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## A Research Note

# Growth Temperatures of Ropy Slime-Producing Lactic Acid Bacteria

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### ABSTRACT

The minimum, optimum, and maximum growth temperatures of ropy slime-producing lactic acid bacteria able to spoil vacuum-packed cooked meat products were determined on MRS-agar with temperature-gradient incubator Gradiplate<sup>®</sup> W10. The minimum growth temperatures of slime-producing lactobacilli and *Leuconostoc mesenteroides* strain D1 were below -1°C and 4°C, respectively. The low minimum growth temperature allows these bacteria to compete with other bacteria in meat processing plants and in meat products causing ropiness problems. The maximum growth temperatures varied between 36.6-39.8°C. The maximum growth temperature of lactobacilli seemed to be an unstable character. Single lactobacilli colonies were able to grow above the actual maximum growth temperature, which is determined as the edge of continuous growth of the bacteria. The significance of this phenomenon needs further study.

A phenomenon sometimes observed in Finland is the spoilage of vacuum-packed cooked meat products due to the formation of ropy slime (2,3). The appearance of such slimy meat products is highly undesirable and consumers consider it offensive. Korkeala et al. (3) have isolated three different types of lactic acid bacteria able to produce slime on vacuum-packed cooked sausages. Two strains were classified as belonging to the genus *Lactobacillus* and one to the genus *Leuconostoc*. The most important strain seems to be a homofermentative *Lactobacillus* belonging to group 1 of Korkeala et al. (3). This strain has been isolated from various meat products produced by different manufacturers (2,3).

The spoilage caused by ropy slime formation has influenced the marketing of vacuum-packed meat products and the use of vacuum-packaging technology, and the financial losses involved in some cases have been quite high. In order to control and prevent the growth of ropy slime-producing lactic acid bacteria, information concerning different growth limits is needed. The growth tempera-

ture is one of the most important factors influencing bacterial growth. The minimum, optimum, and maximum growth temperatures of ropy slime-producing lactic acid bacteria strains were therefore studied in this work.

### MATERIALS AND METHODS

#### Lactic acid bacteria strains

The study used a total of 10 lactic acid bacteria strains isolated from vacuum-packed meat products. All the strains were able to cause ropiness on vacuum-packed cooked meat products. The strains A1, A210, B1, L1, MN1, PP1, R1, and T1 belonged to group 1; strain C1 to group 2; and strain D1 to group 3 of Korkeala et al. (3). The strains of groups 1 and 2 were homofermentative lactobacilli, and the strain of group 3 resembled *Leuconostoc mesenteroides*.

#### Determination of growth temperatures

Temperature-gradient incubator Gradiplate<sup>®</sup> W10 (Biodata Oy, Helsinki, Finland) was used (1,4).

Each bacterial strain was cultured in MRS broth at 20°C for 24 h. After incubation, 0.1 ml of culture was mixed with 100 ml of 0.1% peptone water; 50 µl of this suspension was inoculated onto a GRADICUVETTE dish containing MRS agar (Oxoid, Hampshire, England) using the droplet-run technique described by Niemelä (4). The cuvettes were incubated in the temperature-gradient incubator for 25 d for the determination of the minimum growth temperature, 2-3 d for the determination of the maximum growth temperature, and 15 h for the determination of the optimum growth temperature. At least six parallel determinations were carried out for each strain studied and for each growth temperature.

#### Reading the results

Given the dimensions and proportions of the gradient plate, the slope of the thermal gradient is defined by formula (1):

$$(1) G = (T_H - T_L) / 60^\circ\text{C mm}^{-1}.$$

To determine the temperature at a defined point on the gradient plate, the distance A of the point is measured from a fixed

baseline. This line, 19 mm from the low-temperature side of the incubation plate, parallel to the isotherms, is defined by the site of the measurement of the lower temperature (reference point). The actual temperature,  $T_A$ , at distance A (mm) from the baseline is obtained from (2):

$$(2) T_A = T_L \pm G \times A = T_L \pm (T_H - T_L)/60 \times A$$

$T_A$  = temperature at distance A from the baseline (°C)

$T_L$  = temperature at lower reference point (°C)

$T_H$  = temperature at higher reference point (°C)

G = the slope of the temperature gradient (°C/mm)

The edge of continuous growth of the microbes was also detected through a microscope.

## RESULTS

The density of bacterial growth diminished gradually towards the low temperature in all strains studied. The boundary of minimum growth was not sharply defined, and growth was observable microscopically below the limit visible to the naked eye. The determination of the minimum growth temperature therefore had to be verified through a microscope. The strains of groups 1 and 2 grew during 25 d of incubation across the whole temperature gradient used, from -1 to 15°C. Thus, the minimum growth temperatures of these roping lactobacilli strains, as determined microscopically, were <-1°C. The minimum growth temperatures, as observed by naked eye, varied between -1 and +1°C.

The minimum growth temperature of strain D1 (group 3) was higher compared to the other strains. Continuous growth clearly decreased at approximately 4°C, and the weak growth of microcolonies observed through the microscope was not found below 3°C.

The optimum and maximum growth temperatures are presented in Table 1. The maximum growth boundaries of the strains of groups 1 and 2 were sharp. The maximum of D1 was difficult to interpret due to its gradually decreasing growth towards the high temperature. Above the boundary of 37.8°C, further growth was observable microscopically. The limit of this growth was 38.1°C.

Above the maximum boundary, single colonies were found in many of the strains studied. The size of the single colonies was not smaller than that of the other colonies (Fig. 1). When the colonies growing above the maximum boundary were incubated in the gradient, their growth reached a higher temperature than the corresponding growth of the initial strain. The maximum growth temperature of such colonies was at least as high as the temperature at the point at which the colony was picked up (Fig. 1). Similar single colonies above the boundary of the continuous growth may also be observed in these cultures. The maximum growth temperatures of these isolates were considered to be reached when single colonies above the boundary were no longer seen (Table 1). Such single isolated colonies growing above the edge of continuous growth were generated in particular by strain C1.

TABLE 1. Optimum and maximum growth temperatures of lactic acid bacteria strains causing ropiness in vacuum-packed meat products.

Strain	Optimum temperature (°C)	Maximum temperature <sup>a</sup> (°C)	Maximum temperature of thermoresistant isolates <sup>b</sup> (°C)
Group 1			
A1	26-31	39.8 ± 0.2 <sup>c</sup>	ND <sup>d</sup>
A210	26-31	38.4 ± 0.1	40.0
B1	27-31	39.1 ± 0.1	39.6
L1	26-31	37.5 ± 0.2	39.6
MN1	26-30	38.7 ± 0.1	39.3
PP1	26-30	38.7 ± 0.1	40.0
R1	27-30	38.6 ± 0.2	39.4
T1	27-30	38.6 ± 0.1	39.3
Group 2			
C1	30-34	36.6 ± 0.3	40.7
Group 3			
D1	27-32	37.8 ± 0.4	ND

<sup>a</sup>Measured from the boundary of continuous growth.

<sup>b</sup>Single colonies growing at higher temperatures than the parent strain.

<sup>c</sup>Mean and standard deviation.

<sup>d</sup>No single colonies observed above continuous growth boundary of the parent strain.

## DISCUSSION

The minimum growth temperatures of all strains studied were low. It was noticeable that all ropy slime-producing lactobacilli strains were able to multiply on MRS agar at temperatures below 0°C. This low minimum growth temperature allows these bacteria to survive and compete with other bacteria in meat-processing plants and in meat products. Ropy slime-producing lactobacilli belong to the atypical streptobacteria, i.e., to homofermentative psychrotrophic lactobacilli (3). Atypical streptobacteria are characterized by a low minimum growth temperature; this is one important difference between atypical streptobacteria and other streptobacteria (5,6,7). Reuter (5,6,7) has reported that the minimum growth temperature of atypical streptobacteria over 10-12 d is 4-2°C.

The present results suggest that the use of low temperatures in the preparation and storage of meat products cannot totally prevent the formation of ropy slime, although a decrease in the storage temperature will result in a longer shelflife for the products.

The optimum temperatures of ropy slime-producing lactic acid bacteria are near 30°C. Such high temperatures are not generally reached during the storage of vacuum-packed meat products in spite of temperature abuses.

The presence of isolates able to grow at higher temperatures than those indicated for continuous growth was observed in lactobacilli. This phenomenon was not found in *L. mesenteroides* strain D1. These thermoresistant isolates were found in all ropy slime-producing lactobacilli

Cont. on p. 803