STUDIES ON THE ACTIVITY OF MICROCOCCI IN VIEW OF THE SELECTION OF STARTER CULTURES.
II. NITRATE REDUCTASE ACTIVITY OF MICROCOCCUS VARIANS STRAINS M6R, M115, M83

Brankova R., E. Markov, Ts. Tsvetkov, B. Dineva

ABSTRACT

The effect of different versions of nutritious media, pH values(5.Ø, 5.5, 6.Ø, 6.8), temperature (2Ø, 25, 3Ø°C) and broth culture age (24, 48 or 72 h) on characterizing the nitrate reductase activity of micrococcus strains in view of their application as starter cultures was studied.

The dependance of nitrate reductase activity on different conditions of incubation was studied as well. The development of microbial cultures and enzymatic activity depend on the content of nutrients in the cultivation medium.

The optimum pH value of the cultivation medium was found to be 6.8 and the optimum temperature 25°C for all three strains studied.

Retaining or enhancement of nitrate reductase activity with culture aging upto 72 hours was observed.

The pH optimum of the incubation solution for all three strains investigated was determined to be a value of 6.Ø.

The reduction of nitrates with the three substrate concentrations studied was nearly the same.

INTRODUCTION

In order to select active starter cultures distinct criteria have been established (Niinivaara 1955, Nurmi 1966, Kuusela 1978). The ability of the starter cultures, applied in production of raw-dried meats, to reduce the nitrate content is of great importance(Pohja 196Ø, Junker & Liepe 1981, Pfleil & Liepe 1974, Liepe 1978), as well as the favorable effect exerted in case of nitrite treatment, since a certain part of nitrite is transformed into nitrate (Mohler 1974). The nitrate reductase activity (NRA) is important at treatment with reduced nitrite quantities, otherwise the absence of reduction will raise the nitrate content during the production process and the nitrite will be insufficient for the colour formation.

The NRA depends on pH, temperature, substrate concentration, etc. (Puolanne, 1977).

The goal of the present study is to investigate NRA of micrococci employed as starter cultures.

MATERIALS AND METHODS

Micrococcus M115, M83 and M6R isolated from specific raw-dried meat products, were used.

The investigated strains were cultivated in six versions of nutritional media, which differ in component composition, as it was described in our preceding study (Dineva et al. 1987). The culture age influence (24, 48 and 72 hours) on enzyme activity of the different versions was studied.

For the nutrition medium version I the influence of pH values (5.Ø, 5.5, 6.Ø, 6.8) and temperature (2Ø, 25 and 3Ø°C) on NRA was investigated.

NRA was determined according to the method described by Puolanne (1977), which is a modification of the method described by Egami and Taniguchi (197Ø). NRA is expressed in terms of μmol NO_2/ml related to the number of microorganisms, determined by means of MSA.

The ability of microorganisms to reduce the nitrate content at different substrate concentrations (4Ø mg%, 8Ø mg% and 16Ø mg%), pH values (5.5, 6.Ø and 6.5), and temperatures (25, 37 and 44°C) was determined.

RESULTS AND DISCUSSION

Results obtained for the influence of the nutrition medium composition and the culture age on microorganism number and NRA are shown in figs 1 and 2.

It could be well seen (fig 1) that the largest number of microorganisms ($9.5*1Ø^9$, at 24th hour) for strain M6R was obtained in nutrition medium containing sucrose (version V) or dried milk (version II). A decrease in in microorganism number at 48th hour compared with that at 24th hour for all nutrition media was observed. The smallest drop in magnitude was obtained for the microorganisms cultivated in sucrose containing medium ($4.5*1Ø^9$).

The microorganism number is kept constant at 72nd hour for three versions of nutrition media (IV, V and VI) or there was a slight decrease for the rest (I, II and III).

The highest NRA (fig 2) was achieved at 24th hour (Ø.363 μmol NO_2/ml) in MPB (version I of nutrition medium) and in medium containing Na-citrate (Ø.358 μmol NO_2/ml).

There was an increase in activity at 48th hour compared with that at 24th hour for all versions of nutrition media, instead of MPB for which considerable decrease in enzyme activity was observed. The most active were the microorganisms cultivated in medium containing Na-citrate (Ø.6Ø5 μmol NO_2/ml).

It was observed an increase in ability of microorganisms to reduce the nitrate content at 72nd hour compared with that at 48th hour as well as at 24th hour. Cultivated in MPB microorganisms retain their enzyme activity unchanged at 48th hour. The highest NRA was observed for microorganisms cultivated in medium containing Na-citrate and ascorbic acid.

The results obtained for M83 showed equal number of microorganisms at 24th hour for all versions of nutrition media (fig 1). The largest number of cells was observed at 48th hour in medium containing sucrose and Na-citrate, and at 72nd hour in medium containing sucrose.

The NRA was highest at 24th and 72nd hour for microorganisms cultivated in medium containing Na-citrate (fig 2). When the cultivation medium was MPB, NRA at 48th and 72nd hours was lower compared with that at 24th hour. This was in contrast to the rest of nutrition media, where the activity was increased with the age of the cultures. The strain micrococcus M115 revealed higher enzyme activity compared to the other two strains.

The largest number of cells (fig 1) at 24th hour was obtained using cultivation medium containing 1% NaCl ($4.5*1Ø^{1Ø}$). At 48th hour the number of cells is lower than at 24th hour and almost the same for the different versions of nutrition media. The number of microorganisms went on decreasing upto 72nd hour.

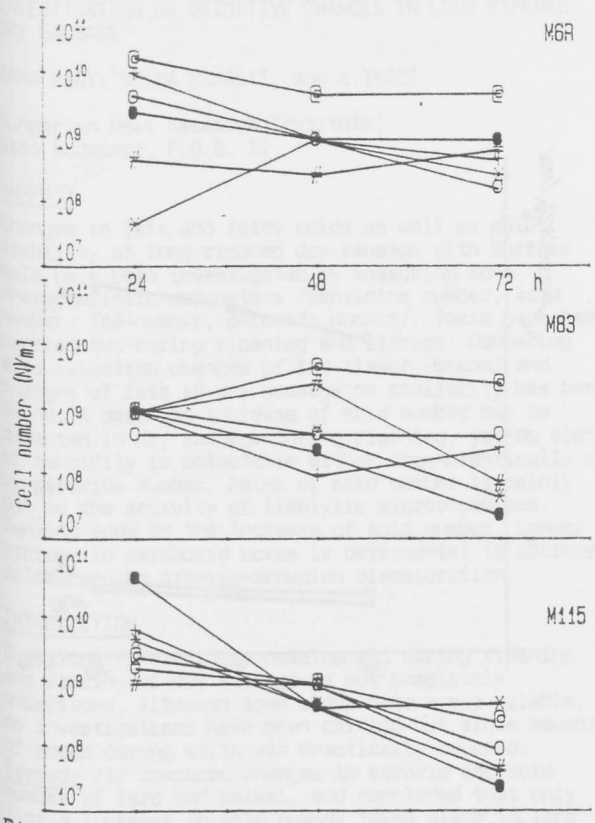

Fig. 1 Influence of the culture age and nutrition media composition on the cell number: * - I; + - II; o - III; ▪ - IV; @ - V; # - VI.

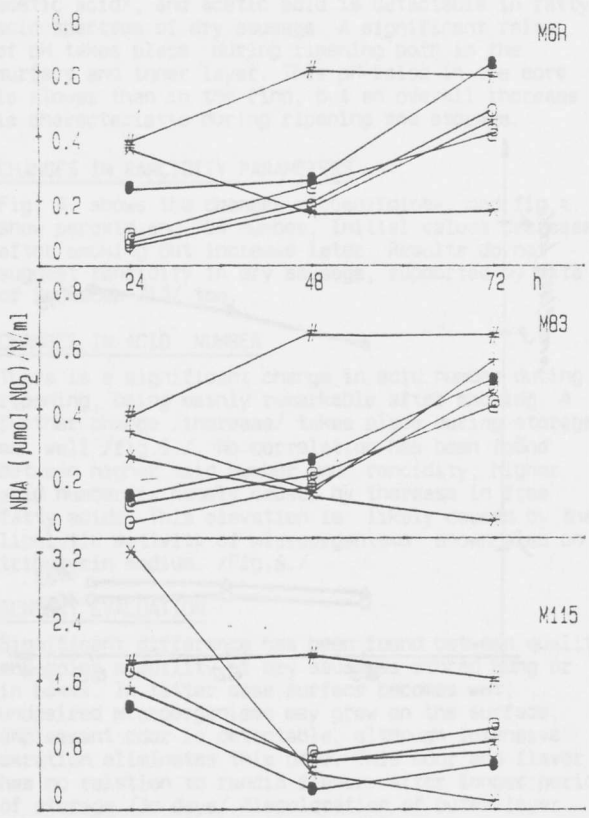

Fig. 2 Influence of the culture age and nutrition media composition on the NRA: * - I; + - II; o - III; ▪ - IV; @ - V; # - VI.

The NRA of M115 for 24 hour broth cultures had it highest value in MPB and went down gradually at 48th and 72nd hour. This reduction in enzyme activity was observed in all versions of nutrition media. The only exception was the increase in activity from 24th to 72nd hour for microorganisms cultivated with Na-citrate. It was generally observed higher NRA for all three strains cultivated in medium containing Na-citrate.

The optimum pH value regarding the number of the cells and the extent NRA was 6.8 for all three strains. After comparing cultivation temperatures, optimum temperature was determined to be 25°C.

The ability of micrococcus M6R, M115, and M83 to reduce the nitrate content was investigated at three substrate concentrations (Table 1). Microorganisms were cultivated in three versions of nutritional media.

According to Puolanne (1977) the velocity of the enzyme reaction is not substantially changed at substrate concentrations from 100 to 600 ppm. This was the reason to use substrate concentrations of 400 to 1600 ppm. We found that at a nitrate concentration of 40 mg%, NRA of M6R was 70-75% of that at the nitrate concentration of 160 mg% at cultivation in MPB or MPB + Na-citrate. The same trend was observed for M83 and M115 as well.

It is necessary to determine optimum conditions for each strain separately in order to specify their activity (Kuusela et al. 1978, Puolanne 1977).

The influence of pH values on NRA of studied strains is shown in fig 3. The optimum was observed at pH of 6.0 for M6R and

Table 1. NRA (µmol NO_2/ml) related to the substrate concentration of the incubation solution (pH 6.0, temperature 44oC, and 108 cells/ml). The values are averaged from the results of six experiments.

M	C mg %	NUTRITION MEDIUM I	IV	VI
M6R	160	0.363	0.348	0.358
	80	0.363	0.358	0.248
	40	0.275	0.358	0.248
M83	160	0.353	0.338	0.385
	80	0.318	0.330	0.275
	40	0.330	0.302	0.248
M115	160	3.220	1.400	1.815
	80	3.190	1.650	1.402
	40	2.920	1.200	1.265

M - 24 hour micrococci broth solution
C - substrate concentration

M115 and at pH of 6.5 for M83. There was a slight reduction in NRA at pH of 5.5. The rate of the enzyme reaction at pH of 5.5 was 85-90% of that at pH of 6.0.

The optimum pH value for studied micrococci was less than that obtained for other strains (9, 13). Results obtained for the studied strains in the pH range mentioned, are favorable for raw-dried meats where pH values in the beginning are 6.0 to 5.5.

The temperature dependance of NRA is represented in fig 4, being different for the separate strains as well as for the investigated temperatures. The temperature

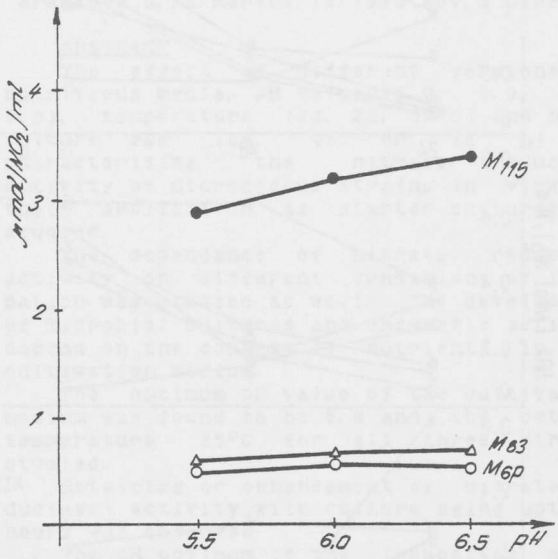

Fig. 3 Influence of the pH values of the incubation solution on the NRA (10^9 cell/ml, temperature 44°C).

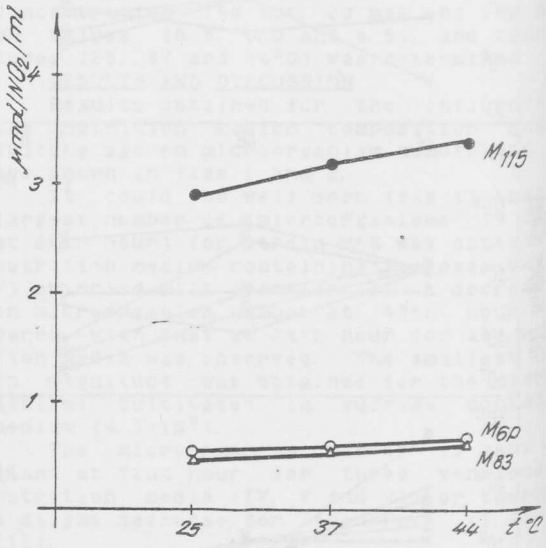

Fig. 4 Influence of the temperature of the incubation solution on the NRA (10^9 cell/ml, pH - 6.0).

rise from 25 upto 37 and 44°C leads to an increase in enzyme activity for all three strains studied. Schormuller and Schilling (1961) determined the optimum temperature for NRA to be 30°C for M. caseoliticus. In their further investigations (14) they found out that the optimum temperature for NRA of M. epidermis var. alleus was 44°C.

REFERENCES

1. Egammi P. S., S. Taniguchi (1970). Nitrat in Bergmeyer H. U.: Methoden der Enzymatischen Analyse, Band II, 2179.
2. Junker M., H. U. Liepe (1981). Fleischvirtschaft, 61, 5, 791.
3. Kuusela K., E. Puolanne, E. Petaja und F. P. Niinivaara (1978). Kulmbach, 24 Eur. Kongres G6.
4. Liepe H. U. (1978). Ernahrungswirtschaft Lebensmitteltechnic 4, 26-30.
5. Moller K. (1974). Proc. ISNMP I Pudoc, Wageningen.
6. Niinivaara F. P. (1955). Acta Agr. Fenn. 84, 128 S, Helsinki.
7. Nurmi E. (1966). Acta Agr. Fenn. 108, 77 p, Helsinki.
8. Pfeil E., H. U. Liepe (1974). Fleischvirt. 54, 11, 1717.
9. Pfeil E., H. U. Liepe (1973). Fleischvirt. 53, 17, 45.
10. Pohja M. S. (1960). Acta Agr. Fenn. 90
11. Puolanne E., P. Torma, G. Djedjeva (1977). Lebensm. Wiss. und Technol 10, 7-11.
12. Puolanne E. (1977). J. of the Sci. Agr. Soc, Fin., 49, 1-106.
13. Schormuller J., M. Schilling (1961). Nahrung 5, 18.
14. Schormuller J., M. Schilling (1963). Zeit. Lebensm. Unt. Forsch., 118, 492.