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UTILIZATION OF PENICILLINASE-PRODUCING *MICROCOCCUS* STRAINS IN THE TECHNOLOGY OF FERMENTED MILK PRODUCTS. II. EFFECT OF PENICILLINASE-PRODUCING *MICROCOCCUS* STRAINS ON THE FERMENTATIVE ACTIVITY OF LACTIC STARTER BACTERIA

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Key words: *Micrococcus*, penicillinase, lactic starters.

A stimulating effect of selected penicillinase-producing *Micrococcus* strains on the fermentative activity of lactic acid bacteria was observed. The strains enabled a normal growth of lactic acid bacteria in milk containing 0.3-6.0 penicillin/cm^3.

INTRODUCTION

Micrococci commonly occur in milk and in milk products as epiphytic microflora [5, 7, 8]. Their role in fermentation processes is connected with a stimulating effect of numerous strains on the activity of streptococci and lactobacilli. Desmazeaud and Devoyod [6] have demonstrated the stimulating effect of micrococci on the growth of *Streptococcus thermophilus*. Ritter (quoted by Desmazeaud and Devoyod [6]) found that these bacteria stimulate lactobacilli. Studies performed in the Department of Industrial Microbiology of the Technical-Agricultural Academy in Olsztyn [13] confirmed these observations and also demonstrated that the micrococci have a more profound effect on the activity of lactobacilli (*Lactobacillus casei*) than on the activity of streptococci. It was also demonstrated that numerous *Micrococcus* strains stimulate propionic fermentation [11] and the biosynthesis of vitamin B_{12} [3].

A separate problem is the occurrence of penicillinase-producing *Micrococcus* strains which can be used to decompose penicillin remains in milk and make possible the growth of lactic acid bacteria [10]. In our previous

studies we have selected strains demonstrating a high activity of decomposition of penicillin in milk [4]. The present study was aimed at determining their effect on the fermentative activity of streptococci and lactobacilli.

MATERIALS AND METHODS

Three selected *Micrococcus* strains were used. They belong to the following subgroups in the classification system of Baird-Parker [1]: subgroup 1 — *Micrococcus sp.* 51p, subgroup 2 — *Micrococcus sp.* 64p, subgroup 8 — *Micrococcus sp.* 26p. The above strains exhibit a high and constant activity of penicillin decomposition, requiring 2-4 h of incubation to decompose the antibiotic present in concentrations of up to 6.0 IU/cm^3 [4].

Experiments were performed with six strains of lactic acid bacteria from the collection of the Department of Industrial Microbiology: *Lactobacillus casei* 49, *Lactobacillus helveticus* 78, *Lactobacillus bulgaricus* 142, *Streptococcus thermophilus* 149, *Streptococcus lactis* 153, *Streptococcus diacetilactis* 157 and also on cheese starter 06 (a mixture of cultures of mesophylic lactic acid streptococci) and a yoghurt starter. The strains were obtained from the Milk Biopreparation Production Plant in Olsztyn.

The effect of the selected *Micrococcus* strains on the fermentative activity of lactic acid bacteria was determined in mixed cultures maintained on milk with and without the antibiotic addition. There were two variants of the mixed cultures with penicillin:

— cultures with preliminary incubation of micrococci applied in order to decompose penicillin (*Micrococcus sp.* 26p and sp. 64p at 44°C and *Micrococcus sp.* 51p at 37°C),

— cultures simultaneously inoculated with micrococci and milk fermentation bacteria.

The milk was inoculated with a 1% dose of 18-h micrococci culture of a known cell number and a 2% dose of lactic bacteria (5% dose in the case of yoghurt starter).

The mixed cultures were incubated at 25°C (cheese starter 06), 30°C (*Lactobacillus casei* 49, *Streptococcus lactis* 153, *Streptococcus diacetilactis* 157) and 44°C (yoghurt starter, *Lactobacillus helveticus* 78, *Lactobacillus bulgaricus* 142 and *Streptococcus thermophilus* 149). The mixed cultures with *Lbc. casei* and *Str. diacetilactis* were maintained for 48 h and all the others for 24 h. Acidification activity was determined after 2, 5, 8, 11, 14, 24 and 48 h; after 24 and 48 h the ability to produce aromatizing compounds was checked by the V-P test (cultures with heterofermentative streptococci of the starters) [2]. For lactic starters cell activity was also determined with the reductase test according to Lober [9].

RESULTS AND DISCUSSION

CULTURES ON MILK WITHOUT PENICILLIN

The studied *Micrococcus* strains stimulated the fermentative activity of lactic acid bacteria or had no effect on milk fermentation. A clearly stimulating effect of micrococci was observed in mixed cultures with *Lbc. helveticus* and *Lbc. casei* (increase of milk acidity by 10.5% and 36.8%, respectively). The acidity in mixed cultures with *Streptococcus lactis*, on the other hand, remained on the same level as in control. *Micrococcus*

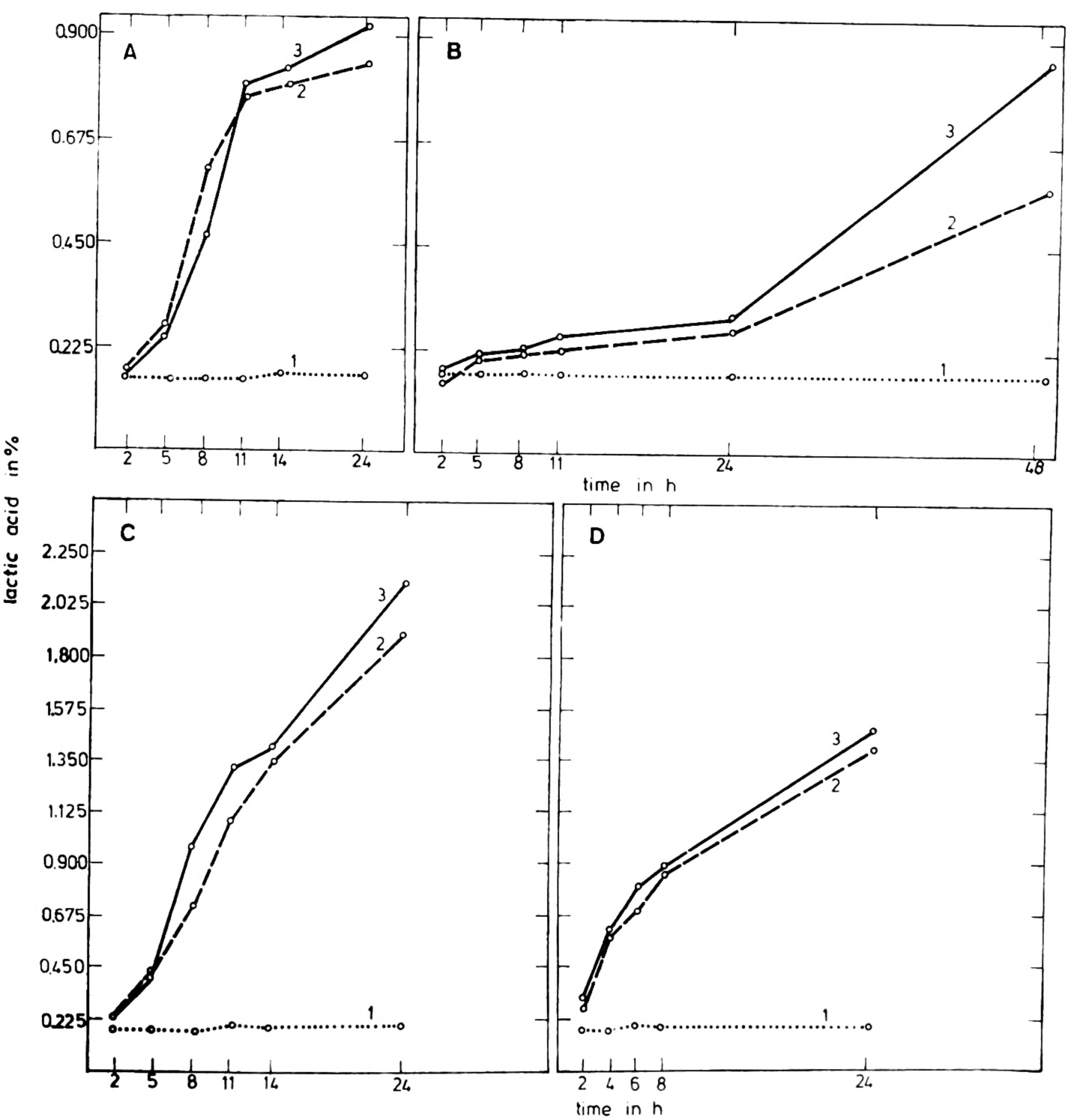

Fig. 1. Acidity increase in milk in mixed cultures of micrococci and lactis acid bacteria; A:1 — *Micrococcus sp.* 64p, 2 — cheese starter mixed culture 06, 3 — *M. sp.* 64p and cheese starter 06; B: 1 — *M. sp.* 51p, 2 — *Lactobacillus casei*, 3 — *M. sp.* 51p and *Lbc. casei*; C: 1 — *M. sp.* 51p, 2 — *Lbc. helveticus*, 3 — *M. sp.* 51p and *Lbc. helveticus*; D: 1 — *M. sp.* 26p, 2 — yoghurt starter, 3 — *M. sp.* 26p and yoghurt starter

strains increased acidity increments in cultures of single lactic acid bacteria strains and of yoghurt microflora already in the first two hours of incubation. This effect was lacking in the case of cheese starter 06 which showed differences in acidity only after 11 hours of incubation (Fig. 1).

Of all the studied strains only *Micrococcus sp.* 64p produced aromatizing compounds. There was no negative effect on the production of aromatizing compounds by heterofermentative lactic acid streptococci in mixed cultures. The reductase test revealed that micrococci stimulated the activity of yoghurt microflora. In a 24-h mixed culture with micrococci the time of resazurine decolourization was 45 min while in control this time was 60 min. No significant effect of micrococci on the results of the reductase test was observed in the remaining cultures in which the time of resazurine decolourization was about 30 min.

CULTURES WITH PENICILLIN

After 3-4 h preincubation in milk containing penicillin (0.3-6.0 IU/cm^3 *Micrococcus sp.* 26p, sp. 64p and sp. 51p enabled a normal growth of lactic acid bacteria and proper fermentation. The increase of acidity in these cultures did not differ from that in cultures devoid of this antibiotic. In control cultures (without micrococci) the growth of lactic acid bacteria was completely arrested in all cases of the studied antibiotic concentrations. The results are illustrated in Fig. 2 on the example of the culture with 6 IU penicillin/cm^3.

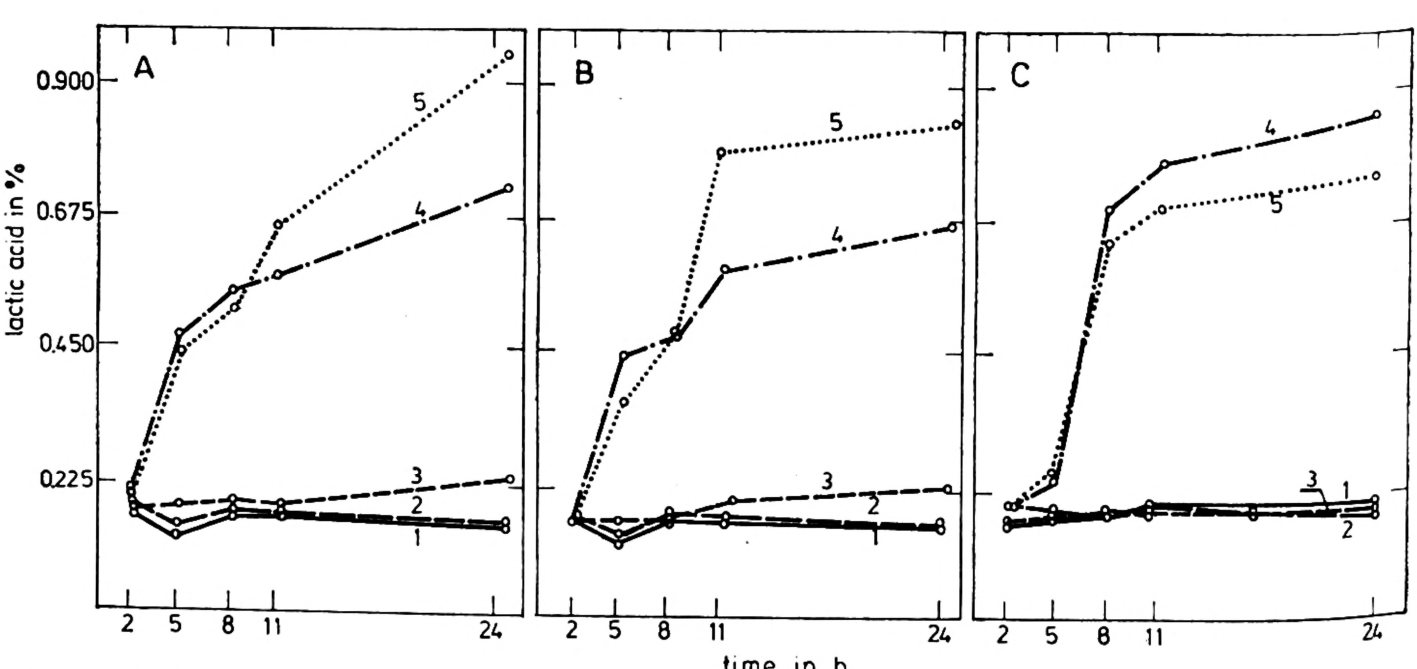

Fig. 2. Acidity changes in milk containing penicillin (6.0 IU/cm^3) in mixed culture after preincubation with micrococci; A: 1 — *Streptococcus thermophilus*, 2 — *Lactobacillus bulgaricus*, 3 — *Micrococcus sp.* 26p, 4 — *M. sp.* 26p and *Str. thermophilus*, 5 — *M. sp.* 26p and *Lbc. bulgaricus*; B: 1 — *Str. thermophilus*, 2 — *Lbc. bulgaricus*, 3 — *M. sp.* 64p, 4 — *M. sp.* 64p and *Str. thermophilus*, 5 — M. sp.. 64p and *Lbc. bulgaricus*; C: 1 — *M. sp.* 51p, 2 — *M. sp.* 64p, 3 — cheese starter mixed culture 0,6, 4 — *M. sp.* 64p and cheese starter 06, 5 — *M. sp.* 51p and cheese starter 06

The high activity of lactic acid bacteria cultured in media containing penicillin and micrococci was confirmed by the reductase test and, for heterofermentative streptoocci, also by the V-P test.

Worse results were obtained when the milk containing penicillin was inoculated with both micrococci and lactic acid bacteria. In such conditions the growth of lactic acid bacteria was markedly impeded. However, the presence of micrococci enabled these bacteria to grow at lower concentrations of penicillin in milk (0.3-1.0 IU/cm³). The best results were obtained with *Micrococcus sp.* 51p and the cheese starter 06 in milk with 0.3 IU penicillin per cm³: the milk coagulated in 11 h like in cultures without penicillin. When *Micrococcus sp.* 64p was used, the coagulation occurred between the 11th and the 24th hour of incubation. In mixed cultures of both these strains with yoghurt starter, the coagulation of milk was delayed and occurred after 8-24 h (Fig. 3).

These observations were confirmed by results of the reductase test which demonstrated that when cheese starter was used the time of resazurine reduction was extended to 60 and 90 min (as compared with 30 min in control) and to 160 min when youghurt starter was applied (60 min in control). Although the activity of milk fermentation bacteria was re-

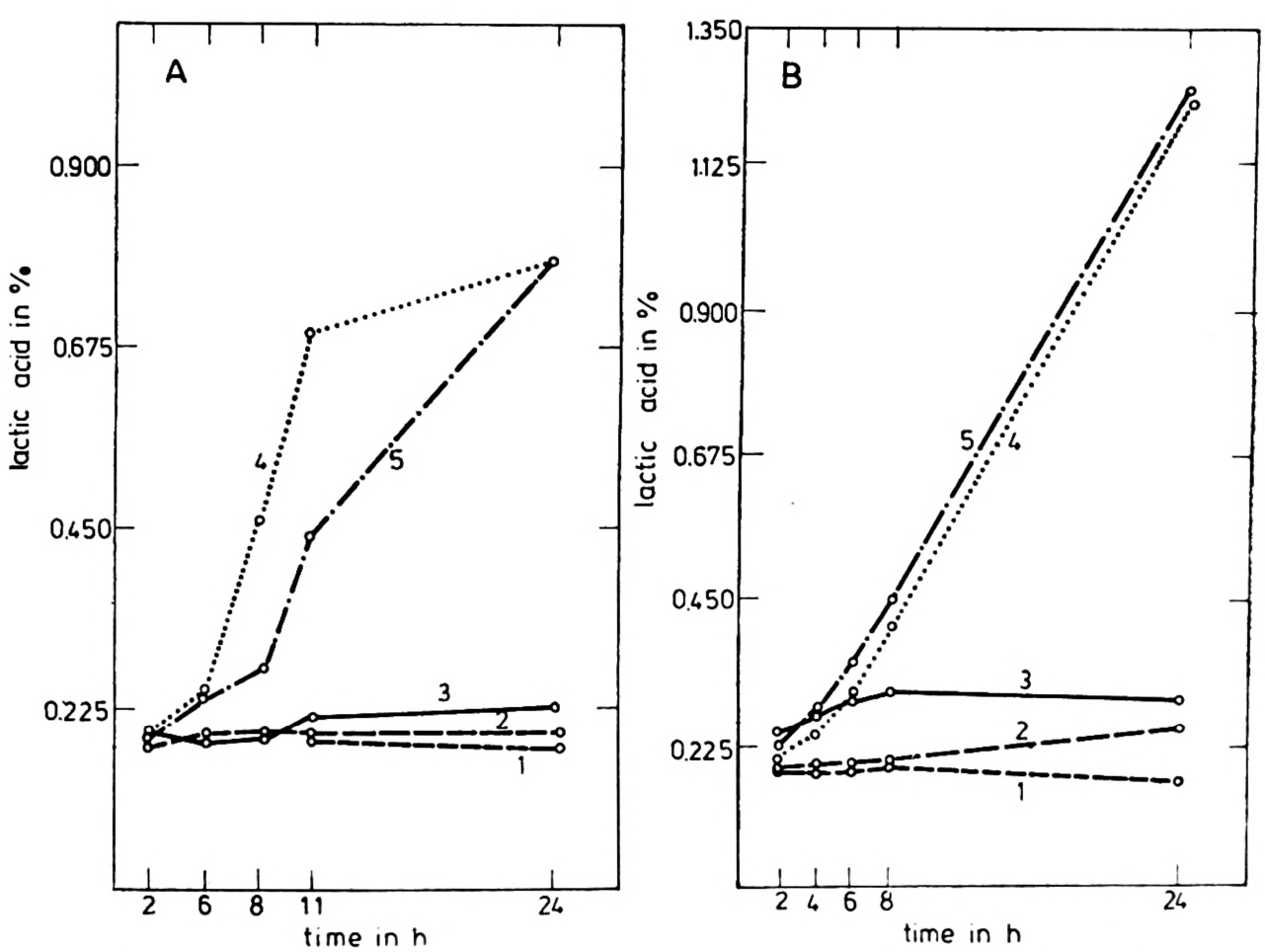

Fig. 3. Acidity changes in milk containing penicillin (0.3 IU/cm³) in mixed culture of micrococci and lactic acid bacteria (no preincubation); A: 1 — *Micrococcus sp.* 51p, 2 — *M. sp.* 64p, 3 — cheese starter mixed culture 06, 4 — *M. sp.* 51p and cheese starter 06, 5 — *M. sp.* 64p and cheese starter 06; B: 1 — *M. sp.* 51p, 2 — *M. sp.* 64p, 3 — yoghurt starter, 4 — *M. sp.* 51p and yoghurt starter, 5 — *M. sp.* 64p and yoghurt starter

duced, the presence of micrococci nevertheless permitted their growth when the penicillin concentration was as high as 3.0 IU/cm^3 (*Micrococcus sp.* 51p) and 6.0 IU/cm^3 (*Micrococcus sp.* 64p).

The experiments show that simultaneous inoculation with both micrococcus and lactic acid bacteria cultures gives good results only when the penicillin concentration in milk is low (0.3-1.0 IU/cm^3). It thus seems advisable to inoculate milk with lactic acid bacteria only after the decomposition of penicillin by micrococci during preincubation.

The stimulating effect of selected *Micrococcus* strains on milk fermentation increases their technological applicability. It seems that it would be worthwhile to use strains 26p and 64p, with no proteolytic, lipolytic and caseolytic properties, in, for example, yogurt production. It goes without saying that the application of penicillinase-producing *Micrococcus* strains in milk technology does not excuse milk producers from strictly observing the waiting period required by antibiotic administration.

Also noteworthy is the strain 51p with lipolytic, proteolytic and caseolytic properties, and a temperature of penicillin decomposition (30-37°C) that is lower than in the remaining strains [4]. Given its biochemical properties it is possible that it can be useful in cheese production, perhaps even reducing the period of cheese ripening and improving the taste of cheese. This is suggested by the studies of Robertson and Perry [12] who have considerably improved the taste of cheddar cheese using a selected *Micrococcus* strain. The authors explained this fact by the proteolytic and lipolytic properties of the strain they studied.

CONCLUSION

1. In the applied experimental conditions the stimulating effect of *Micrococcus* strains on milk fermentation was confirmed.
2. The application of selected penicillinase-producing *Micrococcus* strains, briefly preincubated in milk containing penicillin (0.3-6.0 IU/cm^3) ensures normal growth of streptococci and lactic acid bacteria.
3. The penicillinase-producing *Micrococcus* strains also make possible the development of lactic acid bacteria in milk with lower concentrations of penicillin (0.3-1.0 IU/cm^3) in cultures without preincubation.

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MOŻLIWOŚCI WYKORZYSTANIA PENICYLINAZO-DODATNICH SZCZEPÓW RODZAJU *MICROCOCCUS* W TECHNOLOGII FERMENTOWANYCH PRODUKTÓW MLECZARSKICH. II. WPŁYW PENICYLINAZO-DODATNICH SZCZEPÓW RODZAJU *MICROCOCCUS* NA AKTYWNOŚĆ FERMENTACYJNĄ BAKTERII ZAKWASÓW MLECZARSKICH

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S t r e s z c z e n i e

Stosowano penicylinazo-dodatnie szczepy rodzaju *Micrococcus* w hodowlach mieszanych z bakteriami fermentacji mlekowej w mleku bez antybiotyku i w mleku zawierającym penicylinę (0,3-6,0 j.m./cm^3). Stwierdzono stymulujące działanie ziarenkowców na aktywność fermentacyjną bakterii mlekowych. Stosując krótką wstępną inkubację ziarenkowców w mleku zawierającym penicylinę uzyskano normalny rozwój bakterii mlekowych i właściwy przebieg fermentacji. Gorsze efekty uzyskiwano w hodowlach mieszanych jednocześnie szczepionych ziarenkowcami i kulturami bakterii mlekowych.