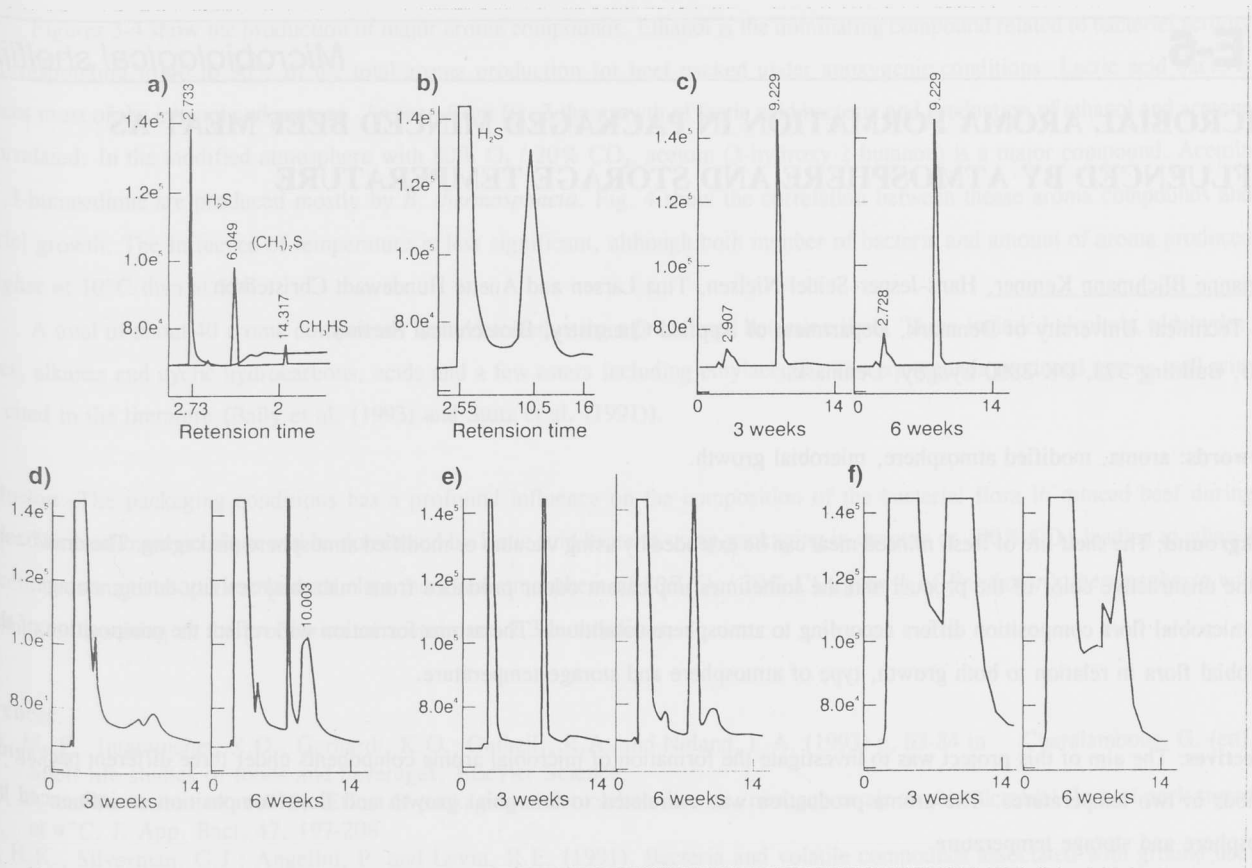

Figure 1 Gas chromatography of **a)** Reference gases: H_2S and $(CH_3)_2S$, **b)** Head space of *L. sake* 225 grown in broth. **c-f)** Head space of inoculated and controls of vacuum-packed pork, **c)** *L. sake* 706, **d)** *L. sake* 225, *L. sake* 2714, f. uninoculated

Conclusion

All the 30 strains of *L. sake* produced sulphide in cysteine-medium under anaerobic conditions, except one (706). The ability of the strains to produce H_2S was the same in medium and in packaged meat. In the strains we tested, the production seemed to be independent of the plasmid content.

Acknowledgement

We thank Anne Gulliksen and Maria Sarmiento Kreutz for able technical assistance.

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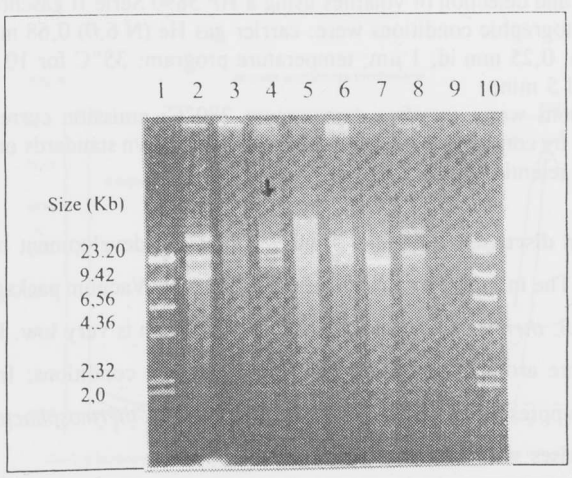

Figure 2 Plasmid profiles of different *L. sake* strains, obtained after agarose gel electrophoresis. Lane 1 and 10: hind 3, 2: 225, 3: 120, 4: 217, 5: 217 E (after curing), 6: 159, 7: 159E (after curing), 8: 706, 9: 706E (after curing)