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UTILIZATION OF PENICILLINASE-PRODUCING *MICROCOCCUS* STRAINS IN THE TECHNOLOGY OF FERMENTED MILK PRODUCTS. I. DECOMPOSITION OF PENICILLIN IN MILK BY SELECTED *MICROCOCCUS* STRAINS

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Key words: *Micrococcus*, antibiotics in milk, penicillinase.

Three of the isolated *Micrococcus* strains demonstrated a high and constant rate of decomposition of penicillin in milk. The strains decomposed penicillin in concentrations ranging from 0.1 to 6.0 IU/cm^3 in 2-4 h.

INTRODUCTION

Penicillin was one of the first antibiotics used in treating inflammatory conditions of the mammary gland and continues to be the most frequently applied medicament [4, 6]. Its remains in milk seriously impede the production of ripening cheeses, fermented milk and several other milk products given the considerable sensitivity of milk fermentation bacteria to this antibiotic.

A penicillin concentration in milk of the order of 0.025-0.3 IU/cm^3 completely inhibits the development of streptococci and lactobacilli; the most sensitive species, *Streptococcus thermophilus*, ceases to develop already when the concentration is 0.0017 IU/cm^3 [5]. Penicillin remains in milk not only lower the quality of milk products but also pose a health hazard to human beings [7]. The danger of penicillin presence in milk may be neutralized to some extent by additions of penicillinase but the application of this enzyme on an industrial scale is uneconomic [6]. Reiter and Vazquez [6, 8] suggested the use of penicillinase-producing *Micrococcus* strains in the manufacture of fermented milk products. They obtained good results in the laboratory production of Cheddar cheese and yoghurt from milk containing 0.3 IU penicillin/cm^3.

The present research was meant to provide further information about penicillinase-producing *Microccoccus* strains and about the changes in penicillin content in milk due to their action.

MATERIALS AND METHODS

The quantitative changes of penicillin in milk due to six selected *Micrococcus* strains isolated from milk and from laboratory air were studied. The strains were chosen from among 73 isolated cultures on the basis of preliminary studies demonstrating their high resistance to penicillin. Diagnostic studies of the selected strains were performed according to the method of Baird-Parker [1].

In order to determine the technological properties of the strains, their proteolytic, caseolytic and lipolytic properties were also investigated, as was their heat-resistance (treatment at 63°C for 30 min) and reaction to litmus milk [9]. The milk inoculated with the studied *Micrococcus* cultures was evaluated organoleptically.

The activity of penicillin decomposition was determined using. sterile milk containing 0.1, 0.3, 0.6, 1.0, 3.0 and 6.0 IP penicillin/cm^3. The milk was inoculated with 18-h cultures of micrococci with a known number of cells (1% of milk volume) and incubated at 25, 30, 37 and 44°C. The inoculum was either a *Micrococcus* culture on broth without penicillin, or a culture on broth with a 1.0 IU/cm^3 addition of antibiotic.

Penicillin content in milk was determined after 2, 4, 6, 8, 10, 12, 14 and 24 h. The assay was done with the plate method using *Bacillus stearothermophilus* BBL 12018 as test organism [2]. Potassium salt of penicillin G (100 000 IU activity, series number 1 040 182) was used in the experiments.

RESULTS AND DISCUSSION

On the basis of the performed studies, in agreement with the classification system of Baird-Parker [1] (Table 1), the strains were classified in the following subgroups: subgroup 1 — *Micrococcus sp.* 51p; subgroup 2 — *Micrococcus sp.* 64p; subgroup 6 — *Micrococcus sp.* 57p, sp. 29, sp. 48; subgroup 8 — *Micrococcus sp.* 26p.

The investigated strains were not heat-resistant and differed biochemically. Strains 26p and 64p did not exhibit proteolytic, lipolytic and caseolytic properties, contrary to strain 51p which exhibited all of them. There were also differences in reaction to litmus milk. For example, strain 26p did not cause changes in the milk while the remaining strains reduced the litmus either partly or completely and produced coagulation (Table 2).

T a b l e 1. Diagnostic scheme for classifying staphylococci and micrococci

	Group I *Staphylococcus*						Group II *Micrococcus*								
Subgroup	I	II	III	IV	V	VI	1	2	3	4	5	6	7	8	
Pink pigment	−	−	−	−	−	−	−	−	−	−	−	−	−	+	
Acid from glucose:															
1) aerobic	+	+	+	+	+	+	+	+	+	+	+	+	+/−	+/−	
2) anaerobic	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
Coagulase	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Phosphatase	+	+	+	−	−	−	−	−	−	−	−	+	−	−	
Acetoin	+	+	−	+	+	+	+	+	+	+	−	−	−	−	
Acid from:															
1) arabinose	−	−	−	−	−	−	−	−	−	+	v	+	−	−	
2) lactose	+	+	v	−	+	v	−	+	v	+	+	+	−	−	
3) maltose	+	+	−	v	+	v	v	+	+	+	+	+	−	+/−	
4) mannitol	+	−	−	−	−	+	−	−	+	+	+	+	−	−	

x/− = weak/negative v = variable

T a b l e 2. Characteristic of penicillinase producing *Micrococcus* strains

Strains	Proteolytic properties gelatine 3 days	Proteolytic properties gelatine 6 days	Caseolytic properties milk agar	Lipolytic properties	Heat-resistance test	Reaction of litmus milk
26p	−	−	−	−	−	No changes after 7 days
51p	+	+	+	+	−	Complete reduction after 7 days Coagulation after 5 days
64p	−	−	−	−	−	Complete reduction after 3 days Coagulation after 3 days
57p	−	+	−	−	−	Incomplete reduction after 4 days Coagulation after 7 days
29p	−	+	+	−	−	Incomplete reduction after 4 days Lack of coagulation after 7 days
48p	−	+	−	+	−	Incomplete reduction after 7 days coagulation after 4 days

Micrococcus sp. 26p and sp. 29 did not produce milk coagulation after seven days of incubation. The remaining strains gave a gelatinous or compact coagulation with a slightly sour taste and aroma the exception being strain 51p which produced a coagulation that had a sterile taste and aroma.

As to the activity of penicillin decomposition, the studies showed that the strains decompose the antibiotic most rapidly at 44°C. The only exception was *Micrococcus sp.* 51p which exhibited the highest penicillin decomposition activity at 37°C. In all cases there was a manifest dependence between the rate of penicillin decomposition and the concentration of the antibiotic. The rate clearly decreased with the increase of concentration. A constant and high activity of penicillin decomposition was demonstrated by *Micrococcus sp.* 64p and sp. 51p. When the former strain was used, the total decomposition of penicillin in milk (6.0 IU/cm³) occurred during 2 h of incubation, while in the case of *Micrococcus sp.* 51p decomposition lasted 6 h although only traces of penicillin occurred in the milk after 4 h. *Micrococcus sp.* 26p decomposed the same amount of antibiotic during 8 h of incubation, while *Micrococcus sp.* 29 needed 12 h. The complete decomposition of penicillin (6.0 IU/cm³) by *Micrococcus sp.* 57p and sp. 48 occurred only after 14 h of incubation (Figs 1 and 2).

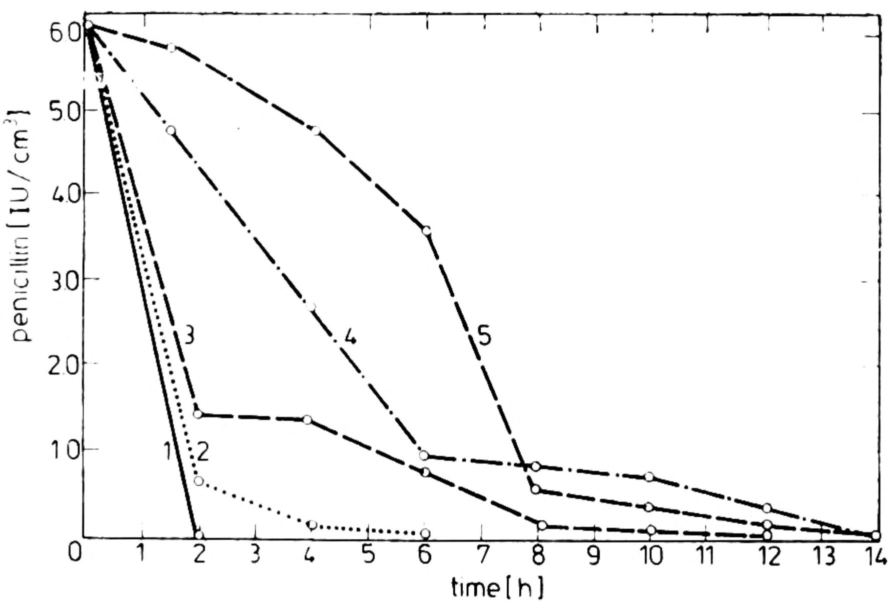

Fig. 1. Changes in penicillin content in milk due to development of selected *Micrococcus* strains; 1 — *Micrococcus sp.* 64p, 2 — *M. sp.* 51p, 3 — *M. sp.* 29, 4 — *M. sp.* 57p, 5 — *M. sp.* 48

The passaging of micrococci in a medium containing penicillin (1.0 IU/cm³) proved inhibitory to the activity of antibiotic decomposition by *Micrococcus sp.* 57p, sp. 48, sp. 29 and sp. 51p but did not affect the activity of *Micrococcus sp.* 64p. A clearly positive effect of this process, expressed by an increased rate of penicillin decomposition, was observed only in the case of *Micrococcus sp.* 26p. This strain was characterized by a constant and high activity of antibiotic decomposition: it decomposed even the highest dose of penicillin (6.0 IU/cm^3) during 2.5 h of incubation (Fig. 2).

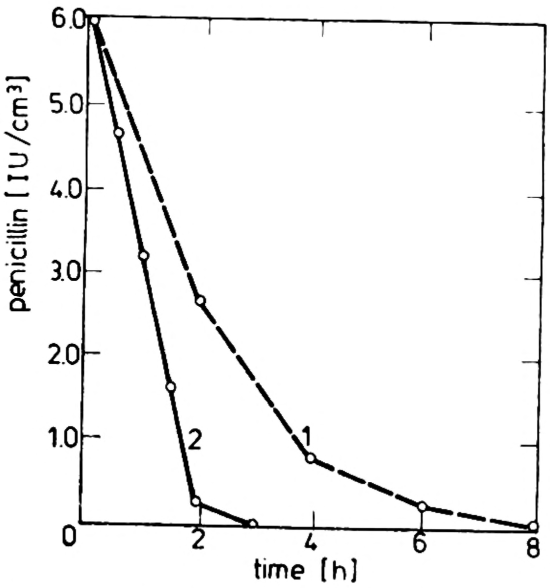

Fig. 2. The effect of passaging with penicillin on the activity of antibiotic decomposition; 1 — *Micrococcus sp.* 26p, 2 — *M. sp.* 26 passaged with penicillin addition

The literature contains abundant information about the occurrence of penicillinase-producing *Staphylococcus* strains by pays scant attention to micrococci which are commonly present in milk and milk products [3]. Our own studies have shown that within this genus there are strains naturally resistant to penicillin, the resistance being strictly connected with the production of penicillinase by these bacteria. The strains differ considerably as regards biochemical properties and activity in the milk, and this makes it possible to utilize appropriate cultures for technological purposes. Such cultures could counteract fermentation disturbances caused by penicillin in milk. It seems particularly advisable to inoculate the milk with lactic acid bacteria cultures after the decomposition of penicillin by micrococci during preliminary incubation. The utilization of selected strains as a component of starters requires further study.

CONCLUSIONS

1. The *Micrococcus* genus includes penicillinase-producing strains belonging to different Baird-Parker systematic groups.

2. Penicillinase-producing *Micrococcus* strains differ considerably as to the activity of penicillin decomposition in milk.

3. The rate of penicillin decomposition in milk depends on the initial concentraton of the antibiotic.

4. The most intense decomposition of penicillin occurs at 44°C, and in one exceptional case at 37°C.

5. The demonstrated biochemical differences between the various strains make it possible to select the most adequate strain for the given technology.

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MOŻLIWOŚCI WYKORZYSTANIA PENICYLINAZO-DODATNICH SZCZEPÓW RODZAJU *MICROCOCCUS* W TECHNOLOGII FERMENTOWANYCH PRODUKTÓW MLECZARSKICH.
I. ROZKŁAD PENICYLINY W MLEKU PRZEZ WYBRANE SZCZEPY RODZAJU *MICROCOCCUS*

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Streszczenie

Badano zmiany zawartości penicyliny w mleku pod wpływem wyselekcjonowanych penicylinazo-dodatnich szczepów ziarenkowców. Stałą i wysoką aktywnością rozkładu antybiotyku charakteryzowały się szczepy: *Micrococcus sp.* 51p, 64p i 26p. Pozostałe szczepy ziarenkowców charakteryzowały się niższą i zmienną aktywnością rozkładu penicyliny.