Stimulierender Effekt von Micrococcus sp. auf Wachstum und Säurebildung von Lactobacillus plantarum

INGER ERICHSEN

Schwedisches Fleischforschungsinstitut, Kävlinge, Schweden

Die Frage ist untersucht worden ob eine stimulierende Wirkung von Micrococcus sp. auf Wachstum und Säurebildung von Lactobacillus plantarum vorliegt, wenn die beiden Bakterienstämme in Mischkulturen und in verschiedenen Zellkonzentrationen bezüchtet wurden.

Prachtische Versuche wurden ausgeführt in denen die beiden Bakterienstämme in denselben Zellkonzentrationen als Starterkulturen bei der Herstellung von Rohwurst verwendet wurden.

Keine stimulierende Wirkung durch Micrococcus sp. auf Wachstum und Säurebildung von L. Plantarum konnte festgestellt werden.

Stimulatory effect of Micrococcus sp. on growth and acid production of Lactobacillus plantarum

INGER ERICHSEN

Swedish Meat Research Centre, Kävlinge, Sweden

To find out whether some mutual stimulatory effect exists between Micrococcus sp. and Lactobacillus plantarum when grown together in a mixed culture, the growth and acid production of the two strains were studied when grown in mixed cultures at varying cell-concentrations.

In practical experiments the influence of varying cell-concentrations of a mixed starter culture containing the same two strains on the quality of raw fermented sausage was examined.

No stimulatory effect of Micrococcus sp. on growth and acid production of L. plantarum was found.

G 12:2

Interaction stimulante du Micrococcus sp. sur la croissance et la production d'acide du Lactobacillus plantarum

INGER ERICHSEN

L'Institut Suédois de Recherche sur les Viandes de Boucherie, Kävlinge, Suède

Afin de savoir s'il se produit une interaction stimulante du Micrococcus sp. et du Lactobacillus plantarum quand on les fait se developper ensemble dans une culture mixte, l'auteur a étudié la croissance et la production d'acide des deux souches quand ils se multiplient dans des cultures mixtes aux concentrations cellulaires variées en processus discontinu.

Lors des expériences pratiques l'auteur a examiné l'influence exercée par des concentrations cellulaires d'une culture de départ mixte contenant les mêmes deux souches sur la qualité du saucisson fermenté.

Aucun effet stimulant du Micrococcus sp. sur la croissance et la production d'acide du Lactobacillus plantarum ne fut découvert.

Стимулирующее воздействие Micrococcus sp. на рост и кислотообразование Lactobacillus plantarum

ИНГЕР ЭРИХСЭН

Шведский научно-исследовательский институт по мясным продуктам, Чевлинге, Швеция

Чтобы узнать, имеет ли место какое-нибудь стимулирующее взаимодействие между Micrococcus sp. и Lactobacillus plantarum когда они выращиваются вместе в смешанной культуре, изучались рост и кислотообразование у этих двух штаммов, развивающихся в смешанных заквасках при меняющихся клеточных концентрациях партиями.

На практических опытах исследовалось воздействие меняющихся клеточных концентраций в смешанной закваске, содержащей те же два штамма, на качество сброженной колбасы.

Не было найдено никакого стимулирующего воздействия Micrococcus sp. на рост и кислотообразование Lactobacillus plantarum.

Stimulating effect of Micrococcus sp on growth and acid production of Lactobacillus plantarum

INGER ERICHSEN

Swedish Meat Research Centre, Kävlinge, Sweden

Introduction

It is a well known fact that bacteria strains grown together in a mixed culture can have mutual inhibitory or stimulatory effects on several parameters applying to growth and metabolic activities ((Daly et al., 1972; Hurst, 1972; Branen & Keenan, 1969,1970; Nieuwenhof et al., 1969; Niskanen & Nurmi, 1976; Noon & Reinbild, 1976).

Growth and toxin production of patogenic bacteria are in some cases inhibited by the use of starter cultures in fermented sausage products (Daly et al, 1973; Genogeorgis, 1976; Smith et al., 1975 b)).

A stimulatory effect on acid production and growth of 6 strains of lactic acid bacteria in the presence of a Micrococcus strain has been reported by Nath and Wagner (1973). These authors found that the amount of hydrogen peroxide produced by lactic acid bacteria was greatly reduced in mixed cultures with micrococci and also by the addition of catalase. The catalase addition stimulated growth and acid production by lactic acid bacteria, but not to the same extent as Micrococcus sp. The authors postulate a second means of stimulation by Micrococcus sp. in addition to stimulation by removal of hydrogen peroxide. This stimulatory factor is connected with the living cell of Micrococcus sp. and has not yet been characterized.

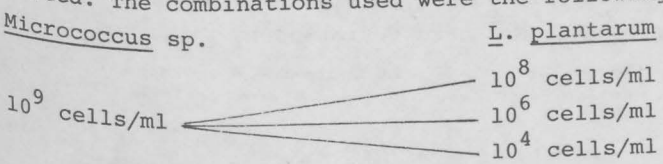
One of the most commonly used starter cultures for fermented sausages in many countries in Europe is a combined starter culture consisting of Lactobacillus plantarum and a Micrococcus sp. (Coretti, 1977; Niinivaara, 1955; Reuter, 1972 ; Nurmi, 1966).

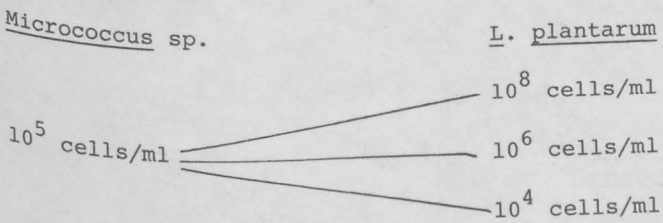
In order to try to find out whether the micrococcus strain in this starter culture had a stimulating effect on the growth and acid production of Lactobacillus plantarum some experiments have been carried out in which varying cell concentration combinations of the two strains involved were tested when used as starter cultures in the production of a Swedish type of fermented sausage. Simultaneously experiments were carried out in which the two strains were grown together in batch cultures using similar cell concentration combinations as in the practical experiments.

Material and Methods

Practical experiments

For the practical experiments seven different series of test productions of sausage were carried out at the Meat Research Centre pilot plant. In each series six different cell concentration combinations of the two starter cultures L. plantarum and Micrococcus sp. were tested. The combinations used were the following.





G 12:4

The micrococcus strain used was received from Finland by the courtesy of prof. F.P. Niinivaa and the L. plantarum strain was isolated from fermented sausage.
The sausages were produced according to the formula used for this type of sausage in Sweden. Beside beef and pork meat the formula contains 2.8% NaCl (with nitrite 120 ppm), 2% sucrose and spices. The water content of the final product was 42%.
The starter cultures were cultivated for 24 hours at 30°C and transferred daily for 3 days, centrifuged, washed, centrifuged again and finally suspended in sterile reconstituted non-fat-dry-milk-solids in the concentrations wanted. They were frozen and held at -45°C until used. To the batches of 6 kg of the sausage mix was added 100 ml of the different starter culture combinations. The different batches were filled in casings and the sausages were ripened in a fermenting chamber for 6 days at 24°C and a rel. humidity of 85%. Smoking was executed for 6 hours on the second and sixth day of fermentation.
Microbiological examinations of the sausages were carried out on the third and sixth day of fermentation and comprised number of lactic acid bacteria on MRS-agar (Man,de, et al, 1960) and number of micrococci on a peptone-meat-extract agar containing 6% NaCl.

Batch culture experiments

The batch culture experiments were carried out in a water bath at 30°C.
The following cell concentration combinations were used:

Experiment 1: Micrococcus sp. 10^8 cells/ml in combination with L. plantarum 10^7 cells/ml
Micrococcus sp. 10^8 cells/ml in single culture
L. plantarum 10^7 cells/ml in single culture.

Experiment 2: Micrococcus sp. 10^8 cells/ml in combination with L. plantarum 10^4 cells/ml
Micrococcus sp. 10^8 cells/ml in single culture
L. plantarum 10^4 cells/ml in single culture.

Experiment 3: Micrococcus sp. 10^5 cells/ml in combination with L. plantarum 10^7 cells/ml
Micrococcus sp. 10^5 cells/ml in single culture
L. plantarum 10^7 cells/ml in single culture.

Three batch cultures were tested simultaneously, one containing the mixed cultures and two containing each of the pure cultures. The medium used for the cultivation was APT (Evans & Niven, 1951) with a pH of 6.8. Changes in pH were followed continuously on a pH meter (Radiometer E 512) connected to a multichannel plotter (Radiometer REA 160).
Samples for microbiological examinations were removed every hour during the first eight hours and finally after 24 hours of incubation. The media used in the microbiological examinations were the same as those used in the practical experiments.

Results and Discussion

Practical experiments

Fig. 1 and 2 show growth of lactic acid bacteria during fermentation of the sausages. The values shown are mean values of 7 experiments. Standard deviation is indicated in the diagram.

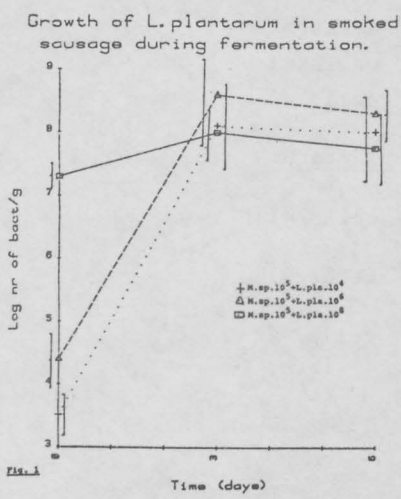


Fig. 1

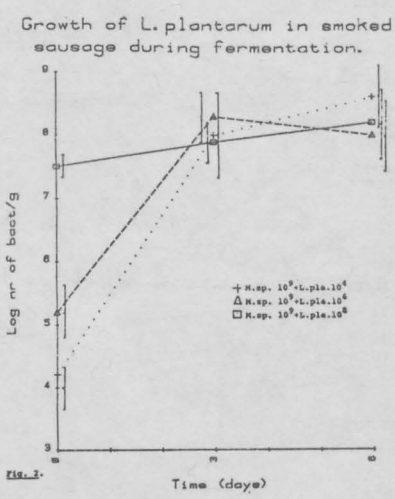


Fig. 2.

Low initial cell concentrations of lactic acid bacteria result in a rapid increase in cell numbers during the first three days of fermentation and in most cases a restriction of growth during the last three days of the fermentation period.

At high initial cell concentrations of lactic acid bacteria the increase in cell numbers during the fermentation period is very small. The number of lactic cid bacteria at the end of the 6 day fermentation period does not significantly differ even though large variations occurred in the initial cell numbers added.

Growth of micrococci during fermentation is fairly good at low initial cell concentrations (Fig. 3). Micrococcus sp. is more sensible to lower pH values and in combination with higher cell numbers of lactic acid bacteria growth is clearly inhibited. No growth occurred with high initial cell numbers of Micrococcus sp. (Fig. 4).

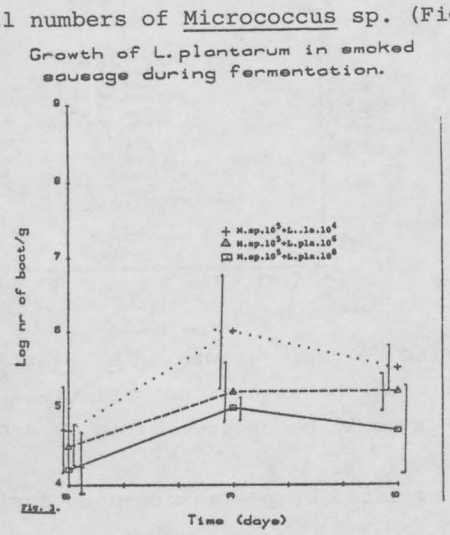


Fig. 3.

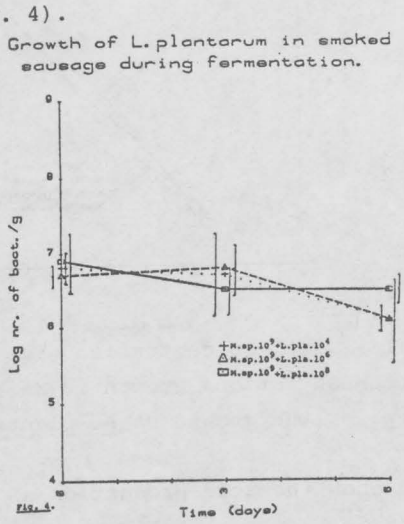


Fig. 4.

The lack of growth at high cell concentrations of both lactic acid bacteria and micrococci can be explained by several factors among which the most important would be exhaustion of nutrients, especially carbohydrate sources, and the rapid formation of large amounts of acid followed by a lowering of pH to inhibiting values. No stimulating effect of different cell concentrations of Micrococcus sp. on growth of lactic acid bacteria in the fermented sausages was observed (Fig. 1 and 2).

Changes in pH values during fermentation are shown in Fig. 5 and 6.

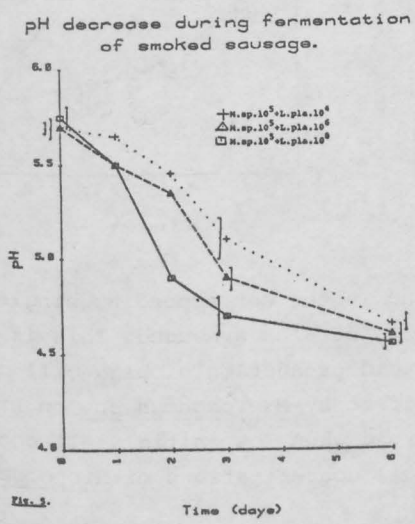


Fig. 5.

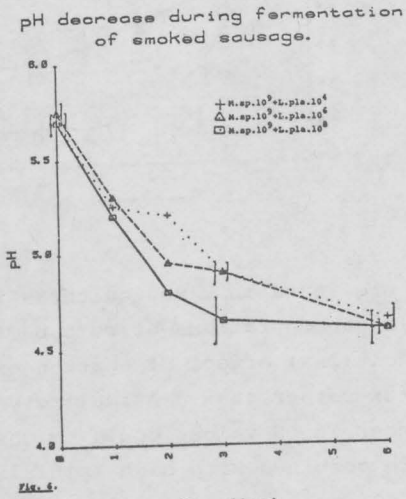


Fig. 6.

During the first two days of fermentation pH falls more rapidly in sausage samples with high initial cell concentrations of Micrococcus sp. added. This is probably not caused by a stimulating effect by Micrococcus sp. on acid production by lactic acid bacteria, but is more likely a result of a faster acid production at the early stages of fermentation at high cell concentrations of Microcuccus sp. The final pH values in the sausages are about the same irrespective of differences in initial cell concentrations.

G 12:6

Pure culture experiments

In laboratory experiments carried out on Micrococcus sp. and Lactobacillus plantarum in pure cultures the results show the same tendency as did the practical experiments. Fig. 7 shows growth curves of the two bacterial strains in single and in mixed batch cultures at high initial cell concentration of Micrococcus sp. combined with low initial cell concentration of L. plantarum. The values presented are mean values of 5 experiments.

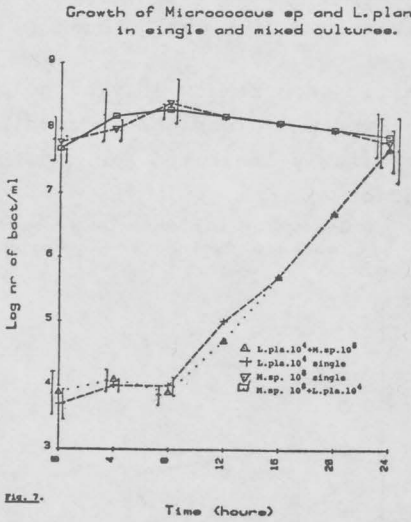


Fig. 7.

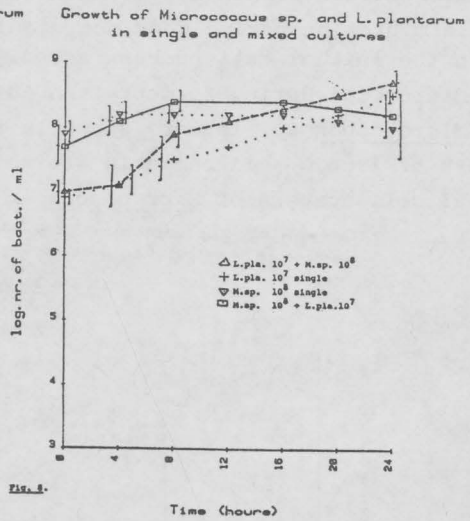


Fig. 8.

Again low initial cell concentration give rise to rapid growth of L. plantarum while at higher initial cell concentrations growth is restricted. No significant stimulating effect caused by Micrococcus sp. on the growth of L. plantarum could be observed (Fig. 7 and 8).

Fig. 9 and 10 show the acid production as indicated by pH measurements during 24 hours incubation at 30°C.

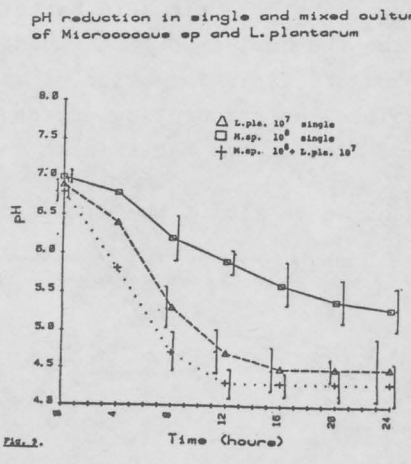


Fig. 9.

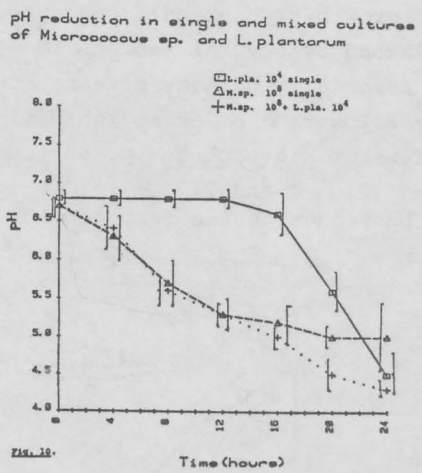


Fig. 10.

Lower pH values are found in mixed cultures than in the corresponding single cultures when high initial cell concentrations of both bacteria strains are used. This is probably the result of the additional effect of a stronger acid production of high cell concentration in the mixed cultures rather than a stimulatory effect by Micrococcus sp. on L. plantarum. No such differences in pH values could be observed when low initial cell concentrations of L. plantarum were combined with high initial cell concentrations of Micrococcus sp.

A third experiment where low initial cell concentrations of Micrococcus sp. in combination with somewhat higher initial cell concentrations of L. plantarum were used, shows that no stimulation of acid production measured by reduction in pH could be found. The growth curves show a clear inhibition of Micrococcus sp. in mixed culture with L. plantarum

(Fig. 11 and 12).

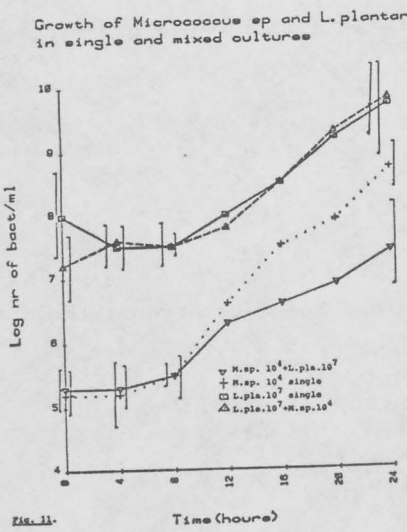


Fig. 11.

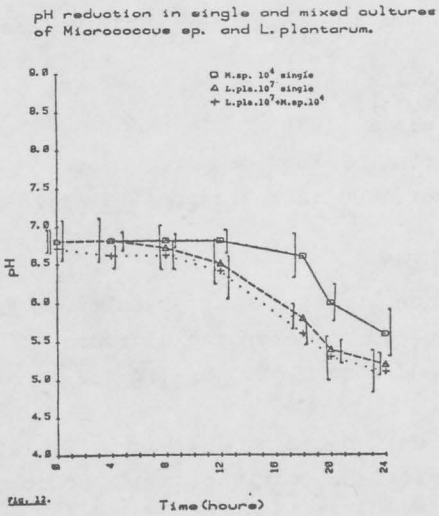


Fig. 12.

Conclusions

In these experiments no evidence could be found which would support the theory that live cells of Micrococcus sp. have a stimulating effect on growth and acid production of Lactobacillus plantarum.

References

Branen A.L. and Keenan T.W. 1969

Growth stimulation of Lactobacillus species by Lactic streptococci

Applied Microbiology 17 (2) 280-285.

Branen AlL. and Keenan T.W. 1970
Identification of a stimulant for Lactobacillus casei produced by Streptococcus lactis
Applied Microbiology 20 (5) 757-760.

Coretti K. 1977
Starterkulturen in der Fleischwirtschaft
Fleischwirtschaft 57 386-94

Daly C., LaChance M., Sandine W.E. & Elliker P.R. 1973

Control of Staphylococcus aureus in sausage by starter cultures and chemical acidulation
J. Food Science 38, 426-430.

Daly C., Sandine W.E. and Elliker P.R. 1972
Interactions of food starter cultures and food borne pathogens Streptococcus diacetilactis versus food pathogens
J. Milk Food Tech. 35 349-357

Evans J.B. and Niven C.F., jr. 1951
Nutrition of the heterofermentative Lactobacilli that cause greening of cured meat products
J. Bact. 62, 559-603.

Genigeorgis T. 1976
Quality control for fermented meats
J. Am. Vet. Med. Association 169, 1220-1228.

Hurst A. 1972
Interaction of food starter cultures and food borne pathogens: the antagonism between S. lactis and sporeforming microbes.
J. Mild and Food Tech. 35, 418-423.

Man J.C. de, Rogosa M. and Sharpe E. 1960
A medium for the cultivation of Lactobacilli
J. Appl. Bact. 23 130-138.

Nath K.R. and Wagner B.J. 1973
Stimulation of Lactic Acid Bacteria by a Micrococcus Isolate: Evidence for Multiple Effects
Applied Microbiology 26 (1) 49-55.

Nieuwenhof F.F. j., Stadhouders J. and Hup G. 1969
Stimulating effect of Lactobacilli on the growth of Propionibacteria in cheese
Neth. Milk and Dairy J. 23 287-289.

Niinivaara F.P. 1955
The influence of pure cultures of bacteria on the maturing and reddening of raw sausage
Acta Agral. Fennica 85 95-101.

Niskanen F.P. and Nurmi E. 1976
Effect of starter culture in Staphylococcal Enterotoxin and Thermonuclease Production in Dry Sausage
Applied and Environmental Microbiology 31 (1) 11-20.

Noon, Nancy and Reinbild R.W. 1976
Commensialism and Competition in mixed cultures of Lactobacillus bulgaricus and Streptococcus thermophilus
J. Milk and Food Tech. 39 (5) 337-341.

Nurmi E. 1966
Effect of Bacterial Inoculations on Characteristics and Microbial Flora of Dry Sausage
Dissertation, Helsinki 1966.

Reuter G. 1972
Versuche zur Rohwurstreifung mit Laktobazillen - und Mikrokokken - Starterkulturen
Die Fleischwirtschaft 52 465-73.

Smith J.L., Palumbo S.A. And Kissinger J.C. 1975 b, Huhtanen C.N.
Survival of Salmonella dublin and Salmonella typhimurium in Lebanon Bologna
J. Milk Food Tech 38 150-154.