

## ANTAGONISM OF STARTER CULTURES TOWARDS SOME MICROBIAL SPECIES

R. Brankova

Meat Technology Research Institute, Sofia

Summary

The interaction between some starter cultures (micrococci and lactic acid bacteria) and species from the undesirable microflora of sausages was studied under laboratory and industrial conditions. Starter cultures were found to have an antimicrobial activity of a different intensity in relation to all the test microorganisms used in the experiment. No growth of enterococci was found during the ageing of raw-dried sausages in which a starter of lactic acid bacterium and micrococcus was introduced. With a sufficient quantity of lactic acid microorganisms available in the beginning of the production process ( $10^7$  cells/g of sausage mass), the growth of enterobacteria is inhibited.

## ANTAGONISME DE CULTURES STARTERS A L'EGARD DE CERTAINES ESPECES MICROBIENNES

R. Brankova

Institut de recherches sur la viande, Sofia

Résumé

On a étudié l'action réciproque entre des cultures starters (microcoques et bactéries lactiques) et certaines espèces de la microflore indésirable des saucissons dans des conditions industrielles et de laboratoire. On a établi que les cultures starters possédaient une activité antimicrobienne, ayant une force différente à l'égard de tous les tests microorganismes, utilisés dans l'expérience. On n'a pas constaté de développement d'entérocoques lors de la maturation des saucissons secs dans lesquels a été introduite une culture starter de bactérie lactique et de microcoque. Lorsqu'il y avait une quantité suffisante de microorganismes lactiques au début du procès de fabrication ( $10^7$  de cellules/g de pâte de saucisson), le développement des entérobactéries était inhibée.

## ANTAGONISMUS DER STARTERKULTUREN GEGEN EINIGE MIKROBENARTEN

R. Brankova

Institut für Fleischwirtschaft - Sofia

Zusammenfassung

Es wurde die Wechselwirkung zwischen einige Starterkulturen /Mikrokokken und Laktobakterien/ und Arten der unerwünschten Mikroflora auf Würsten in Labor- und Produktionsbedingungen untersucht. Es wurde festgestellt, dass die Starterkulturen eine verschieden starke antimikrobielle Aktivität gegen alle im Versuch verwendeten Mikroorganismen aufweisen. Es wurde keine Entwicklung von Enterokokken während der Reifung von rohgetrockneten Würsten, welche mit Laktobakterien und Mikrokokken angesäuert wurden, festgestellt. Bei Anwesenheit von einer genügenden Menge milchsaurer Mikroorganismen bei Beginn des Produktionsprozesses / $10^7$  Zellen/g Wurstmasse/ wird die Entwicklung von Enterobakterien gehemmt.

## АНТАГОНИЗМ ЗАКВАСОЧНЫХ КУЛЬТУР К НЕКОТОРЫМ МИКРОБНЫМ ВИДАМ

Р. Бранкова

Институт мясной промышленности, София

Резюме

Исследовано взаимодействие между некоторыми заквасочными культурами (микрококками и молочнокислыми бактериями) и видами из нежелательной микрофлоры колбасных изделий, в лабораторных и производственных условиях. Установлено, что заквасочные культуры обладают антимикробной активностью различной силы ко всем использованным в опыте тест-микроорганизмам. Не обнаружено развитие энтерококков во время созревания сыро-вяленых колбас, в которых внесена закваска из молочнокислой бактерии и микрококка. При наличии достаточного количества молочнокислых микроорганизмов в начале производственного процесса ( $10^7$  клеток/г колбасной массы), развитие энтеробактерий подавляется.

## ANTAGONISM OF STARTER CULTURES TOWARDS SOME MICROBIAL TYPES

R. BRANKOVA

Meat Technology Research Institute - Sofia

In the filling mass for sausages, rich of different kinds of microorganisms /1,2/ are developed complex interactions between the separate kinds of microbial associations. The technological processes also lead to some readjustments in the contents of the initial microflora /3/, with gradually predominant microorganisms to become the micrococci and the acid lactic bacteria /4,5/. After the writings of Nurmi /6/ lactic bacteria inhibit the growth of micrococci, and both groups together - of the rest.

By the introduction of starter cultures, necessary for the correct ripening of the meat products, are obtained changes in the quantitative ratios between the different types of microorganisms /7/. It is established that there exist an antagonistic action of some kinds of starter cultures towards different pathogenic microorganisms /8, 9, 10, 11/. It is believed that one of the factors effecting the antagonistic action is the formation of oxydes, which can be inactivated by catalasae /12, 13/. The different sensibility for the different microorganisms is due to their ability to form catalasae.

The microbial antagonism could well be harnessed to help the keeping qualities of foods. Dry raw sausages represent the best example for this application /14, 15/. Our present work is a study of the interactions between some starter cultures and types of undesired microflora in sausages under laboratory and production conditions.

## MATERIAL AND METHODIC

We worked with 5 strains lactic acid microorganisms, 5 strains micrococci, and 10 types test microorganisms from the general microflora in sausages, for the tests under laboratory conditions. Under production conditions, is followed the change in the total count of the lactic acid microorganisms, the micrococci, the enterococci and enterobacteria in raw dried and fast ripening sausages during ripening and rying.

The interactions between the separate kinds are studied in the following way:

- introduction of culture in the agar prior to pouring into petri and after cooling of the media - surface inoculation of the studied microorganisms;
- stripe inoculation of the studied for antagonism strain on the surface of the nutritive media following the diameter of the petri, cultivation for 24 hours, so that the strain could develop and the products from its development to diffuse into the agar, and perpendicularly to the already grown stripe are inoculated the test microorganisms /16/. After 24 hours is seen the sterile zone between the studied for antagonism strain and the test microorganism;
- the count for the different kinds of microorganisms in the sausages has been determined following the allaccepted methods/degree dilutions in physiological solution and inoculations on differentiating hard nutritive media/.

The catalasae activity was determined qualitatively by hydrogen dioxyde.

## RESULTS AND DISCUSSION

The results from the antagonistic action of the micrococci towards the studied test microorganisms are given on table 1. The figures present mean values from 6 to 8 repetitions. The inoculations are made on meatpepton agar with a neutral pH. If we accept a sterile zone with a length of or under 8 mm for weak antimicrobial activity, and a zone above 15 mm for strong activity, we could say that strain M<sub>1</sub> has a strong activity towards one half of the studied test microorganisms. Micrococcus flavus is most sensitive to the action of the micrococci. Most ending are Candida albicans and Bac. idosus.

On table 2 are presented the results from the antimicrobial activity of 5 lactic acid strains. Most active from these is Lb<sub>3</sub>, and most sensitive from the used test microorganisms are Micrococcus flavus, and Staph. aureus. The big sensitivity of M. flavus towards the antagonistic activity of the starter cultures is due to the fact that it is catalase negative, while Bac. idosus has a strong catalase activity. A lower catalase activity have also Bac. mesentericus, and Sarcina lutea, with them the inhibition is more evident.

Data from the laboratory studies on the activity of micrococci and lactic acid bacteria demonstrate that all used in the tests starter cultures exhibit an inhibitory action on the development of the 10 test microorganisms, while the strength of this inhibition is different - from negligible /2-3 mm/ to 22 mm sterile zone between two investigated strains.

Tests with introduction of starter cultures in nutritive media were made with 2 strains of micrococci /M<sub>1</sub> and D<sub>11</sub>/ and 3 strains lactic acid bacteria /Lb<sub>2</sub>, Lb<sub>3</sub>, T<sub>5</sub>/ while as test organisms were used the same strains from the above cited reference. On places where in the middle is inoculated strain D<sub>11</sub>, do not develop 6 from the inoculated on the surface strains of test organisms, while 3 of them develop but very slightly. Under the conditions of the study good activity show strain T<sub>5</sub> and Lb<sub>3</sub>. It is possible that the microanaerophile conditions in the media assure the better development and activity of the starter cultures, as it is done under the conditions in the filling meatmass for the sausages.



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In a series of tests with introduction of mixed culture of lactic bacteria and micrococcus in the production of raw dried sausages is followed the development of the enterococci. It is established while in the control they attain a figure of  $10^7$  cl/g during the ripening period, with the presence of starter cultures in the media, it is seldom that enterococci could be isolated and this only during the initial stages of the production process.

In many test regimes with introduction of lactic acid microorganisms and increase of the temperature for ripening of the sausages during the first days, which leads to a rapid decrease in pH values is established that no enterobacteria could be isolated after two days of ripening in spite of the fact that in the beginning of the process existed  $10^7-10^8$  cl/g product.

Some trials were made for introduction of different quantities of lactic acid bacteria in the filling mass for sausages, and it was found that in the presence of glucose, with the smaller quantity of lactic bacteria and higher temperature, the respective quantity of the enterobacteria increases significantly. On fig 1 could be seen the curves reflecting the changes in the count of enterobacteria in 4 tests 1 and 2 are with introduced lactic acid culture in a quantity of  $10^7$  cl/g sausage mass, while 3 and 4 - with  $10^8$ ; 1 and 3 ripe at  $24^\circ\text{C}$ , while 2 and 4 - at  $27^\circ\text{C}$  in the presence of glucose. The absence of glucose does not inhibit the growth of bacterial mass from the lactic bacteria, but act upon their activity.

## CONCLUSIONS

1. The starter cultures used in laboratory tests /5 strains micrococci and 5 strains lacticbacteria/ possess an antagonistic activity towards all studied test organisms, exhibiting different degrees of activity - from 2-3 mm to 22 mm sterile zones.
2. Most sensible to the inhibiting activity of the starter cultures are *Micr. flavus* and *St. aureus*, which is explained with lack or weak catalase activity. Most important in a practical way is the inhibition of the growth of the spore forms and *E. coli* and *Staph. aureus*.
3. No development of enterococci was encountered during the ripening of raw dried sausages, when in the filling mass was introduced a mixed culture of micrococcus and lacticbacteria.
4. The starter cultures inhibit the development of the enterobacteria, when they are in a predominant quantity / $10^7$ / and with higher temperatures during the ripening / $24-27^\circ\text{C}$ /.

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Table 1

Antagonistic activity of micrococci towards test microorganisms from the generally existing microflora, expressed in mm sterile zone.

No	Test microorganism	Starter Culture			
		Str M <sub>1</sub>	Str P <sub>4</sub>	Str 1/N	Str 3N
1	<i>Bacillus subtilis</i>	16	11	10	13
2	<i>Bacillus idosus</i>	9	8	8	7
3	<i>Bacillus cereus</i>	9	7	9	10
4	<i>Bacillus mesentericus</i>	11	10	9	11
5	<i>Micrococcus flavus</i>	21	16	14	12
6	<i>Escherichia coli</i>	18	7	7	11
7	<i>Proteus OX<sub>19</sub></i>	14	9	8	12
8	<i>Staph. aureus</i>	15	9	15	5
9	<i>sarcina lutea</i>	13	12	8	10
10	<i>Candida albicans</i> *	9	6	7	9

Table 2

Antagonistic activity of lactic acid bacteria towards test microorganisms from the general flora, expressed in mm sterile zone.

No	Test microorganism	Starter Culture				
		Str Lb <sub>2</sub>	Str Lb <sub>3</sub>	Str Lb <sub>4</sub>	Str T <sub>5</sub>	Str D <sub>7</sub>
1.	Bacillus subtilis	10	17	11	9	5
2.	Bacillus idosus	4	18	9	7	6
3.	Bacillus cereus	7	19	8	7	7
4.	Bacillus mesentericus	3	16	9	9	6
5.	Micrococcus flavus	17	14	22	14	18
6.	Escherichia coli	16	14	9	8	4
7.	Proteus OX <sub>19</sub>	8	17	7	8	7
8.	Staph. aureus	14	14	15	12	5
9.	Sarcina lutea	9	21	12	7	6
10.	Candida albicans	3	4	3	2	3

