5-28

A STUDY ON THE ANTIBIOTIC SENSITIVITY OF PEDIOCOCCUS CEREVISIAE STRAINS 136 AND 167 USED AS STARTER CULTURE

K.Boschkova, St.Danchev
Higher Institute of Food and Flavour Industry, Plovdiv, Bulgaria

The growth and activity of starter cultures used in meat industry depend on many factors as pH-value, cultivation temperature, a_w-value e.t.c. One of the less studied factors in this point of wiew are the resudues of antibiotics in meat. They can be detected in meat when the animals are treated with the same antibiotics for a long time as well as when they are used a few days before slaughter (Billon, 1980). There is also a possibility for antibiotic resudues in meat if the antibiotics are used as stimulators. According to Billon(1980), Shotors(1978), Daubert(1983) from 1 to 37% resudues of antibiotics and other substances with antimicrobial action can be found in meat. The aim of this work is to study the antibiotic sensitivity of a starter culture of Pediococcus cerevisiae strains 136 and 167(1:1) used in the production of raw-dried non-comminuted meat products and to determine the minimal inhibitory concentrations of the antibiotics.

Material and Methods

Studies were carried out in model systems. The starter culture of P.cerevisiae strains 136 and 167 was used in the form of 24-hour boullion suspension (Hottinger yeast boullon-HYB) in inoculum level 10^6-10^7 viable cells/cm^3 culture media. The sensitivity of strains to various antibiotics is determined by the papier disk method (Skerman, Guschterov, 1977). Antibiotics tested were streptomycin, tetracyclin, ampicillin, penicillin, gentamycin, oxacillin, oleandomycin, erythromycin, kanamycin, chloramphenicol and carbenicillin at concentrations 10 and 30 mg/disk. Antibiotic sensitivity test was performed using Hottinger yeast agar with pH-values of 5,5, 6.0, 6.5. The zones of inhibition (sterile zones) was recorded in mm after incubation for 48 and 72 hours at cultivation temperatures of 16°C and 26°C. The antibiotic sensitivity of the strains was classified in one of the three categories, based mainly on Kirby-Bauer's categorization (from Tjagonenko, 1973). 1. The culture is sensitive to the antibiotics if the diameter of the sterile zone is more than 22mm 2. Resistance- the diameter is less than 10mm 3. Intermediate- the diameter is between 12-19mm. Minimal inhibitory concentrations (MIC) of the antibiotics were determined by the method of serial dilutions using HYB with pH-value 6.5 (Guschterov, 1977). MIC were read after incubation for 48-72 hours at cultivation temperature of 26°C.

Results and Discussion

The results obtained on the antibiotic sensitivity of starter culture P.cerevisiae strains 136 and 167(1:1) are presented in Tables 1, 2 and 3. The zones of inhibition are listed in Table 1 and 2 as mean values ($\bar{X}$) from n=45 measurements.

The data in Table 1 show that the starter culture is sensitive towards the antibiotics streptomycin, gentamycin, tetracyclin, chloramphenicol, ampicillin and kanamycin. A less zones of inhibition is observed in respect to the antibiotics oxacillin erythromycin, oleandomycin, penicillin and carbenicillin. It is clear that under experimental conditions P. cerevisiae strains 136 167(1:1) is resistance to these antibiotics. The results obtained show also that the antibiotic sensitivity of the investigated strains is greater at lower pH value 5.5 at both cultivation temperatures of 16°C and 26°C. The higher sensitivity at these conditios is probably the result of the weaker bacterial growth at low pH values. Therefore it could be expected that with the decrease in pH values in meat products with starter culture of P.cerevisiae strains 136 and 167 (1:1) during technological process especially during ripening and drying its sensitivity to resudual quantities of antibiotics increases. From Table 1 it is seen that the higher pH values (6.0 and 6.5), which are favourable for bacterial growth the zones of inhibition are nearly equal, but less than at pH value 5.5.

Independently of that the sizes of the sterile zones at both cultivation temperatures show any significant differences, they are higher at the lower cultivation temperature. This result is of interest to the production of raw-dried meat products. It indicates that the conditions close to those in industrial production especially the ripening and drying temperature of 16°C will effect more on P.cerevisiae to be sensitive towards resudues of the antibiotics.

The application of higher concentration of the antibiotics (30 mg/disk) inhibited

Y61

the growth of investigated bacteria in greater extent (Table 2).

P.cerevisiae growth inhibition zones to different antibiotics with a concentration of 10 mg/disk,mm

Table 1

Antibiotic	cultivation temperature of 16°C			cultivation temperature of 26°C		
pH	5.5	6.0	6.5	5.5	6.0	6.5
streptomycin	38	33	33	36	32	31
kanamycin	30	28	27	28	26	24
penicillin	10	8	6	10	7	7
tetracyclin	27	24	26	25	20	20
gentamycin	25	23	23	25	24	22
ampicillin	28	24	23	25	24	22
chloramphenicol	24	20	20	22	20	20
erythromycin	10	6	6	8	6	6
oleandomycin	8	6	6	6	6	6
carbenicillin	7	6	6	8	6	6
oxacillin	8	6	6	8	6	6

P.cerevisiae growth inhibition zones to different antibiotics with a concentration of 30 mg/disk,mm

Table 2

Antibiotic	cultivation temperature of 16°C			cultivation temperature of 26°C		
pH	5.5	6.0	6.5	5.5	6.0	6.5
streptomycin	38	33	33	36	32	31
kanamycin	30	28	27	28	26	24
penicillin	10	8	6	10	7	7
tetraciclyn	33	29	28	30	27	26
gentamycin	32	26	26	30	24	22
ampicillin	36	31	30	34	30	28
chloramphenicol	30	28	26	30	27	25
erythromycin	7	6	6	8	6	6
oleandomycin	11	9	7	10	8	8
carbenicillin	8	6	6	7	6	6
oxacillin	9	7	7	8	7	6

From Table 2 it is seen the starter culture possesses sensitivity to the same antibiotics as to the concentration of 10 mg/disk.The same tendency to higher inhibitory zones at pH value 5.5 and cultivation temperature of 16°C is observed.

Minimal inhibitory concentratios of the antibiotics, j/cm^3

Table 3

Antibiotic	concentration
streptomycin	0.000062
ampicillin	0.000175
gentamycin	0.000937
tetracyclin	0.000482
chloramphenicol	0.086
kanamycin	0.488

From the data given in Table 3 it is noticed that minimal inhibitory concentrations of some antibiotics are very low.The lowest minimal inhibitory concentration(0.000062 j/cm^2)is for a streptomycin.The results obtained for MIC are in accordance with those obtained for the antibiotic sensitivity of P. cerevisiae.It is therefore of importace that the growth and activity of the strains used as starter culture could be inhibited from minimal resudues of some antibiotics which could be detected in raw meat material.

Conclusions

1.P.cerevisiae strais 136 and 167(1:1) was found to be sensitive to the antibiotics streptomycin,gentamycin,ampicillin,chloramphenicol,tetracyclin and kanamycin.The antibiotic sensitivity of the strains was influenced by the pH value of culture medium and cultivation temperature.

2.The minimal inhibitory concentrations of the antibiotics were in the interval of 0.488-0.000062 j/cm^2.

Literature

1.Guschterov G,P.Andonov,T.Todorov,L.Kominkov,M.Starcheva,Prakticum po mikrobiologii Sofia,1977.

2.TjagunenkoU.,Instruktsia za opredeljane na chuvstvitelnosta na bacterii sprjamo

antibiotici,Medicina i fisk.,1979.
3.BillonJ.,S.H.Tac,RTVA,1980,19,164,9-16.
4.Daubert S.,La clinica veterinaria,1983,96,16-23.
5.Skerman V.B.D. Abstracts of Microbiological Methods.
6.Schothors M.,M.F.Lersden,J.F.Nows J.Assoc.Offic.Anal.Chem.,1978,61,5,1209-1213.

preparations, followed by those with GDL. Comparative studies with results similar to those obtained by Krylova et al., were made also by Winter (1982), Kissinger (1981) and by many other authors.
The objective of this work was, by using starter microorganisms, to substitute partially or completely GDL in the manufacture of fast-ripening meat products.

MATERIAL AND METHODS

In the experimental work, use was made of lactic acid microorganisms, micrococci and yeasts: either as 24-hour broth cultures, or freeze-dried. GDL was introduced in control variants or in experimental ones in combination with starter cultures.
The experiments were made while observing the technological requirements towards the manufacture of fast-ripening salami with the introduction of GDL or starter cultures The sausage meat was filled into casings of a diameter of 55 mm or 80-100 mm. Active ripening took place at 24-26°C for 48 hours. Then followed 24 hours of smoking at 18-20°C. The sausages of a casing diameter of 100 mm are rolled in spices after the stripping of the casing. Then drying followed at 14-16°C and a relative air humidity of 90-95% which was gradually reduced to 80%.
The growth of the major groups of microorganisms in the experimental samples was determined by standard microbiological methods. Changes in pH were monitored (potentiometrically). In some of the experiments, pigment level was determined, in terms of per cent nitroso-myoglobin, and residual nitrites (after Bulgarian State Standards /BDS/).

RESULTS AND DISCUSSION

I. Experiments with the combined application of starter cultures and GDL

Those were conducted using a technology for the manufacture of fast-ripening sausages in the following variants: (1) with 0,5% GDL; (2) with 0,5 % GDL + 10^5 cells/g of Lactobacillus plantarum, and M. varians; (3) with 0,2% GDL + 10^5 cells/g of Lactobacillus plantarum, and Micrococcus varians; and (4) 10^6 cells/g of Lactobacillus plantarum, and Micrococcus varians.
Comparative studies were made after active ripening and after two weeks: in finished product. The results of the study of the major groups of microorganisms are shown in Table 1.
It is obvious from the table that the presence of GDL results in the growth of smaller counts of lactobacilli and micrococci which is one of the reasons for the differences in the flavours of those products. Variant 1 sausages were sourer and had an insufficiently good flavour, but the best binding and colour. The best flavour qualities

Table 1. Microorganism counts in fast-ripening sausages made with GDL and starter cultures

Variant No.	Ageing time (days)	Microorganism counts (cells/g)			
		Lactobacilli	Micrococci	Coli-titre	Proteus-titre
1	2	$8,1 \times 10^5$	$5,4 \times 10^3$	10^{-1}	$>10^{-1}$
	14	$5,5 \times 10^7$	$2,4 \times 10^2$	$>10^{-1}$	$>10^{-1}$
2	2	$1,3 \times 10^7$	$2,5 \times 10^3$	10^{-1}	$>10^{-1}$
	14	$8,5 \times 10^7$	$3,1 \times 10^3$	$>10^{-1}$	$>10^{-1}$
3	2	$1,1 \times 10^7$	$4,8 \times 10^4$	10^{-1}	10^{-1}
	14	$1,5 \times 10^8$	$5,4 \times 10^3$	$>10^{-1}$	$>10^{-1}$
4	2	$4,2 \times 10^7$	$3,3 \times 10^5$	10^{-1}	10^{-1}
	14	$3,5 \times 10^8$	$7,2 \times 10^3$	$>10^{-1}$	$>10^{-1}$

were found in variant 4, but colour there was slightly paler than in variant 1.
The total scores of the finished products indicated that it was possible to reduce the GDL level used and, by combination with starter cultures, to compensate the disadvantages of both additives observed on their separate application.

II. Experiments on the substitution of GDL by starter cultures

(1) Experiments with 24-hour broth cultures: The following variants were prepared; (i) with the introduction of Streptococcus diacetilactis at he lavel of $4,0 \times 10^6$ cells/g of sausage meat; (ii) with Candida utilis, $4,0 \times 10^6$ cells/g; (iii) Lactobacillus plantarum, $1,6 \times 10^6$, and Micrococcus varians and Staphylococcus saprophyticus each $8,0 \times 10^5$ cells/g: sugar level was raised to 0,6% in this variant (0,4% being introduced in the remaining variants); (iv) the same levels of microorganisms were introduced as in variant 3, + Candida utilis, $8,0 \times 10^5$ cells/g; (v) control, 0,5% GDL related to the sausage meat without the introduction of starter cultures. Casings of a diameter of 100 mm were used.
Comparative studies were made in finished products after 14 days. The results of the microbiological analyses are shown in Table 2.

Table 2. Microorganism counts in fast-ripening sausages with starter cultures compared to controls with GDL

Variant No.	Microorganism counts (cells/g of product)				
	Lactobacilli	Micrococci	Yeasts	Coli-titre	Proteus-titre
1	$3,2 \times 10^7$	$1,3 \times 10^2$	below 10	$>10^{-1}$	$>10^{-1}$
2	$4,0 \times 10^7$	$7,8 \times 10^3$	$1,1 \times 10^3$	10^{-1}	$>10^{-1}$
3	$1,0 \times 10^8$	$7,2 \times 10^2$	$3,0 \times 10^2$	$>10^{-1}$	$>10^{-1}$
4	$8,0 \times 10^7$	$6,0 \times 10^3$	$1,0 \times 10^3$	10^{-1}	$>10^{-1}$
5	$7,0 \times 10^7$	$1,8 \times 10^2$	below 10	$>10^{-1}$	$>10^{-1}$

As obvious from the table, the highest lactobacilli counts were found in variant 3 where the level of carbohydrates was higher. The presence of micrococci was affected by the presence of lactic acid streptococci, GDL and a higher level of sugar when acid production is enhanced.
Finished product pH values and also the per cent nitroso-myoglobin and residual nitrites are shown in Table 3.

Table 3. Comparative data by some indices of finished experimental products

Variant No.	% sugar introduced	Indices		
		pH	Nitroso-myoglobin (% total pigment)	Residual nitrites (mg%)
1				
2	0,4	4,85	75,60	0,90
3	0,4	4,80	74,13	0,72
4	0,6	4,60	76,12	0,54
5	0,4	4,70	75,50	0,90
	0,4	4,50	76,90	0,90

It can be seen that the lowest pH values were obtained in the variants with GDL (4,50), followed by the one with 0,6% sugar (4,60). Nitroso-myoglobin level is similar, the highest percentages being found in variants with GDL and with 0,6% sugar. Residual nitrites are lowest in variant 3: with 0,6% sugar.
Variants 3 and 4 possessed the best expressed flavour qualities. Control samples with GDL were sourer in taste and had a slight unpleasant smell.

(2) Experiments with freeze-dried starter cultures: The experiments were conducted in three major variants: (1) sausage meat was filled into casings of a diameter of 55 mm; (2) 100 mm dia casings were filled; (3) as in variant 2, only with the addition of 0,6% sugar instead of the 0,4% introduced in the first two variants. The samples were put under the same technological conditions, of the manufacture of the Zakuska salami. Freeze-dried microorganisms were introduced at such levels as to provide $2,5 \times 10^5$ cells of Micrococcus varians/g of product, $2,8 \times 10^4$ cells/g of Candida utilis, and $2,2 \times 10^5$ cells/g of Lactobacillus plantarum.
Figure 1 shows graphically the changes of lactobacilli and micrococci counts and pH values in the three experimental variants. It is obvious that the lactobacilli counts in sausages of a larger diameter are higher than those in the variants of a diameter of 55 mm. This results in reduced micrococci levels. The lowest pH values were obtained upon the additional introduction of sugar.
At sensory evaluations, a good cut surface was found, and also a fine and stable colour, a pleasant sourish taste of the finished products. No flavour differences were found on the application of different levels of sugar. Further, no differences were found attributable to the form of the cultures introduced: a 24-hour broth culture, or a freeze-dried preparation. On the longer refrigerated storage of samples, variants with starter cultures were found to keep, and even to improve in flavour owing to continuing enzymatic processes. At the same time, the storage life of the samples with GDL was more limited.

CONCLUSIONS

(1) Good quality fast-ripening sausages can be manufactures with a reduced GDL level combined with the introduction of starter cultures.
(2) Meat products such as the Zakuska salami can be manufactures using starter cultures in the stead of GDL.
(3) Combinations of lactobacilli and micrococci, or of lactobacilli, micrococci and yeasts, can be applied as broth cultures or as freeze-dried preparations. It is necessary that the inoculation of microorganisms provides counts in the range of $10^6 - 10^7$ cells/g of sausage meat.
(4) The advantages of the application of starter cultures over the usage of GDL are:

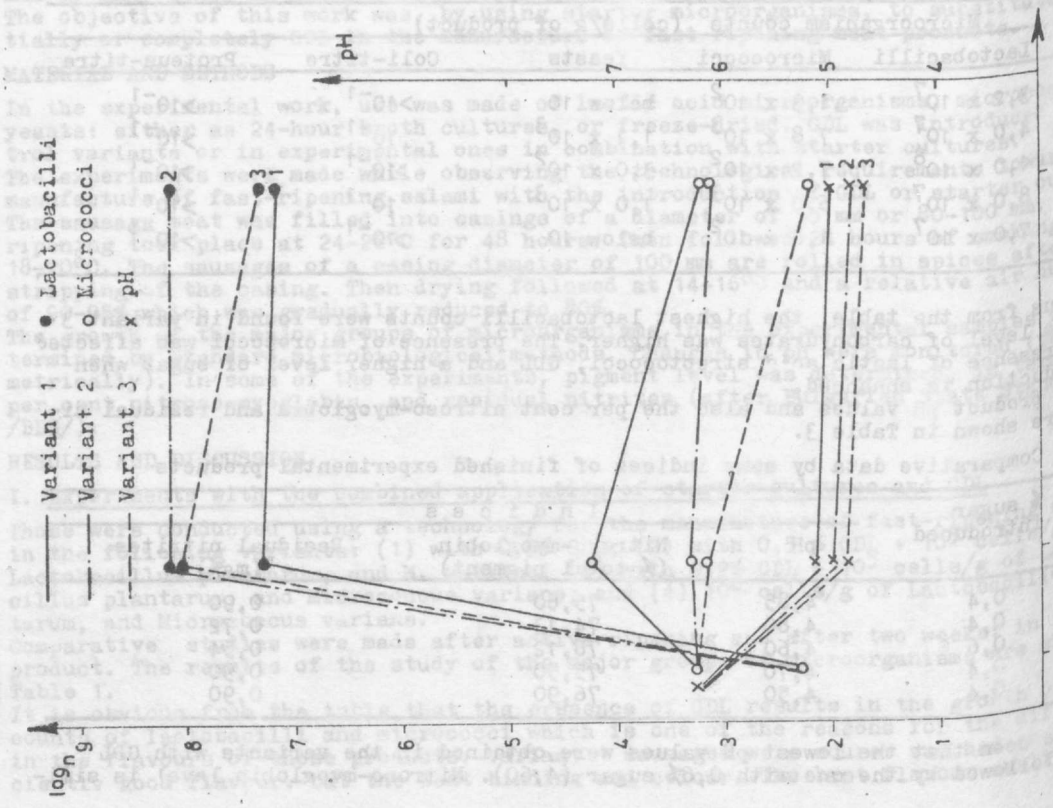

Fig. 1. Changes in lactobacilli and micrococci counts and in pH in ripening sausages

better flavour of the products; a longer storage life; it is not imperative to fill the sausages within a short time of making the sausage meat, as in GDL usage, since meat particle binding is delayed till after 24 hours.

REFERENCES

1. Krylova V. et al., 1970, Trudy VNIIMP, vyp. XXIII.
2. Krylova V. et al., 1976a, XXII Eur. Meet. Meat Res. workers, Malmö, 4:3-6.
3. Krylova V. et al., 1976b, ibid., 5:1-4.
4. Krylova V. et al., 1982, Myasnaya industriya SSSR, 1, 18-20.
5. Marshall, G.A., 1980, Myasnaya industriya SSSR, 6, 35-36.
6. Patent No. 1692 174/73, FRG, A means of improving the quality of raw sausages.
7. Cuvlek, B. et al., 1981, Tehnologija mesa, 22, 5, 135-139.
8. Frey, W., 1983, Fleischerei, 34, 5, 517-520.
9. Gallert, H., 1973, Fleischerei, 24, 6, 29-30.
10. Kissinger, H., 1978, Fleischerei, 29, 3, 41-42.
11. Modić, P., L. Turubatović, 1983, tehnologija mesa, 10, 298-302.
12. Rede, R., T. Lazić, 1983, Tehnologija mesa, XXIV, 9, 247.
13. Sair, L., 1964, US Patent, 3 122,442
14. Winter, F., 1982, Fleischerei, 33, 9, 576-690; 10, 577-691.