A C T A A L I M E N T A R I A P O L O N I C A

Vol. XV (XXXIX), No. 3 1989

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POSSIBILITIES OF UTILIZATION OF PENICILLINASE-POSITIVE STRAINS OF *MICROCOCCUS SP.* IN THE TECHNOLOGY OF FERMENTED MILK PRODUCTS. III. ATTEMPTS TO UTILIZE PENICILLINASE-POSITIVE STRAINS OF *MICROCOCCUS SP.* FOR THE MANUFACTURE OF YOGHURT

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Key words: *Micrococcus sp.*, penicillinase-positive strains, manufacture of yoghurt.

Two penicillinase-producing Micrococcus strains were used in producing yoghurt from milk containing 0.3 IU penicillin/cm^3. Favourable effects were obtained using *Micrococcus sp.* 26 p.

INTRODUCTION

The residues of penicillin in milk cause serious disturbances in the production of ripening cheeses, fermented milk products and some other dairy products which is connected with the high sensitivity of lactic acid bacteria to this antibiotic. Concentrations of penicillin of the order of 0.025-0.3 IU/cm^3 in milk inhibit completely the growth of streptococci and lactobacilli; *Streptococcus thermophilus* present in the yoghurt microflora is especially sensitive. Growth of this strain is already partially inhibited at a concentration of 0.0017 IU/cm^3 [5]. A certain protection against the consequences of the presence of penicillin in milk may be the application of penicillinase. However the use of this enzyme under industrial condition is not economical [7].

Studies of Reiter and Vazquez [7, 8] revealed the possibility of utilizing the penicillinase-positive strains of *Micrococcus sp.* in the production of Cheddar cheese and yoghurt. According to Kalogridou-Vasiliadau [4] micrococci which stimulate the development of lactic acid bacteria, have a favourable influence on the quality of yoghurt and especially, on the rheological properties of this product. In our earlier studies, strains with a high activity of degradation of penicillin in milk, were selected [3, 6].

The aim of the present study was to define the suitability of the selected strains for yoghurt production.

MATERIALS AND METHODS

Strains *Micrococcus sp.* 64p and 26p, used in the studies, were selected from among 73 isolated cultures according to the classification system of Baird-Parker

[1]. They were classified in subgroups: *Micrococcus sp.* 64 — subgroup 2 and *Micrococcus sp.* 26p — subgroup 8. These strains did not reveal any proteolytic, caseolytic and lipolytic properties and they did not survive the temperature of 63°C for 30 min. *Micrococcus sp.* 26p did not cause any changes in litmus milk whereas *Micrococcus sp.* 64p caused a reduction of lithmus and formation of curd after 4 days incubation. *Micrococcus sp.* 64p was cultivated on a brotho while *Micrococcus sp.* 26p was passaged on a broth with the addition of 1.0 IU penicillin/cm^3. Strains cultivated under these conditions demonstrated a constant and high activity, degrading penicillin in concentrations 0.1-6.0 IU/cm^3 during 2-3 h what was stated in earlier studies [3, 6].

For the production of yoghurt, pasteurized milk with an addition of 2% milk powder, containing 0.3 IU/penicillin/cm^3, was used. Milk was inoculated with the cultures of micrococci in an amount of 1% and with 5% yoghurt culture. Yoghurt was incubated at 44°C. The studies covered:

Yoghurt no 1 — from milk without penicillin and without the share of micrococci (natural yoghurt — control);
Yoghurt no 2 — from milk without penicillin, with the share of micrococci — strain 26p;
Yoghurt no 3 — from milk without penicillin, with the share of micrococci — strain 64p;
Yoghurt no 4 — from milk containing penicillin, with the share of micrococci — strain 26p;
Yoghurt no 5 — from milk containing penicillin, with the share of micrococci — strain 64p;

milk containing penicillin (0.3 IU/cm^3) inoculated with yoghurt culture only.

After formation of curd, yoghurts were stored at a temperature of 4°C. After 10 hours and then after 3 and 7 days, they were organoleptically evaluated and active titratable acidity were determined. In yoghurts no 1, 4 and 5, the content of total nitrogen, protein nitrogen, non-protein nitrogen (NPN), peptide nitrogen (2) and aminoacids, were determined. In order to determine the content of amino acids, acid hydrolysis was conducted at 110°C for 36 hours. Separation of amino acids was carried out in a two-column system, using the automatic analyzer of aminoacids type JLC-6AH of JEOL, under the standrd conditions recommended by the producer. The results were calculated on the basis of data from the two-channel integrator.

Besides, the yoghurts were examined under a microscope and the quantitative relation between lactobacilli and lactic streptococci was determined which is of importance in the evaluation of the final product.

RESULTS AND DISCUSSION

The curd was not formed only in case of milk containing 0.3 IU penicillin/cm^3, inoculated with yoghurt starter without the share of micrococci.

In yoghurts no 1, 2 and 3, the curd was formed during the same period of time

after 3 hours and 30 minutes. In yoghurt no 4, produced from milk containing penicillin (0.3 IU/cm^3) with the share of *Micrococcus sp.* 26p, the curd was formed after 5 hours and in case of *Micrococcus sp.* 64p (yoghurt no 5) — after 8 hours and 30 minutes.

Organoleptic evaluation pointed to a lower taste value of yoghurt no 5, produced from penicillin containing milk, with the participation of *Micrococcus sp.* 64p. In all remaining cases, the curd had a uniform, firm consistency typical for yoghurt, a pure taste, characteristic for yoghurt without foreign off-flavours. Among fresh yoghurts (10-hour storage at 4°C), the highest evaluation was obtained by yoghurt with an addition of *Micrococcus sp.* 64p culture (yoghurt no 3). After 3 and 7 days of storage, the highest evaluation went to yoghurt no 4 produced from penicillin containing milk (0.3 IU/cm^3), with an addition of *Micrococcus sp.* 26p and yoghurt no 1 — the control one. Acidity of yoghurt produced with the use of micrococci (yoghurts no 2 and 3) and of the control yoghurt (no 1) did not differ. On the other hand, the acidity of yoghurts pro duced from milk containing penicillin, with an addition of micrococci (yoghurts no 4 and no 5) was lower than in the control sample (yoghurt no 1) but complied with the standard. In all yoghurts, there was a distinct increase in acidity during 3 and 7 days of storge at 4°C (Table 1).

Tests of the content of nitrogen compounds did not demonstrate significant differences between the examined yoghurts (Table 2).

No significant changes were stated in the qualitative compositation of amino acids. After 7 days storage, a higher level of amino acids was found in the control yoghurt in comparison with yoghurts made from penicillin containing milk and penicillinase-positive strains of *Micrococcus sp.*

In the control yoghurt during storage, the relation between lactobacilli and streptococci varied within the limits of 1:1.1-1:1.4. In yoghurt with the addition of *Micrococcus sp.* 26p (yoghurt no 4), there was an insignificant dominancy of streptococci (1:1.6-1:2.1) while in yoghurt no 5 with the share of strain 64p, streptococci were the dominating microflora.

The attempt to produce yoghurt from milk containing penicillin (0.3 IU/cm^3) gave satisfactory results when strain 26p was applied. When this strain, was used, the curd was formed with a slight daley (1-1.5 h). The same delay in formation of curd was obtained by Vazquez and Reiter [7, 8]. However, among the strains used by them, two strains revealed distinct haemolytic properties (haemolysis α and β) which could point to their potential pathogenicity. Using strain 64p in the production of yoghurt from penicillin containing milk, the results were less satisfactory — there was a considerable delay in curd formation (5h) and a distinctly lower taste standard.

However, the application of this strain in the production of yoghurt from milk without antibiotic, increased the taste value of the product. It was a confirmation of the assumptions contained in the study of Kalogridou-Vasiliadau [4] who pointed out that micrococci of subgroups 1, 2 and 3 are mostly found in yoghurt and can affect its organoleptic properties. The author suggests also that

Table 1. Changes of acidity, lactic acid content, organoleptic evaluation and relation between streptococci and lactobacilli during storage of yoghurt

Yoghurt	Storage time	pH	Acidity SH	Lactic acid content in %	Organoleptic evaluation	Relation between streptococci and lactobacilli
Natural yoghurt	10 hours	4.36	44.0	0.99	firm curd, pure aromatic flavour	1 : 1.3
	3 days	4.24	45.2	1.02	firm curd, pure aromatic flavour – the highest estimation	1 : 1.1
	7 days	4.18	49.3	1.11	firm curd, pure aromatic flavour — the highest estimation	1 : 1.4
Yoghurt N° 2	10 hours	4.40	43.6	0.98	firm curd, pure aromatic flavour	not studied
	3 days	4.29	44.8	1.01	firm curd, pure aromatic flavour	not studied
	7 days	4.20	48.8	1.10	firm curd, pure aromatic flavour	not studied
Yoghurt N° 3	10 hours	4.39	43.9	0.99	firm curd, pure aromatic flavour — the highest estimation	not studied
	3 days	4.25	45.0	1.01	firm curd, pure aromatic flavour — the highest estimation	not studied
	7 days	4.19	49.0	1.10	firm curd, pure aromatic flavour — the highest estimation	not studied
Yoghurt N° 4	10 hours	4.65	36.5	0.82	firm curd, pure aromatic flavour – the highest estimation	1 : 1.9
	3 days	4.54	38.9	0.88	firm curd, pure aromatic flavour – the highest estimation	1 : 2.1
	7 days	4.47	42.3	0.95	firm curd, pure aromatic flavour — the highest estimation	1 : 1.6
Yoghurt N° 5	10 hours	4.76	35.5	0.80	firm curd, light synersis of whey bitter taste palpable	lost stability — supremacy of streptococci
	3 days	4.72	36.0	0.81	firm curd, light synersis of whey bitter taste palpable	lost stability — supremacy of streptococci
	7 days	4.61	39.1	0.88	firm curd, light synersis of whey bitter taste palpable	lost stability — supremacy of streptococci

Table 2. Changes of nitrogen compounds content and total amino acids in yoghurts during storage

Yoghurt	Storage time	Content in %				Total amino acids mg/16 g N
		total N	protein N	non protein N	peptides	
Natural yoghurt	10 hours	0.763	0.568	0.196	0.0387	535.9
	3 days	0.734	0.563	0.171	0.0507	579.2
	7 days	0.744	0.372	0.372	0.0594	658.5
Yoghurt N = 4	10 hours	0.797	0.562	0.235	0.0375	566.1
	3 days	0.794	0.549	0.251	0.0467	569.9
	7 days	0.764	0.339	0.425	0.0526	570.4
Yoghurt N = 5	10 hours	0.779	0.580	0.199	0.0359	530.5
	3 days	0.778	0.571	0.207	0.0509	529.7
	7 days	0.779	0.325	0.454	0.0526	531.5

micrococci take part in the formation of curd cause an increase in yoghurt acidity. In our studies, the curd in the control yoghurt (yoghurt no 1) and in yoghurts with micrococci addition, produced from milk without antibiotic, was produced in the same period of time and there were no distinct differences in the acidity of the products (Table 1). Higher increases of acidity, compared to control yoghurt, were obtained as late as after 24-hour incubation.

The obtained results demonstrate the possibility to apply strains of micrococci in the production of fermented drinks. Special attention should be paid to *Micrococcus sp.* 26p which facilitates growth of yoghurt microflora in milk containing. 0.3 IU penicillin/cm^3, without simultaneously affecting the sensoric properties of this product. The conducted characteristics of micrococci in our earlier studies [3, 6] points to the need of conducting further investigations of their application in the technology of fermented milk products, both milk drinks and ripening cheeses.

CONCLUSIONS

1. Application of properly selected strains of *Micrococcus sp.* allows to produce high quality yoghurt from milk containing 0.3 IU penicillin/cm^3.
2. Presence of proper strains of *Micrococcus sp.* does not cause changes of nitrogen compounds during ripening and storage of yoghurt.
3. Favourable effect of micrococci on the taste standard of yoghurt produced from milk without antibiotic (penicillin) was confirmed.

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Manuscript received: December, 1987
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MOŻLIWOŚCI WYKORZYSTANIA PENICYLINAZO-DODATNICH SZCZEPÓW RODZAJU *MICROCOCCUS* W TECHNOLOGII FERMENTOWANYCH PRODUKTÓW MLECZARSKICH. III. PRÓBA ZASTOSOWANIA PENICYLINAZO-DODATNICH SZCZEPÓW RODZAJU *MICROCOCCUS* W PRODUKCJI JOGURTU

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Streszczenie

Podjęto próbę otrzymania jogurtu z mleka zawierającego 0,3 j.m. penicyliny/cm^3 przy zastosowaniu wyselekcjonowanych penicylinazo-dodatnich szczepów mikrokoków. Przy użyciu szczepu 26p skrzep powstawał z opóźnieniem (1-1,5 h) w porównaniu z jogurtem kontrolnym (bez penicyliny), jednak ocena organoleptyczna wykazała wysoką jakość obydwóch jogurtów. Nie stwierdzono istotnych różnic w przemianach związków azotowych, zachodzących podczas 7-dniowego przechowywania. Zawartość azotu niebiałkowego wzrastała podczas przetrzymywania jogurtów w niskiej temperaturze z 0,196 do 0,372% (jogurt kontrolny) oraz z 0,235 do 0,425% (jogurt wyprodukowany z mleka zawierającego penicylinę z zastosowaniem szczepu *Micrococcus sp.* 26p). Nie występowały również różnice w składzie jakościowym aminokwasów. Drugi, aktywnie rozkładający penicylinę szczep *Micrococcus sp.* 64p okazał się nieprzydatny w produkcji jogurtu ze względu na 5 h opóźnienie w powstawaniu skrzepu oraz obniżenie wartości smakowej gotowego produktu.