THE EFFECT OF NITRITE ON THE GROWTH CURVE OF LACTOBACILLI AND MICROCOCCI

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The effects exerted on microbes b y curing agents, such as sodium chlorid, potässium nitrite, and sodium nitrite, in the first place, have been rather extensively examined. The invesigations have, however, been mainly concerned upon microbes food poisonings. Among these be mentioned e.g. staphylococci (Castellani and Niven 1965, Lechovich et al. 1956, Peterson et al. 1963), or clostridia and their spores (Pivnick and Barnett 1965, Roberts and Ingram 1966, Perigo and Roberts 1968, Emodi and Lechowich 1969).

Numerous interpretations have been presented in regard to the mechanism of the bacteriostatic effect of nitrite. The reaction of nitrous acid with α- amino groups brings about disturbances in the activity of dehydrogenase enzymes (Castellani and Niven 1955);Phil pot and Small (1938 a,b) have indicated that nitrous acid can react with monophenols, such as tyrosine, and they attributed the inactivation of pepsin to this reaction. It also is possible that hydroxylamine may be produced from nitrite by a number of organisms (Lindsey and Rhines 1932) and this substance in turn is responsible in some degree for nitrite bacteriostasis.

The bacteriostatic action of nitrite may be explained by the ability of the decomposition product, nitric oxide, to react with heme pigments. The production of nitric oxide myoglobin is the bases for meat curing. According to Ingram (1939), at pH 6 oxygen uptake by *Bacillus cereus* was strongly inhibited by nitrite, which indicated an interference with the cytochrome systems. Tarr (1941) encountered that among two *Achromobacter* strains and two micrococci, the growth of which in broth was distinctly retarded by nitrite, the aerobic respiration of only one _Achromobacter_ strain was inhibited at different pH levels. In his opinion nitrite does not inhibit growth solely by affecting the activity of the aerobic respiratory catalysts. This theory is further supported by the results obtained by Castellani and Niven (1955). They found that nitrite was strongly inhibitory toward some streptococci even though these bacteria are devoid of tne heme-containing respiratory catalysts. Furthermore, nitrite was generally more bacteriostatic in ahaerobic conditions where the cytochrome systems are not important in the metabolism of the microorganisms.

Castellani and Niven (1955) found, moreover, that nitrite may inactivate cetrain bacterial enzyme systems that possess a sulfhydril group. Barron and Singer (1945) reported that among a large number of enzyme systems studied the pyruvatemetabolizing enzymes were the most sensitive to sulfhydrylinhibitors. Bernheim (1943) and Nord and Mull (1945) encountered that the pyruvate - metabolizing enzymes of _Vibrio comma_ and _Fusarium_ were inactivated by nitrite.

Tolerance toward nitrite varies widely among different groups of bacteria. Several factors have been fund to exert an influence on the bacteriostatic action of sodium nitrite. The pH value of the environment influenced the level of nitrite causing inhibition in a manner tends to confirm the hypothesis that undissociated nitrous acid is the active form (Castellani and Niven 1955).

The utilization of microbial starter cultures in the manufacture of fermented meat products is rather common in the meat processing industry. In this wav the ripening process of products can better be controlled and a uniform guality can be maintained. Niven et al. (1955, 1958) and Deibel et al. (1961a, 1961b) have suggested the use of Pediococcus cerevisiae. Niinivaara and Pohja (1957 a, 1957 b, and 1957 c), on the other hand, recommend the use of micrococci, and Nurmi (1965, 1966) has studied the possibilities to employ lactobacilli and mixed cultures of lactobacilli and micrococci in the manufacture of fermented meat products.

Besides sodium chloride, sodium nitrite is in meat products a substance possessing highest microbiostatic effect. In the production of dry sausage the growth of certain microbes, lactobacilli and micrococci in the first place, is important, however, from the standpoint of the ripening process of sausages. For this reason attempts have been made in the present study to elucidate the influence of nitrite on the growth of lactobacilli and micrococci and likewise to examine the basis for the selection of the strains to be used as starter cultures.

Material and methods

The object of the study comprised lactic acid bacteria and micrococci. They included the following genera :
Lactobacilus (78 different strains, mainly homofermentative),
Micrococcus and Staphylococcus (24 different strains),
and Pediococcus (one species P. cerevisiae). From the standpoint of manufacturing technique these microbes form the most important part of the microbial flora of dry sausages.

The composition of the nutritient medium emplyed in the cultivation of the microbes under examination was as follows :

Peptone	(Oxoid)	10.0 g
Meat extract	(")	10.0 g
Yeast extract	(")	5.0 g

Glucose		20,0 g
Tween 80		1.0 ml
NaCl		5.0 g or 40 g
Distilled water	ad.	1000.0 ml

The pH value of the nutrient broth was adjusted to 6.0 with lactic acid. The different sodium nitrite contents of the broth were produced by aseptically adding suitable amounts of solutions diluted from the 10 % basic solution.

The strains under examination were inoculated as follows: The lactobacilli inoculum consisted of 0.1 ml of a 10^{-5} dilution and the micrococci inoculum of 0.1 ml of 10^{-4} dilution. The strains had been incubated for one day at 30°C. The volume of broth in the culture bulb of the biophotometer was 10 ml.

The growth of the microbes was followed by using a biophotometer (manufactured by Jouan, Paris, France), in which the culture was kept at 25°C and continuously stirred. The instrument measured the turbidity of the growth medium every 20 th second and the reading was registered by a recorder.

The instrument was capable of simultaneously holding 6 buibs so that at the same time either the effects of six different nitrite concentrations could be followed or the growth of six different bacterial strains, respectively. The lag phase and logarithmic phase of the microbial growth curve can well be followed using this instrument. From the standpoint of the industrial use of microbes these two phases are of greatest importance.

Results and discussion

When the effect of sodium nitrite on the shape of the growth curve of lactobacilli at 0.5 % NaCl concentration is examined, it can be found that the growth curves obtained were mostly about similar to the curves presented in Fig.1.

The highest concentration emplyed (0.5%) has generally inhibited the growth completely or the growth has been remarkably retarded. The next concentration (0.1%) has still considerbably retarded the growth, and it has brought about a delay of the start of the growth, the delay being _ - 10 hours on the average. Furthermore, the growth curve does not rise in the same way as it does when other concentrations are used. The concentration 0.02% has still caused retardation of the growth, whereas the concentration 0.004% and the control have generally been very similar. On the other hand, when 0.0008% of nitrite was emplyed, in all series a distinct increase in growth could be observed. This phenomenon is interesting but the elucidation of the growth-promoting factor is still uncompleted.

When comparing the growth in the cases where MRS-broth (De Man et al.1960), a lyophilized strain or a smaller dilution were used, it was found that they changed the shape or location of the growth curve, but the differences caused by different concentrations remained about the same. Instead, sodium nitrite seemed to interfere distinctly more with the growth of heterofermentative lactobacilli than with that of homofermentative lactobacilli. However, the number of heterofermentative strains examined was only four. The difference mainly appeared in the cases where the nitrite concentration was 0.1%.

When 4 or 5% of sodium chloride was added to nutrient broth, it was observed that the start of the growth was delayed but otherwise the differences caused by nitrite remained similar. When the salt tolerance was only examined, it was encountered that hetero fermentative lactobacilli seemed to grow with the 4% concentration better than did homofermentative.

The effect of sodium nitrite on the growth of micrococci is illustrated by Fig.2.

Two highest concentrations (0.5% and 0.1%) completely inhibited the growth. The concentration 0.02% also retarded considerably the growth, whereas the lower concentrations were rather near each other in this respect. The concentration 0.0008% also promoted the growth in the case of micrococci, it is true, but this was not as noticeable as it was in the case of lactobacilli. Lyophilization markedly decreased the lag phase. Instead, an increase of sodium chloride content to 4% only influenced the growth in the case of the 0.02% nitrite concentration.

Regarding the Pediococcus examined, different nitrite concentrations affected the growth about similarly as they affected that of lactobacilli. Instead, when the salt content was increased to 4 or 5%, the growth was markedly retarded at the concentration of of 5% by about 48 hours. Likewise the two highest nitrite concentrations (0.5 and 0.1%) completely inhibited the growth when the sodium chloride concentration was 5%.

From the standpoint of the industry, a microbial strain to be used in inoculation should meet the following main requirement : it must be capable of growing in conditions corresponding with those in dry sausage, in other words, it must be able to tolerate about 4% salt and 0.0008 - 0.02% nitrite concentrations. On account of this, experimental series were done in which 6 different strains were run at one time, the salt concentration being 4% and that of nitrite 0.02% and 0.0008%

When the nitrite concentration was 0.02%, among lactobacilli five strains were growing best (strains 10/8, 28/li, 340/2i H.K., 340/iH, and 331/iC), the growth of which started after about 20 hours. A decrease of the concentration to 0.0008% did not accelerate the start of the growth. At a 0.02% nitrite concentration, three strains (28/2 B.f., 44/1, and 549/2a) among micrococci were growing best. When the concentration was decreased to 0.0008%, the lag phase was reduced from 17 hours (0.02%) to 14 hours so that the micrococci started to grow earlier at a 0.0008% nitrite concentration.

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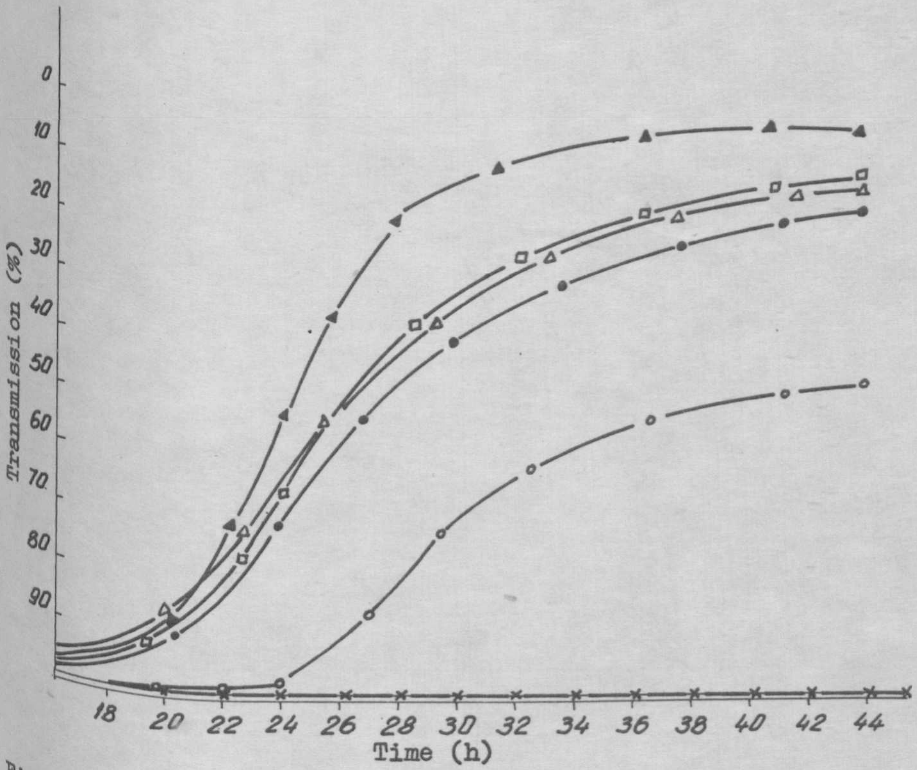

Figure 1. Effect of different concentrations of sodium nitrite on the growth of Lactobacillus plantarum strain 344/2 g measured with a biophotometer. NaCl concentration of culture broth 0.5 % and growth temperature 25°C. Nitrite concentrations :

0.5 % (x-x) 0.004 % (△-△)

0.1 % (o-o) 0.0008 % (▲-▲)

0.02 % (•-•) control (□-□)

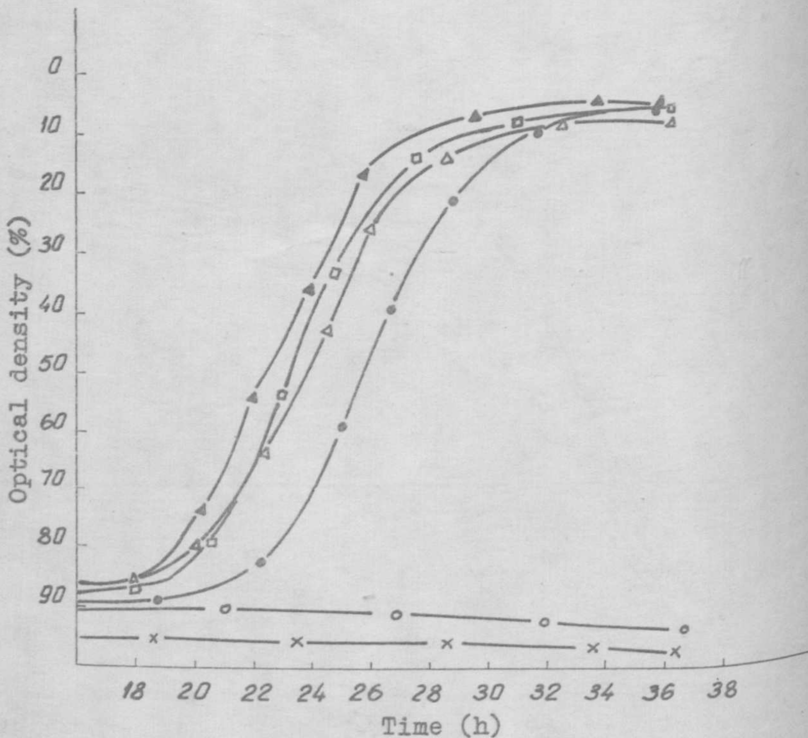

Figure 2. Effect of different concentrations of sodium nitrite on the growth of a Micrococcus, strain 62/6a B.f., measured with a biophotometer. NaCl concentration of a culture broth 0.5% and growth temperature 25°C. Nitrite concentrations :

0.5 % (x-x) 0.004 % (△ - △)

0.1 % (o-o) 0.0008 % (▲ - ▲)

0.02 % (•-•) kontrolli (□ - □)