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Effect of Sodium Nitrite and Sodium Chloride on Growth of Lactic Acid Bacteria

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Korkeala, H., T. Alanko and T. Tiusanen: Effect of sodium nitrite and sodium chloride on growth of lactic acid bacteria. Acta vet. scand. 1992, 33, 27-32. – The effect of $NaNO_2$ and NaCl on the growth of 24 lactic acid bacteria strains isolated from vacuum-packed cooked ring sausages were examined by analyzing different growth parameters with Bioscreen. $NaNO_2$ had a very limited effect on the growth of lactic acid bacteria at 50 and 100 mg/l but at 400 mg/l a more pronounced inhibitory effect was found. Bacterial growth was enhanced by 1-2% (w/v) of added NaCl, while NaCl concentrations above 3% (w/v) had a clear inhibitory effect. *Leuconostoc* isolates seemed to be more sensitive to sodium nitrite and sodium chloride than homofermentative lactobacilli strains. Among homofermentative lactobacilli, the strains resembling *Lactobacillus curvatus* were more sensitive to NaCl than those resembling *Lactobacillus sake.*

Lactobacillus curvatus; Lactobacillus sake; growth; growth inhibition; bioscreen; lag phase; delta absorbance; gradient.

Introduction

Lactic acid bacteria have been reported to be quite resistant to nitrite *(Castellani & Niven* 1955). *Dodds & Collins-Thompson* (1984), determining both growth rates and cell yields, have found that some lactic acid bacteria are sensitive to 200 µg/ml of nitrite under anaerobic conditions. They also found that the sensitivity of 2 *Lactobacillus viridescens* strains differed from other *L. viridescens* strains by showing a significant decrease in growth rate only. It thus appears that the mode of the inhibitory action can vary between different lactic acid bacteria strains.

Lactic acid bacteria are able to grow at high NaCl concentrations. Most of the atypical streptobacteria isolated from vacuum-packed meat and meat products tolerate 8 % of added NaCl in the medium *(Reuter* 1970, *Mol et al.* 1971). However, there is little information available concerning the effect of low concentrations of sodium nitrite and sodium chloride separately or together on lactic acid bacteria. This study was undertaken to examine the effect of $NaNO_2$ and NaCl concentrations on the growth of various strains of lactic acid bacteria isolated from vacuum-packed cooked sausages by analyzing different growth parameters.

Materials and methods

Bacterial strains

The 24 strains of lactic acid bacteria used in the study were isolated from vacuum-packed cooked ring sausages. Twenty of the strains studied were homofermentative lactobacilli belonging to groups 1, 2, 3, 4, 5 and 7, and 4

were leuconostocs belonging to groups 9 and 11 *(Korkeala & Mäkelä* 1989). Of the homofermentative lactobacilli 5 strains resembled *L. curvatus* and 10 strains *L. sake* (*Niemand & Holzapfel* 1984, *Kandler & Weiss* 1986, *Schillinger & Lücke* 1987). In the following, they are denoted as the *L. curvatus* group and the *L. sake* group.

Monitoring of bacterial growth

Bacterial growth was monitored using an automated turbidometer (Bioscreen, Labsystems Oy, Finland). Absorbances were measured every 10 min by vertical pathway filter-photometry (at 580 nm) through optically high-quality cuvettes and the data were transferred to the computer (*Korkeala & Männistö* 1988).

Overnight bacterial cultures in MRS broth (Difco) were used for inoculation (10 µl of culture in 390 µl of MRS broth). The cultures were exposed to all combinations of sodium nitrite: 0, 50, 100, 200 and 400 mg/l and NaCl: 0, 1, 2 ,3, 4, 5, 6 and 7 % (w/v) in MRS broth. $NaNO_2$ and NaCl stock solutions were filter-sterilized. In the preparation of the final solutions MRS broth was used to maintain a constant nutrient level in the medium. The incubation temperature was 37°C and the incubation period 50 h.

The delta absorbance (difference between the first and last absorbance reading) the gradient (slope of the logarithmic growth phase) and the lag phase (time when the bacterial growth started) were calculated to characterize growth. If no bacterial growth was observed, the lag phase was assessed as 50 h. The procedure was carried out according to the system developed by Labsystems (Helsinki, Finland).

Statistical analysis

The differences in growth parameters between different lactic acid bacteria groups were examined by ordinary t-tests, using Satterthwaite's approximation whenever there was evidence of unequal variances between groups. Differences in responses to increasing NaCl and $NaNO_2$ concentrations were examined graphically, holding the other concentration at zero and plotting the group means against levels of the other factor.

The SAS/STAT package *(SAS Institute Inc.* 1987a) was used for statistical analysis. All the figures were produced using the SAS/GRAPH package *(SAS Institute Inc.* 1987b).

Results

Descriptive analysis

The effects of different sodium nitrite concentrations on delta absorbance, gradient and lag phase in lactic acid bacteria in different NaCl concentrations are presented in Fig. 1. All concentrations of $NaNO_2$ without added NaCl had an inhibitory effect on the growth of lactic acid bacteria, but at $NaNO_2$ concentrations of 50 and 100 mg/l the effect was very low.

The effects of different sodium chloride concentrations on delta absorbance, gradient and lag phase in lactic acid bacteria at different $NaNO_2$ concentrations are illustrated in Fig. 2. The growth of lactic acid bacteria was enhanced with 1 - 2 % of added NaCl compared to 0 % of added NaCl. NaCl concentrations above 3% had a clear inhibitory effect on the growth of lactic acid bacteria. The sensitivity of lactic acid bacteria to NaCl did not change markedly as a function of the $NaNO_2$ concentration.

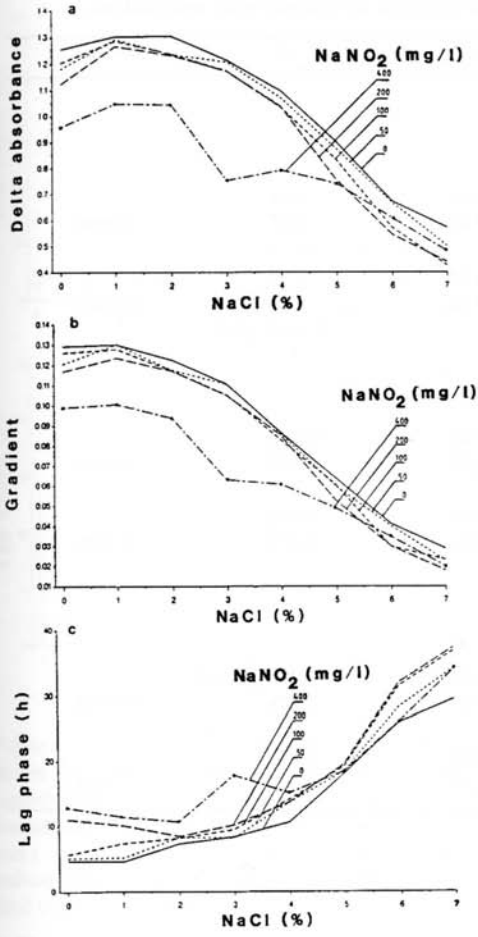

Figure 1. Average values for effects of different sodium nitrite concentrations on the growth of 24 lactic acid bacteria strains in different sodium chloride concentrations, determined by delta absorbance (a), gradient (b) and lag phase (c).

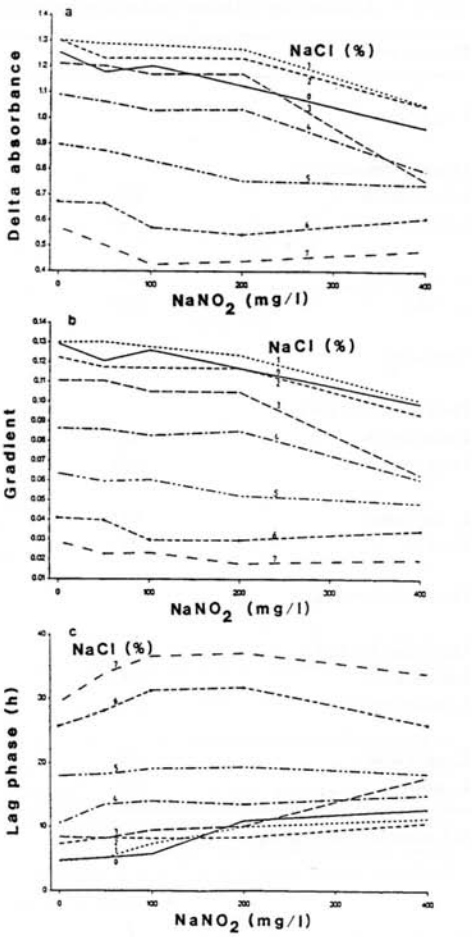

Figure 2. Average values for effects of different sodium chloride concentrations on the growth of 24 lactic acid bacteria strains in different sodium nitrite concentrations, determined by delta absorbance (a), gradient (b) and lag phase (c).

Differences between bacterial groups

The overall differences both between the homofermentative lactobacilli and *Leuconostoc* isolates on the one hand and between the *L. sake* and *L. curvatus* groups on the other are statistically significant ($p < 0.001$) for all 3 growth parameters as shown in Table 1. *The Leuconostoc* strains had longer mean lags, lower gradients and lower delta absorbances than the homofermentative lactobacilli strains. Similarly, *L. curvatus* strains had longer lags, lower gradients and lower delta absorbances than *L. sake* strains.

Table 1. Differences between bacterial groups in terms of lag phase, delta absorbance and gradient.

Bacterial group	N	Mean	SD	p-values
Lag phase				
Homofermentative				
Lactobacilli	800	11.96	15.5	
Leuconostocs	159	37.49	16.7	0.0000
L. curvatus	400	14.09	17.3	
L. sake	160	3.11	4.47	0.0001
Gradient				
Homofermentative				
Lactobacilli	800	0.089	0.068	
Leuconostocs	160	0.037	0.045	0.0000
L. curvatus	400	0.076	0.056	
L. sake	160	0.144	0.079	0.0000
Delta absorbance				
Homofermentative				
Lactobacilli	800	1.020	0.414	
Leuconostocs	160	0.595	0.378	0.0000
L. curvatus	400	0.986	0.392	
L. sake	160	1.239	0.376	0.0000

SD=standard deviation.

Do then changes in NaCl and $NaNO_2$ concentration levels play any part in producing these overall differences? This question is best answered by plotting separately the average responses of the bacterial groups against $NaNO_2$ and NaCl concentrations while keeping the other concentration at 0.

Sensitivity to $NaNO_2$ levels is examined first. As seen from Fig.3, with respect to the lag phase, leuconostocs are more sensitive to increasing $NaNO_2$ concentrations than homofermentative lactobacilli. No marked differences were found for gradient or delta absorbance. The differences between the *L. sake* and *L.curvatus* groups were not significant for any growth parameter.

Similar examination with respect to NaCl levels showed marked differences in sensitivity for all growth parameters in both pairs of bacterial groups except for delta absorbance between leuconostocs and homofermentative lactobacilli. Leuconostocs are thus more sensitive to increasing NaCl levels than homofermentative lactobacilli as measured by the lag phase and gradient. The differences are observable in the lag phase after 2% of added NaCl. The *L. curvatus* group is more sensitive to increased NaCl level than the *L.*

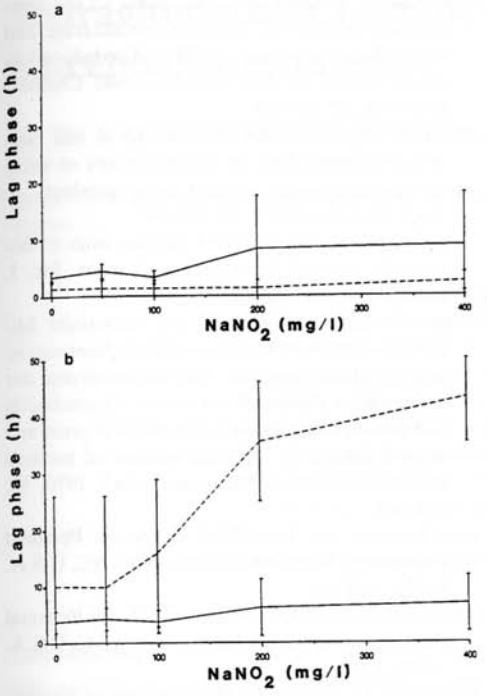

Figure 3. Effect of sodium nitrite on growth of strains resembling *Lactobacillus curvatus* (——) and *Lactobacillus sake* (----) (a), and on growth of homofermentative lactobacilli (——) and leuconostocs (----) (b) determined by lag phase, when added sodium chloride was 0. Vertical bars denote standard errors of the mean.

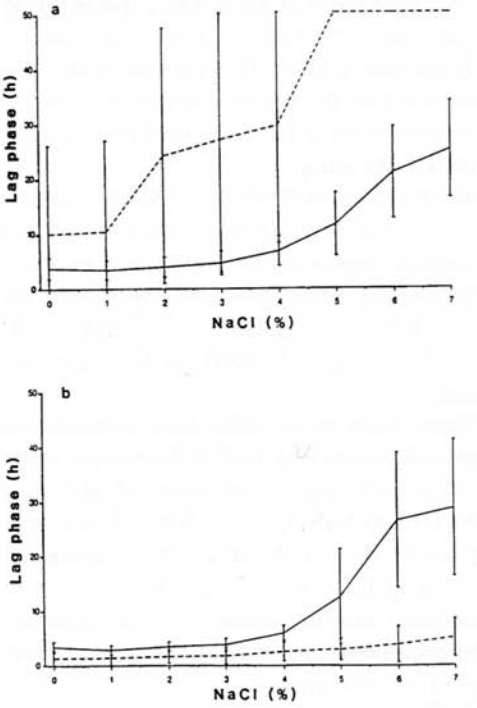

Figure 4. Effect of sodium chloride on growth of strains resembling *Lactobacillus curvatus* (——) and *Lactobacillus sake* (----) (a), and on growth of homofermentative lactobacilli (——) and leuconostocs (----) (b) determined by lag phase, when added sodium nitrite was 0. Vertical bars denote standard errors of the mean.

sake group. This is particularly evident at high NaCl concentrations. After 4 % of added NaCl the length of the lag phase started to increase when compared to *L.sake* group. Fig. 4 illustrates the differences in response for the lag phase.

Discussion

Lactic acid bacteria seem to grow well in MRS broth at the low sodium nitrite concentrations studied. The pronounced inhibitory effects caused by sodium nitrite alone were observed at high concentrations. At 400 mg/l the reduction in growth potential was distinct, but a slight growth inhibition was found already at 200mg/l.

The known growth stimulation caused by NaCl *(Ingram & Kitchell* 1967) was found at NaCl concentrations of 1-2 %. Retardation of growth was observed for all growth parameters at 3% of added NaCl. An increment of NaCl above 3 % caused retardation of growth in spoilage lactic acid bacteria.

Leuconostocs were more sensitive to sodium nitrite and sodium chloride than homofermentative lactobacilli. The sensitivity of het-

erofermentative lactic acid bacteria to nitrite was also observed by *Dodds & Collins-Thompson* (1984). The present study also showed that the strains resembling *L. curvatus* were more sensitive to NaCl than those of the *L. sake* group.

Small amounts of $NaNO_2$ or NaCl especially when used together may select lactic acid bacteria population to multiply e.g. on vacuum-packed meat products. However, for total inhibition of lactic acid bacteria considerable amounts of $NaNO_2$ or NaCl are needed.

There seem to be differences between the growth parameters used in the present study in the detection of sensitivities of lactic acid bacteria to $NaNO_2$ and to NaCl. This is appparently due to the different toxic mechanisms of these compounds. The analysis of different growth parameters with Bioscreen offers a possibility of observing small changes in bacterial growth.

Acknowledgement

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Sammanfattning

Effekten av natriumnitrit och natriumklorid på tillväxten av mjölksyrebakterier.

Effekten av $NaNO_2$ och NaCl på mjölksyrebakteriers tillväxt som hade isolerats från vakuumpackade finska falukorvar undersöktes. $NaNO_2$ hade en mycket begränsad inverkan på mjölksyrebakteriernas tillväxt vid 50 och 100 mg/l, men vid 400 mg/l kunde en betydande inhiberande effekt konstateras. Tillsatsen av 1-2 % salt ökade bakterietillväxten, medan saltkonsentrationer över 3 % hade en klar förhindrande inverkan. *Leuconostoc* stammar verkar vara mera sensitiva för $NaNO_2$ och NaCl än homofermentativa laktobasiller. Bland homofermentativa laktobasillstammar var stammar som liknade *Lactobacillus* curvatus mera sensitiva för NaCl än stammar som liknade *Lactobacillus* sake.

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