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STUDIES ON THE ACTIVITY OF MICROCOCCI IN VIEW OF THE SELECTION OF STARTER CULTURES. I. CATALASE ACTIVITY OF STRAINS ISOLATED FROM RAW-DRIED MEAT PRODUCTS

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SUMMARY

The effects of various factors: different nutritional media, pH-values (5,0, 5,5, 6,0, and 6,8), temperatures (20, 25, 30°C) and broth culture ages (24, 48 or 72 h), on characterizing the catalase activity of micrococcus strains in view of their application as starter cultures, were studied.

Ascorbic acid and Na-citrate stimulated growth and affected catalase activity. In some strains, the addition of dry milk had a positive effect on the counts and the catalase activity of the strains investigated. The greatest count of cells was obtained upon the introduction of sucrose into the media.

Differences in the enzymatic activities of the individual strains were found on the 24th h of cultivation.

Atendency can be observed towards the enhancement or preservation of catalase activity until 72 h of microbial growth.

In the pH range shown, the investigated strains demonstrated an increase in catalase activity at pH 5,5 compared to pH 5,0, which remained the same up to pH 6,8.

The growth temperature of 25°C was found to be more appropriate than 20 or 30°C for catalase activity.

INTRODUCTION

It is well known that the accumulation of peroxides in metabolism causes defects in colour formation and undesirable oxidative changes in fats during the manufacture of raw-dried meat products. Some lactic acid microorganisms produce hydrogen peroxide which oxidizes meat myoglobin to green purine compunds (Tjaberg et al., 1969).

Catalase (ES 1.11.1.6) is an oxidoreductase which is synthesized in animal tissues, vegetable materials and almost all cytochrome-containing aerobic microorganisms. In meat and meat products, it catalyzes the reaction of degrading peroxides to water and oxygen.

Upon the drying of raw sausages the catalase activity of meat decreases (Walters et al., 1974). The presence of a microflora having a catalase activity contributes to the compensation for the reduced activity of meat catalase (Reuter, 1972) since the optimum pH-value of microbial catalases is within the acid range (Rozier, 1971).

The OBJECTIVE of the present work was to study the catalase activity of some micrococcus strains isolated from raw-dried meat products typically found in this country.

MATERIALS AND METHODS

Changes that were followed in the activity of micrococci selected for starter cultures, depended on the composition of the nutritional medium, the age of the culture, the temperature of strain cultivation, and the medium pH.

Three micrococcus strains were used: M_{115}, M_{83}, and M_{6R}, isolated from raw-dried meat products originating from different regions of Bulgaria.

Determinations were made of the volumetric and specific activities of the micrococci by Bergmeyer's spectrophotometric method (1955). Protein was determined according to Bradford (1976) using ready-made reagents of Bio RAD (1979) and the counts of cells (N) per ml of sample, on MSA. 6 different nutritional media were prepared, differing by the presence of individual components: medium I, fundamental medium formulated on the basis of meat peptone broth (MPB); in medium II, peptone was substituted by dry milk; in medium III, the quantity of sodium chloride was increased up to 1%; in medium IV, 0,04% of ascorbic acid was added; in medium V, the carbohydrate source was sucrose (instead of glucose); and 0,5% of sodium citrate was added to medium VI.

In all the media, the effects of culture age (24, 48, and 72 hours) on enzymatic activity were followed.

Nutritional medium I was used for the study on the effects of pH-values (5,0, 5,5, 6,0, 6,8) and of temperature (20°, 25°, 30°C) on the enzymatic activity of all the three strains under investigation.

RESULTS AND DISCUSSION

The formulating of an optimum nutritional medium containing the nutrients necessary for the growth of microorganisms, is one of the major tasks in the preparation of starter cultures.

The effect of nutritional medium composition on the counts and the growth of the micrococcus strains studied is presented in Fig. 1. It is obvious that the requirements of the individual strains towards the composition of the nutritional medium are different. The highest counts for strain M6R were observed on the 24th hour when cultivating with the addition of dry milk (nutritional medium II), and sucrose (medium V). On the 48th and the 72nd hour of growth, a general trend towards a reduction in the counts of microorganisms was found. An exception was provided by the strains cultivated with the addition of sodium citrate (medium VI) where, at 72 h, microorganism counts rose again. This trend was also found in strains M83 and M115. An exception was provided by strain M83 cultivated with sucrose as the carbohydrate source (medium V) and with the introduction of sodium citrate (medium VI), in which the highest counts were observed on the 48th hour.

The growth of strain M115 was favoured by 1% NaCl (medium III) and 0,04% ascorbic acid (medium IV).

In this study, no correlation was found between the counts of microorganisms and the enzymatic activity produced, which is in conformity with literature data (Alford, 1960).

The results obtained on the catalase activity of the strains in the different nutritional media are shown in Table 1. It is obvious that the presence of sucrose (medium V) enhances catalase activity in all the three strains under investigation. Activity also rises with the rise of substrate concentration from 45 nM of H_2O_2 to 90 nM of H_2O_2 at a constant temperature of the experimentation.

Enzymatic activity was also favoured by the addition of 1% of NaCl, as well as of 0,04% of ascorbic acid.

The effects of culture age and nutritional media compositions on enzymatic activity are shown in Fig. 2.

Catalase activity differs not only in individual microorganism species, but also in individual strains within the same species. It depends on the age and the stage of growth, on the physiological condition and a number of further indices (Kultugin, 1926).

The results (Fig. 2) indicate, that there are differences in the catalase activity between the individual strains cultivated in the same nutritional medium, and also with the different nutritional media. The highest enzymatic activity at 24 h was observed in all the three strains cultivated in a medium with sucrose (volumetric activity, 398-448 U/ml of sample). A positive effect was also exerted by the presence of 1% of NaCl, and 0,04% of ascorbic acid. The lowest activity was demonstrated by the strains cultivated in the presence of milk in the culture medium (104 to 133 U/ml of sample).

Culture age affects the catalase activity of the strains likewise. It can be seen in the Figure 2 that, up to the 72nd hour, activity rises to a different extent, depending on the nutritional medium.

The effects of cultivation temperature and medium pH on the counts and the catalase activity of the micrococci under investigation are shown in Fig. 3 and Fig, 4. Even though the counts of microorganisms are affected by pH, their catalase activities are similar with the pH values studied, when microorganism counts in the culture studied are 10^8 cells/ml. In M115 and M6R, the largest counts are found with a temperature of 30°C, and in M83, at 25°C. Catalase activity is the highest for all the three strains at 25-30°C.

The results obtained indicate that, under the conditions in the sausage meat: pH reduction, the presence of NaCl, ascorbic acid and sucrose, and with a definite temperature regime in the period of the ripening of raw-dried meat products, it is possible to ensure the optimum activity of the catalase produced by the micrococci studied.

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Table 1. Effect of the nutritional medium on the catalase activity of micrococci, U/ml of sample

24-h micrococci broth solutions	Substrate concentration (nM H_2O_2)	Nutritional media					
		I	II	III	IV	V	VI
M115	45	296,9	104,1	253,1	327,5	397,7	119,7
	90	463,9	223,0	463,9	543,2	767,9	327,5
M83	45	341,6	163,8	297,0	253,1	445,4	268,3
	90	564,0	NE	618,6	518,0	824,8	428,3
M6R	45	400,5	133,4	297,0	353,5	428,3	371,2
	90	593,9	268,3	618,0	586,1	718,4	530,2

U, catalase units after Bergmeyer.

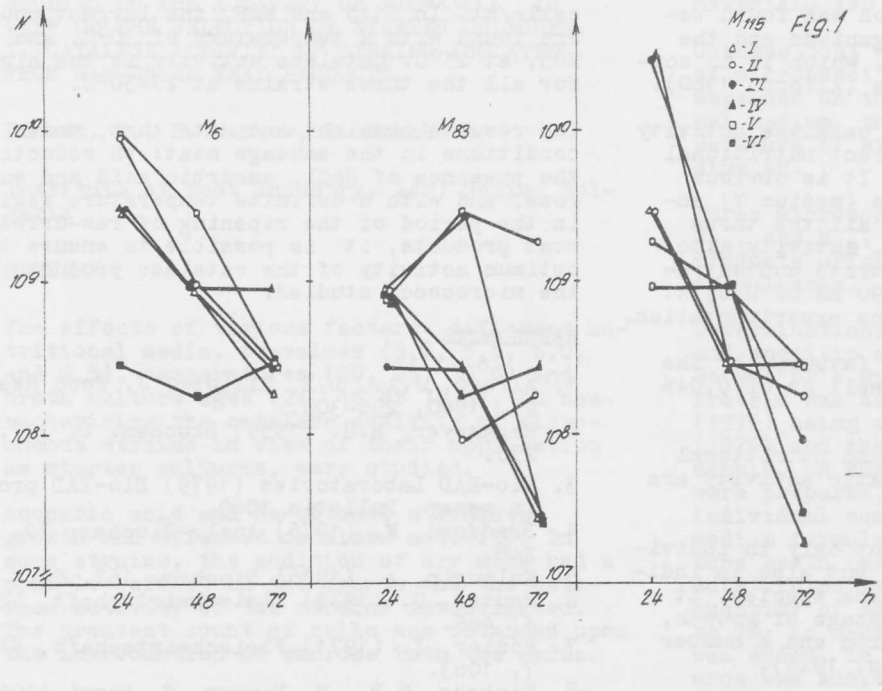


Fig.1

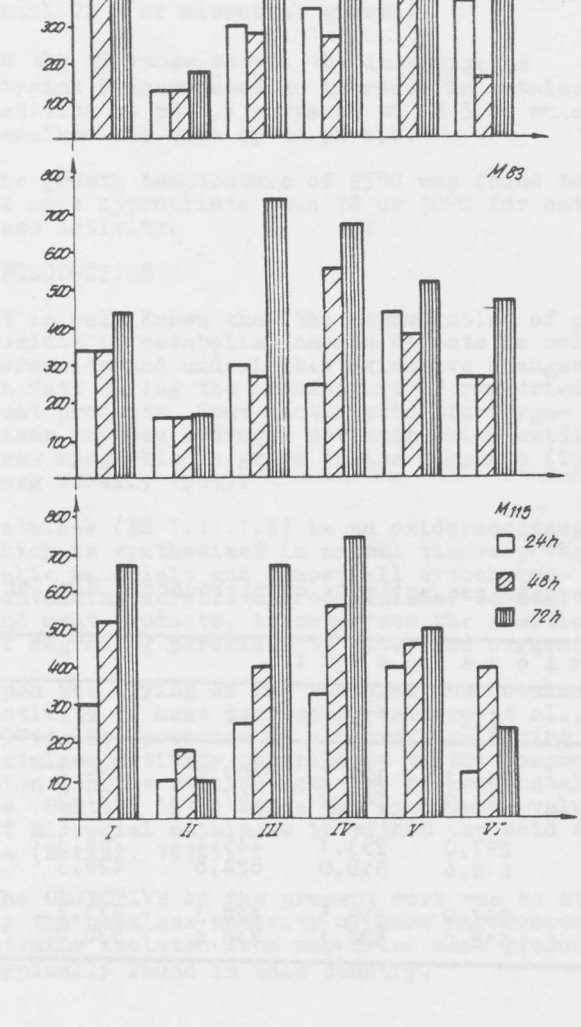
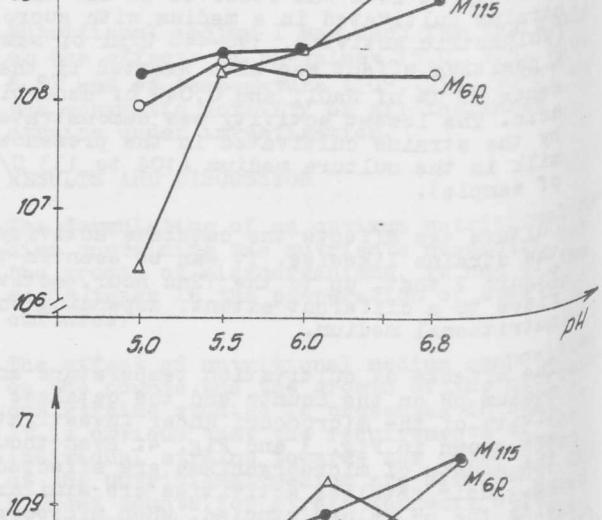


Fig.2



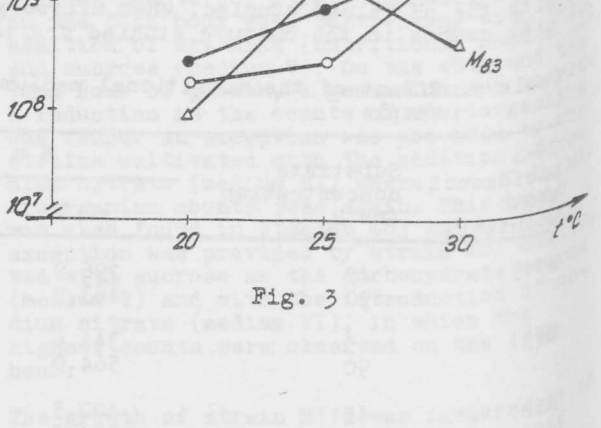


Fig. 3

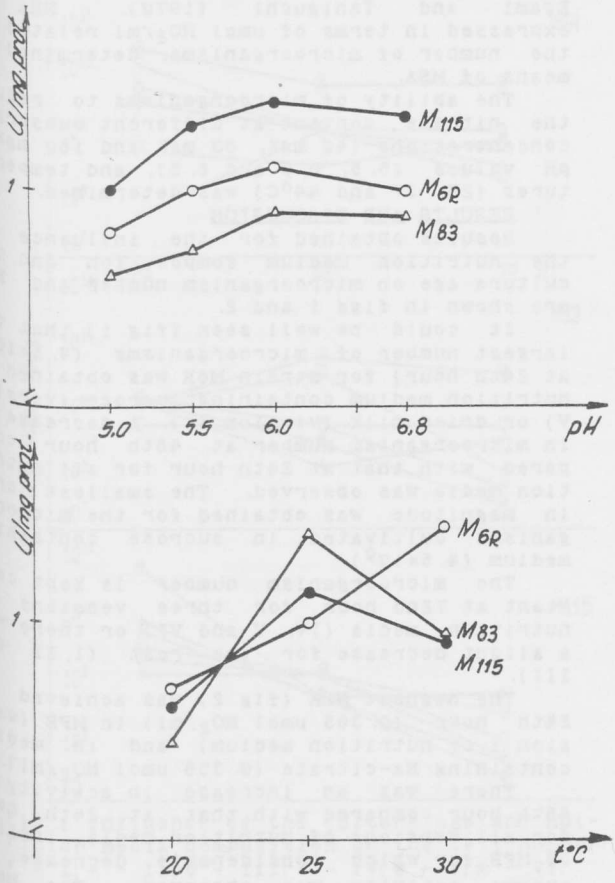

Относ. ед.
1
M 115
M 6R
M 83
5.0
5.5
6.0
6.8
pH
Относ. ед.
1
M 6R
M 83
M 115
20
25
30
t°C

Fig. 4