Fermented Sausage as a Contamination Source of Ropy Slime-Producing Lactic Acid Bacteria

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ABSTRACT

The ability of four ropy slime-producing lactic acid bacteria strains to multiply during the manufacture of Finnish fermented sausages was studied. Two of the three lactobacilli strains studied were able to compete with the starter bacteria used, and these lactobacilli occurred in high levels in the sausages at the end of manufacture. The Leuconostoc strain studied was found only once during the manufacture. Since fermented sausages in Finland are commonly handled in the same rooms as cooked meat products, the fermented sausages form a potential source of the ropy slime-producing lactobacilli for cooked products.

Formation of ropy slime on vacuum-packed cooked meat products has been a common spoilage problem in Finland for some years. This phenomenon has been shown to be caused by lactic acid bacteria (7). Korkeala et al. (7) and Mäkelä and Korkeala (8) isolated from meat and meat products three different lactobacilli groups and one Leuconostoc group capable of producing ropy slime. The general properties of these ropy slime-producing lactic acid bacterial groups resemble those of other lactic acid bacteria isolated from meat and meat products by other workers (7).

Lactic acid bacteria are usually the predominant component of the microbial population in fermented sausages. At Finnish meat processing plants, cooked meat products and fermented sausages are commonly handled in the same rooms. Fermented sausages thus form a possible source of lactic acid bacteria for cooked meat products. Traditional manufacturing methods of fermented sausages rely on natural fermentation, during which lactic acid bacteria proliferate rapidly and dominate the microbial flora (1,10). Lactic acid bacteria, usually species from the genera Pediococcus and Lactobacillus, are also used as starter cultures in the manufacturing of fermented sausages (4,15). By using starter cultures, it is possible to influence the maturation time, aroma and consistency of the sausage; the addition of large numbers of starter organisms also inhibits the growth of spoilage organisms and foodborne pathogenic microorganisms (2,9,12,15). In Finland, fermented sausages are manufactured using commercial starter cultures.

The purpose of this work was to study the ability of ropy slime-producing lactic acid bacteria to multiply during the manufacture of Finnish fermented sausages, and to evaluate whether fermented sausages can form a contamination source of these bacteria affecting other meat products.

MATERIALS AND METHODS

Bacterial strains

Four different ropy slime-producing lactic acid bacteria strains were used. All these strains were able to cause ropiness on vacuum-packed cooked meat products. Homofermentative lactobacilli strains A210 and Cl and a Leuconostoc-strain D1 were isolated from ropy meat products by Korkeala et al. (7). Strain A210 belonged to Group 1, Strain Cl to Group 2 and Strain D1 to Group 3 of Korkeala et al. (7). Strain R51 was a homofermentative Lactobacillus isolated from pork meat by Mäkelä and Korkeala (8). For the inoculations, the bacteria were grown overnight in MRB broth (Difco, Detroit, MI) at 20°C, and the culture was diluted in 0.1% (v/w) peptone water.

Starter culture

A commercial mixed culture of Staphylococcus carnosus and Lactobacillus pentosus (Chr. Hansen's Laboratorium A/S, Horsholm, Denmark) was used. The freeze-dried cultures were diluted in 0.1% peptone water and inoculated into sausage mass to obtain final concentrations of 10^7 CFU/g of both organisms.

Preparation of fermented sausage

The sausages were prepared using normal commercial production method. The sausage had the following composition: beef 56%, pork 40%, salt 3%, spices 0.02%, glucose 0.6%, and sodium nitrite 120 ppm. The meat was ground using a cutter (Saydelman, Stuttgart, FRG), in which salt, sugar, spices, nitrite, and the starter culture were added during rotation. In each trial the sausage mass was divided into five parts, each 1.5 kg in weight. The batches were transferred to a mixer (Hugo Kunzi, Stuttgart, FRG) where the inoculations were made. Each ropy slime-producing bacterial strain was added to two batches to provide final concentrations of 10^7 and 10^8 CFU/g. An uninoculated batch served as control. In the first two trials the inoculation was made with Strains A210 and Cl, and in the 3rd and 4th trials Strains DI and R51 were used. The sausage masses were stuffed into fibrous casings with a diameter of 7 cm (Visko, Hanko, Finland).

The sausages, each weighing about 400 g, were incubated in the Autotherm (Waxweiler, FRG) for 2 d at 23°C and 95% relative humidity, then the sausages were smoked for 5 d at 20-22°C and the humidity was reduced to 80%. After smoking, the
sauces were kept at 18°C and 75-80% humidity for 1 week, followed by 10°C and 50% humidity for another week to com-
plete the 21 d manufacture.

Sausage sampling and isolation of ropy slime-producing bacteria

One sausage of each type was sampled on the 3rd, 7th, 14th,
and 21st d of manufacture. A 10-g cross-sectional sausage sample
was homogenized with 90 ml of 0.1% peptone water. and then
diluted 10-fold in peptone water and plated onto APT agar (Difco)
for the aerobic plate count. The APT plates were incubated
aerobically at 20°C for 5 d. For the isolation of ropy slime-
producing bacterial strains, the following culture methods were
used: Rogosa SL agar (Orion Diagnostica, Espoo, Fin-
land) and MRS agar (Oxoid, Basingstoke, England) at 15°C for Strains
A210 and Cl, and Rogosa SL agar at 15°C and MRS-S plates at
20°C for Strains DI and R51. The methods were chosen on the
basis of the test of the ability of the strains to form ropy colonies
in different culture methods (unpublished data). The MRS-S agar
was prepared according to Korkeala and Lindroth (6). All these
plates were incubated anaerobically for 5 d in an anaerobic jar
using a H₂+C₂CO₃ gas generating kit (Oxoid). All separate colonies
on these plates were tested for their ability to rope with a straight
wire. If ropy colonies were detected, 1 to 5 viscous colonies from
each plate were isolated. The ropy isolates were examined for
Gram reaction and catalase production. The fermentation of glu-
cose, lactose, maltose, D-mannitol, salicin, D-sorbitol, and raffin-
one were tested according to Kandler and Weiss (5) and Sharpe
(13, 14). The method of Gibson and Abdel-Malek (3) was used to
test the ability to produce gas from glucose. Two isolates from
each sample were further confirmed using the API 50 CHL
system (API System, La Balme les Grottes, France).

pH and a_w measurements

A 10-g portion of each sausage sample was mixed with 10
ml of distilled water and the pH was determined using a pH meter.
The a_w measurements were carried out according to the method of the
Nordic Committee on Food Analysis (11) using a hygrometer
(Luft GmbH, Stuttgart, FRG).

RESULTS

The aerobic plate counts and numbers of ropy colonies
in the fermented sausages are presented in Tables 1 and 2.
The aerobic plate counts determined on APT agar were at
the same level in both the uninoculated control sausages
and the sausages inoculated with different ropy slime-
producing bacterial strains. Ropy colonies were detected
from the sausages inoculated with Strains A210 and R51
during the whole 21 d manufacture at both levels of inoculation
(10⁵ and 10⁶ CFU/g), and the colonies formed
m a w

a proportion of the total microbial population. Ropy
colonies were detected less regularly from the sausages
inoculated with Strain R51 than those inoculated with Strain
A210. Ropy colonies were found once in the sausages
inoculated with strain Cl at the beginning of manufacture
(Table 2). No ropy colonies were detected in the
uninoculated control sausages and in sausages inoculated
with strain Cl (Table 1 and 2).

The numbers of ropy colonies on MRS and Rogosa SL
agar did not differ greatly from the samples of sausages
inoculated with Strain A210, while all the ropy colonies
from the sausage inoculated with Strain DI were found on
MRS-S agar plates. In the case of the sausages inoculated
with Strain R51, more ropy colonies were found on Rogosa
SL agar plates than on MRS-S plates.

A total of 126 isolates able to form ropy colonies was
recovered from sausages inoculated with Strain A210. All
these isolates were similar to Strain A210 in the tests
performed. The six isolates from the sausage inoculated
with Strain DI were identified as Strain DI. Of the 52 ropy
isolates from sausages inoculated with Strain R51, 50 iso-
lates were similar to Strain R51, while two isolates from a
sample on the 7th d of manufacture gave a deviant identi-
fication with the API 50 CHL system. Isolates similar to
R51 were also found in this sample.

During the process of fermentation, the pH and a_w
values of the sausages inoculated with ropy slime-produc-
ing bacteria did not differ greatly from the control sausages.
The pH values of the sausages varied on the 3rd d of
manufacture from 5.7 to 5.5, on the 7th d from 5.0 to 5.1
and on the 21st d from 4.6 to 4.9. On the 21st d of
manufacture the a_w values were 0.88 ± 0.01.

No color or consistency faults were detected in the
fermented sausages inoculated with ropy slime-producing
lactic acid bacteria, nor was any slime observed in the
sausages with a high level of the slime-producing bacteria.

DISCUSSION

Two of the three ropy slime-producing lactobacilli strains
studied were able to compete successfully with
starter bacteria in the fermented sausage. Both strains
multiplied from the inoculation level of 10⁵ CFU/g to a
level of 10⁷ CFU/g during the fermentation process. The
bacteria also survived ripening, being found at high levels
at the end of the manufacturing period. The Leuconostoc
strain studied was found only once during fermentation.
These results suggest that ropy slime-producing lactobacilli
strains can occur at high levels in Finnish fermented sau-
sages, if these bacteria are present in the raw materials.
Some ropy slime-producing lactobacilli have been isolated
from pork meat (8), indicating that in practice the meat
used in the manufacturing of the sausages can contain these
bacteria.

The high proportion of ropy slime-producing lactoba-
cilli strains in the fermented sausages seemed not to have
any negative effect on the maturation or the color and
consistency of the sausages. Furthermore, no slime
was detected, although the sausages contained high levels of the
bacteria. This may be due to the low a_w of fermented
sausages; the slime may need some free water to be
detectable.

The method of testing the ropiness of colonies is not
fully reliable in detecting ropy slime-producing bacteria,
since the proportion of ropy colonies formed can vary
greatly according to the culture method, the test time, and
the bacterial strain (unpublished data). Strain R51 proved to
be an especially weak former of ropy colonies, which may
be a reason for the irregular detection of the strain from the
sausages in this work. The methods used in detecting
Strains A210, Cl, and DI were evaluated as best for these
bacteria, and the strains should have been well detected.
The detection limit of the method used was high, about 10⁵-
TABLE 1. Aerobic plate counts and numbers of ropy colonies during 21 d manufacture of Finnish fermented sausages inoculated with ropy slime-producing lactobacilli strains A210 and C1.

<table>
<thead>
<tr>
<th>Day of manufacture</th>
<th>Control</th>
<th>10^6 CFU/g</th>
<th>10^7 CFU/g</th>
<th>10^8 CFU/g</th>
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<td></td>
<td></td>
<td>APC</td>
<td>Ropy</td>
<td>APC</td>
<td>Ropy</td>
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<td>ND</td>
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<td>7.9</td>
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<td>ND</td>
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<td>21st d</td>
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<td>ND</td>
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</tr>
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<th>Day of manufacture</th>
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10^6 CFU/g in the present study, because only separate colonies on the plates can be tested. Thus, the presence of small numbers of the bacteria could not be detected.

In Finland, fermented sausages are commonly stored, sliced, and packed in the same rooms as the cooked whole-meat products and sausages; at some plants the same slicing machines are even used. This may be one reason for the occurrence of the ropiness problem in Finnish cooked meat products. The ropy slime-producing bacteria can be transported from fermented sausages via equipment and personnel to cooked products; or the bacteria may also spread to the air and the rooms of the plant, whence they can further contaminate the products. To avoid contamination with these ropy bacteria, fermented sausages should be stored and handled in totally separate rooms from cooked meat products.

Four ropy slime-producing lactobacilli strains, including Strains A210, C1 and R51, have been identified as Lactobacillus sake in DNA homology studies (unpublished data). L. sake strains are nowadays used in some commercial starter culture preparations (4). The ability to form ropy slime may not be a very rare characteristic among the strains of this species, and it may be beneficial to test the L. sake strains used as starter bacteria for this ability to produce ropy slime.
REFERENCES


