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

Vol. IV (XXVIII), No. 2 1978

HELENA OBERMAN
ZDZISŁAWA LIBUDZISZ

PHYSIOLOGICAL ACTIVITY OF *STREPTOCOCCUS DIACETILACTIS* AND *LACTOBACILLUS CASEI* STRAINS IN A CONTINUOUS CULTURE SYSTEM

Institute of Fermentation Technology and Microbiology, Technical University, Łódź

Key words: *Streptococcus diacetilactis*, *Lactobacillus casei*, continuous culture

The effect of the dilution rate (D) on obtaining maximum production of biomass with high physiological activity expressed as acidifying, proteolytic and aroma producing ability was investigated in a continuous culture of streptococci and lactobacilli in lactose — peptone media.

INTRODUCTION

The application of continuous culture in industrial practice is connected very closely with investigating the physiology of the strains growing in these conditions and with determining the range of the possible changes in the production activity of the populations under study. These problems were the subject of numerous papers presented at international symposia dealing with continuous culture, of which the latest took place in Oxford in 1975.

In the dairy industry continuous culture can be used first of all for the production of biomass. Keen [3, 4], Linklater and Griffin [10, 11] cultivated lactic acid bacteria in milk media. Lewis [6], Lloyd and Pont [12, 13, 14], Jakubowska et al. [2], Oberman and Libudzisz [19], McDonald et al. [16] obtained good results in lactose-peptone media applying different dilution rates. However, the above mentioned authors did not report any correlation between various features of the investigated micro-organisms in conditions of continuous work.

The present paper is concerned with finding optimum conditions in continuous cultivation for chosen strains of lactobacilli and streptococci used as starters in the dairy industry, expecting that the model of cont-

inuous culture will be applied for the production of concentrated and frozen biomass of bacteria as well as for the production of enzymic preparations which accelerate the ripening of cheeses.

EXPERIMENTAL METHODS

Organisms: In continuous culture two strains *Streptococcus diacetilactis* were examined: 239 and UV mutant 20/1 and three strains *Lactobacillus casei*: W-10 and No. 2 mutant obtained as the result of an associated action of nitrozoguanidine (NTG) and UV rays as well as NTC mutant No 60/7 [20]. The parental strains *Str. diacetilactis* 239 and *L. casei* W-10 were obtained from the Institute of Pure Dairy Culture Collection in Olsztyn. The mutants were selected in the Institute of Technology of Fermentation and Microbiology, Technical University, Łódź. The strains were stored routinely in liophilized state. For the continuous culture the populations were activated three times through reconstituted skim milk (10% solids) and through APLC medium (streptococci) or A medium (lactobacilli).

Media: For the culture in a chemostat two kinds of lactosepeptone media were used: a) for streptococci — APLC medium having the following composition: yeast autolyzate (Production Plant of Sera and Vaccines) — 0,5%; peptone-peptobak (Bacutil) — 1,0%; lactose — 1,5%; sodium citrate — 1,0%. b) for lactobacilli — A medium with the following composition: enzymatic hydrolyzate of casein (Plant of Sera and Vaccines) — 0,5%; yeast autolyzate (Plant of Sera and Vaccines) — 0,5%; yeast autolyzate (Plant of Sera and Vaccines) — 0,5%; lactose — 1,5%; sodium citrate — 0,5%. Final pH of the two media was 6.8. The media were sterilized at 117°C for 10-30 min. (depending on volume).

Chemostat: The experiments were carried out in a one — stage apparatus with a working capacity of the fermentor — 800 ml, constructed by the Institute of Chemical Engineering, Technical University in Łódź. The cultures were cultivated at 30°C with mixing at 400 r.p.m. and at a regulated pH level ranging between 6.1-6.3 NaOH was used for neutralization. The system was sterilized at 120°C for 40 min. The cultures were inoculated in the fermentor adding 3% of 12 — hours *Str. diacetilactis* culture or 5% of 24 — hours *L. casei* culture as inoculum. After obtaining the stationary growth phase by the bacteria growing in the fermentor (about 12 horus — streptococci and 24 horus — lactobacilli) the flow of the sterilized APLC or A medium from the feeding flask was started. The initial dilution rate D was different for various strains — 0.20 h^{-1} for streptococci and 0.05 h^{-1} for lactobacilli. The biomass of bacteria was collected for control determinations into a sterile vessel ice-cooled to 2-3°C.

Bacterial growth: The growth of bacteria was determined: a) spectrophotometrically at 540 nm. The obtained values of extinction (E) were plotted against the standard curve of biomass E = f (d.w.); b) by means of the plate method — the amount of bacteria in liquid media and in milk was denoted as "colony forming units" (CFU). For streptococci cultivation, APLC medium with the addition of 1.5% agar, and for lactobacilli — the medium after Elliker et al. [1] were applied. Dilutions for plate method were made in 0.1% peptone solution.

Acid-producing activity: Acid — producing activity was determined by means of titration: for streptococci after 6, 12 and 24 h of incubation at 30°C in milk, and for lactobacilli after 18, 24, 48 and 72 h. The specific acidifying ability was determined in milk after 6 h of incubation of streptococci and after 18 h of incubation of lactobacilli. The indicator of specific acidifying activity was calculated from the formula:

$$\text{spec.acid.act.} = \frac{\text{m}^{\text{M}} \text{ of lactic acid in 1 ml}}{\text{CFU in 1 ml}}$$

Aroma-producing activity: The content of acetoine and diacetyl was determined by means of the distillation method [5].

Proteolytic activity: Proteolytic activity was estimated against the isoelectric casein, α_s-casein, β-casein, κ-casein and γ-casein (for *Str. diacetilactis* 20/1). Cell-free extracts [8] were the source of proteolytic enzymes. The degree of digestion of casein substrates was evaluated after 72, 120 and 168 h of incubation with the enzyme. The amount of decomposed protein by means of tannin method (Mejbaum-Katzenellenbogen, 1955) as well as the amount of the aminoacids liberated according to ninhydrine method [29] allowed to determine the activity of decomposition of proteins. The proteolytic activity was expressed as the amount of μg of casein digested during a given time of incubation with the enzyme or as the amount of the liberated aminoacids per 100 μg of protein of the cell-free extract.

RESULTS

Changes of activity of *Str. diacetilactis* populations in continuous culture at different dilution rates.

Continuous cultivation of *Str. diacetilactis* 239 was carried out for about 300 h. As a result about 110 generations were obtained. The mutant of that strain — *Str. diacetilactis* 20/1 was cultivated for 350 h and during that period of time about 160 generations were obtained. When pH level was regulated to the level of 6.2-6.3; the best growth yield of biomass of *Str. diacetilactis* 239 (about 1 g/l) was obtained with the dilution rates ranging from 0.20 to 0.45 h^{-1} corresponding to the generation time in the

batch culture from 3.4 to 1.5 h. At higher dilution rates (D = 0.5 and 0.7 h^{-1}) the biomass yield decresed by 20 to 27% (Fig. 1).

UV mutant of *Str. diacetilactis* 20/l grew much better in the conditions of continuous culture in comparison with the parental strain. The highest yield of biomass (about 2 g/l), i.e. about 100% more than the amount produced by the mother strain, was attained at dilution rates from 0.30 to 0.65 h^{-1} corresponding to a generation time ranging from 2.3-1.1 h. At a lower flow rate the growth yield of biomass decreased by about 20% and similarly when D values were higher than 0.7 h^{-1} (Fig. 1).

The content of acetoin and diacetyl in continuous culture of *Str. diacetilactis* 239 decreased from 70 mg/100 ml when the flow rate was the smallest, to about 28 mg/100 ml at D = 0.70 h^{-1}. For mutant strain — *Str. diacetilactis* 20/l, the amount of acetoin and diacetyl decreased from 117 mg/100 ml to the values 6 mg/100 ml with the wash-out dilution rate (Fig. 1).

The aroma — producing ability of the population in spite of its explicit lowering was preserved. For it has been proved that after inoculating the populations from continuous culture into milk, there is a normal production of acetoin and diacetyl. The results presented in Fig. 1 and in

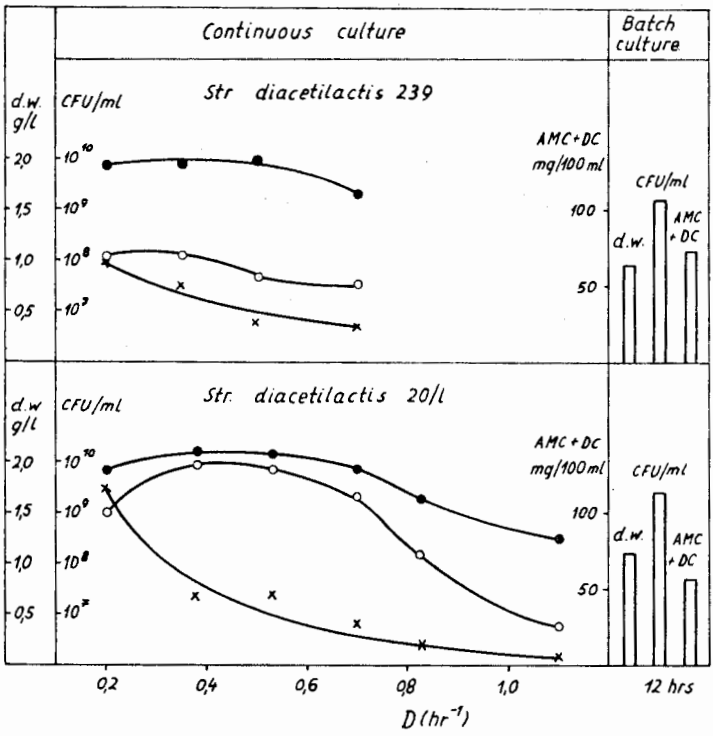

Fig. 1. Growth of *Str. diacetilactis* 239 and 20/I in chemostat (medium APLC). Effect of dilution rate (D, h^{-1}) on viable count [CFU/ml, ●], dry weight [g/l, ○], and aroma — producing ability [mg/100 ml, ×] of cells

Table 1. Proteolytic activity of cells of *Str. diacetilactis* 239 and 20/1 grown in chemostat medium APLC

Strain	D h^{-1}	Protein decrease μg/100 μg protein cell-free extract					$N\text{-}NH_2$ increase μg/100 μg protein cell-free extract				
		total casein	αs^- casein	β^- casein	$\varkappa^-$ casein	γ^- casein	total casein	αs^- casein	β^- casein	$\varkappa^-$ casein	γ^- casein
Str. diacetilactis 239	0.20	96.3	69.8	88.5	74.5	—	4.0	3.4	5.0	3.7	—
	0.35	75.1	72.2	79.3	75.2	—	4.2	3.8	4.6	3.9	—
	0.50	63.8	56.5	68.0	68.9	—	3.1	2.8	3.8	2.8	—
	0.70	53.4	57.1	72.9	68.1	—	3.3	2.6	4.5	3.8	—
Str. diacetilactis 20/1	0.21	44.0	37.0	34.0	36.0	69.0	3.4	3.3	3.1	3.4	6.7
	0.38	39.0	47.0	38.0	41.0	58.0	2.9	2.9	2.6	2.9	4.7
	0.53	46.0	40.0	39.0	49.0	65.0	2.7	2.6	3.3	2.6	4.4
	0.70	46.0	53.0	47.0	51.0	73.0	2.3	2.7	2.6	2.4	4.5
	0.82	36.0	35.0	32.0	36.0	59.0	2.4	2.8	2.7	2.3	3.9
	1.08	37.0	37.0	18.0	18.0	60.0	2.9	4.1	3.0	2.7	4.3

Table 1 also show that there is no direct relationship between the level of biomass production in continuous cultivation, and thus between the dilution rate of cultivation, and the proteolytic activity of the strains under investigation. For the mother strain of *Str. diacetilactis* 239 at $D = 0.02$ h^{-1} the highest ability of digesting isoelectric casein and β-fraction was observed. With $D = 0.35$ h^{-1}, being the optimum value for biomass production, decomposition of those fractions was lowered by 20-25%, whereas the intensity of digesting α_s and $\varkappa$-casein were similar at both mentioned D. The increase of the dilution rate values in continuous culture up to 0.5 and 0.7 h^{-1} results in the achievement of a biomass having an activity of decomposing proteins 10 to 40% lover in comparison with the activity observed at $D = 0.2$ h^{-1} (Table 1).

Mutant 20/1 and its populations digested casein fractions with a similar intensity at dilution rates ranging from $D = 0.21$ to 0.53 h^{-1}. Only at $D = 0.70$ h^{-1} their proteolytic activity was increased by 10-38% (depending on the substrate). A further increase of the dilution rate again lowered the activity of intracellular proteolytic enzymes (Table 1) of these microorganisms.

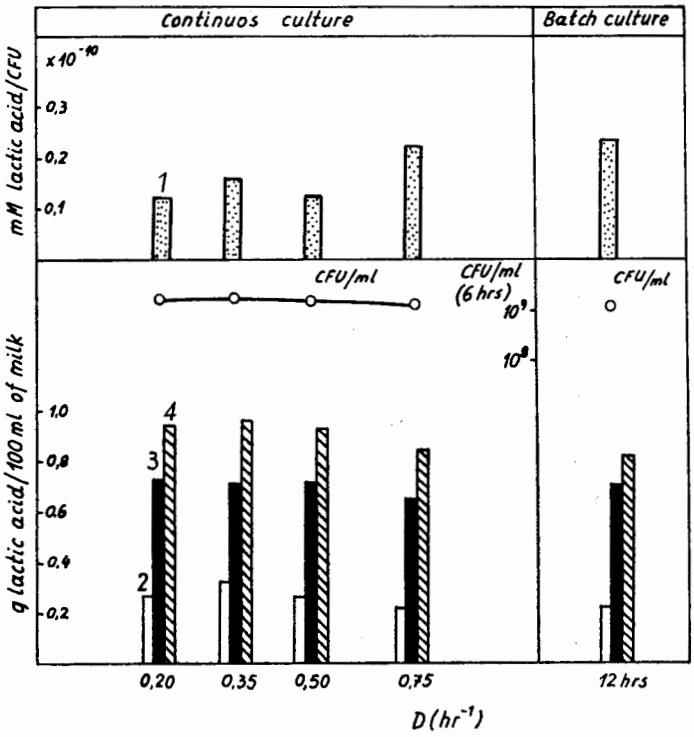

Fig. 2. Lactic acid production activity of *Str. diacetilactis* 239 in milk; 1 — specific lactic acid production activity (6 h); acidification of milk 2 — after 6 h, 3 — after 12 h, 4 — after 24 h of cultivation

The specificity of decomposing casein fractions by *Str. diacetilactis* 20/l expressed by the intensity of digestion of α_s, β, $\varkappa$ and γ-casein was stable with all dilution rates in continuous culture. γ-fraction was digested most strongly whereas α_s, β and $\varkappa$-casein lower from 19 to 39% (Table 1).

The ability of acidifying milk by the populations *Str. diacetilactis* 239 and 20/l originating from a continuous culture was high for all dilution rates, and especially for D ranging from 0.20 to 0.50 h^{-1} correlating with the end of the logarithmic growth phase, and with the early stationary growth phase in batch culture. It was also confirmed by good specific acid producing ability of the populations in continuous cultivation especially at D = 0.5 h^{-1} (Fig. 2 and 3).

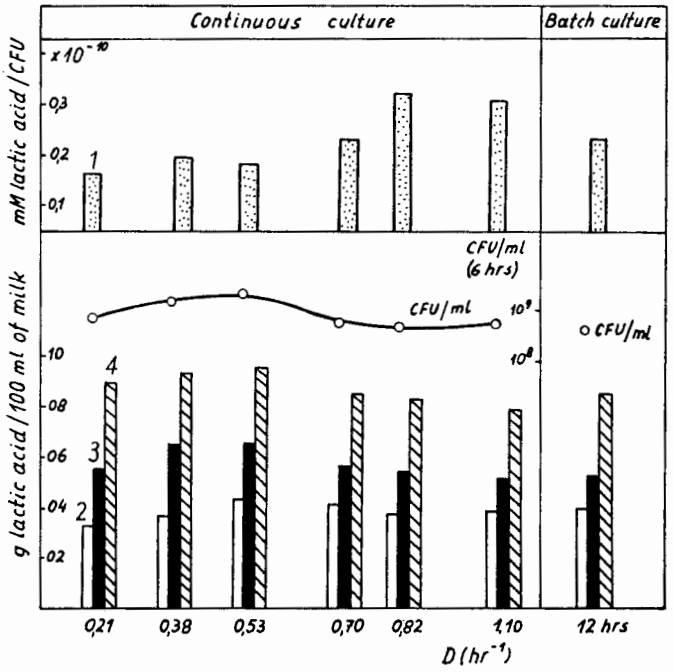

Fig. 3. Lactic acid production activity of *Str. diacetilactis* 20/l in milk; explanations as in Fig. 2

CHANGES OF ACTIVITY OF *L. CASEI* POPULATIONS IN CONTINUOUS CULTURE AT DIFFERENT DILUTIONS RATES

Continuous cultures of lactobacilli were carried out for 180 to 244 h and during that time the growth of about 20 to 30 generations was controlled.

The highest yield of biomass for the mother strain. *L. casei* W-10 amounting to 0.7-08 g/l was obtained at dilution rates ranging from 0.07 to 0.15 h, i.e. with a much slower flow than observed in the case of

streptococci. At the dilution rate of 0.21 h^{-1} the yield of biomass of lactobacilli decreased by about 65% (Fig. 4).

L. casei mutants No. 2 and 60/7 differed in parameters expressing growth and the production of biomass: *L. casei* 2 grew much better than the mother strain and the mutant 60/7. At optimal dilution rates D = 0.07 to 0.15 h^{-1} for *L. casei* 1.0 to 1.1 g of biomass was obtained from 1 l culture. At a higher dilution rate equal to 0.22 h^{-1} the decrease of the biomass yield was about 60% (Fig. 4).

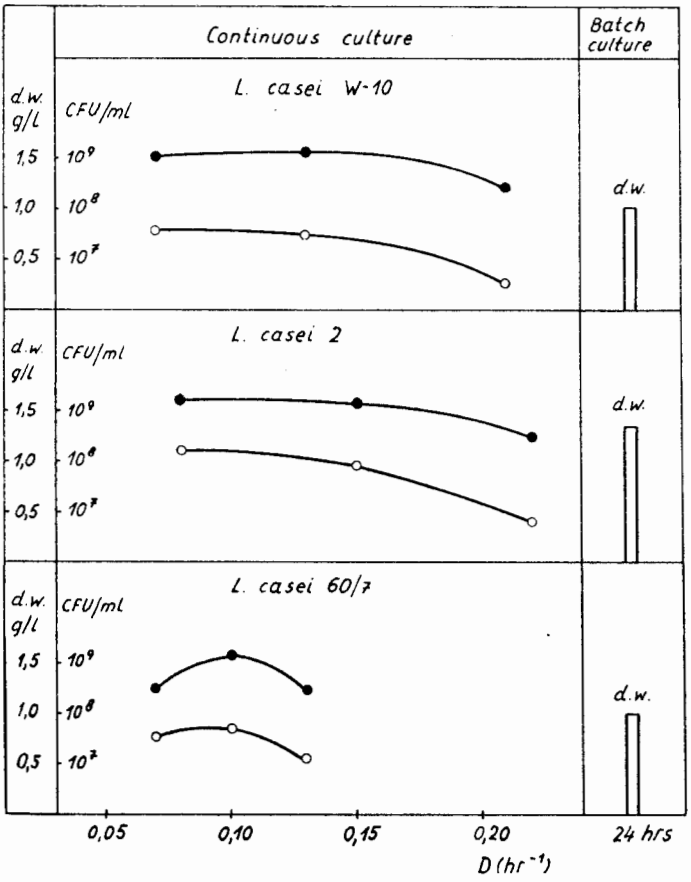

Fig. 4. Growth of *L. casei* W-10, 2 and 60/7 in chemostat (medium A). Effect of dilution rate (D, h^{-1}) on viable count [CFU/ml, ●], dry weight of cells [g/l, ○]

For *L. casei* 60/7 the growth of biomass amounting to 0.8 g/l, i.e. similar to that of the mother strain, was obtained at D ranging from 0.07 to 0.10 h^{-1}. At the faster flow (D-0,13 h^{-1}) there occured a weakening of growth by about 38% in comparison with the optimal cultivation with D = 0.10 h^{-1} (Fig. 4). In continuous culture the highest proteolytic activity was that of the more slowly growing mutant (No. 60/7).

Table 2. Proteolytic acticity of *L. casei* W-10, 2 and 60/7 grown in chemostat (medium A)

Strain	D h^{-1}	Protein decrease μg/100 μg protein cell-free extract				$N\text{-}NH_2$ increase μg/100 μg protein cell-free extract			
		total casein	α_s-casein	β-casein	κ-casein	total casein	α_s-casei	β-casein	κ-casein
L. casei W-10	0.07	117.0	95.0	116.0	137.0	5.5	5.0	7.5	8.3
	0.13	121.0	103.0	116.0	135.0	8.9	8.0	10.4	11.2
	0.21	76.0	79.0	66.0	99.0	5.6	9.5	5.0	7.2
L. casei 2	0.08	73.0	42.0	90.0	120.0	3.7	2.7	6.1	6.9
	0.15	68.0	42.0	75.0	116.0	3.9	2.4	4.8	4.7
	0.22	69.0	47.0	67.0	107.0	2.2	1.1	2.9	2.8
L. casei 60/7	0.07	107.0	106.0	114.0	160.0	7.2	10.8	10.8	11.1
	0.10	106.0	95.0	94.0	139,0	5.8	4.2	6.0	8.5
	0.13	106.0	103.0	113.0	143.0	5.5	4.0	5.6	6.6

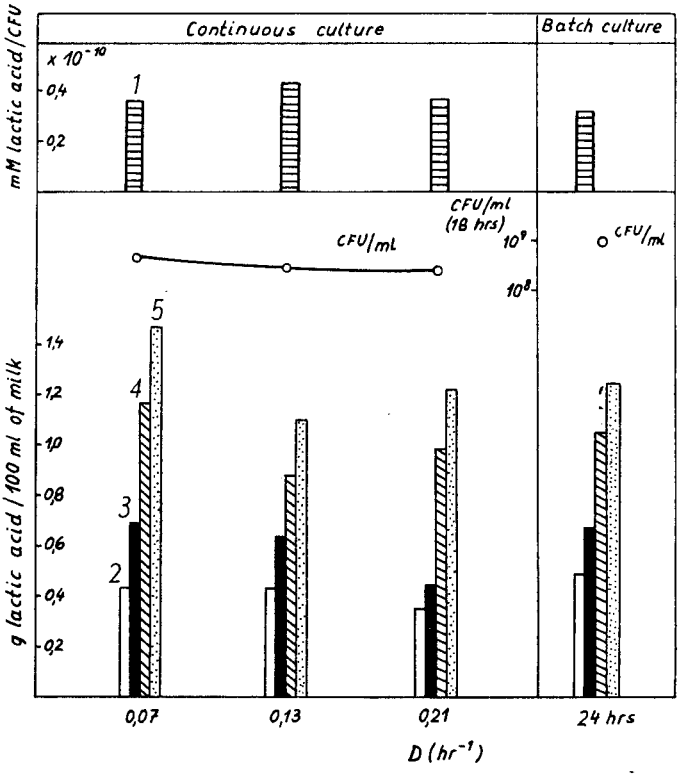

Fig. 5. Lactic acid production activity of *L. casei* W-10, in milk; 1 — specific lactic acid production activity (18 h); acidification of milk 2 — after 18 h, 3 — after 24 h, 4 — after 48 h, 5 — after 72 h of cultivation

L. casei cells obtained at the lowest dilution rates (D = 0.07 h^{-1}) for *L. casei* 60/7 as well as W-10, and 0.08 h^{-1} for *L. casei 2* (Tabl. 2) showed the greatest ability at digesting casein substrates. At higher dilution rates an about 20% drop of proteolytic activity of mother strain *L. casei* W-10 was observed and for the mutant. *L. casei* 2 the activity was decreased by about 10%. The proteolytic activity of *L. casei* 60/7 mutant was similar at dilution rates D = 0.10 and 0.13 h^{-1}, however its value was about 10% lower than when D was 0.07 h^{-1}. The specificity of decomposition of casein fractions by lactobacilli remained stable as in the case of streptococci, the strongest digestion was observed for $\varkappa$-casein, weaker for β-fraction and the weakest for α_s-casein. The amounts of aminoacids liberated from casein substrates were also changeable, according to the strain and dilution rate — similar for *L. casei* 60/7 and W-10 but higher by about 20-70% than *L. casei* 2 (Tabl. 2).

L. casei cells isolated from continuous culture, after inoculation into milk had a high acidifying ability. It has been proved that irrespective of the dilution rate of the continuous culture from which they came. *L. casei* cells produced similar amounts of lactic acid after 18 h of incubation in

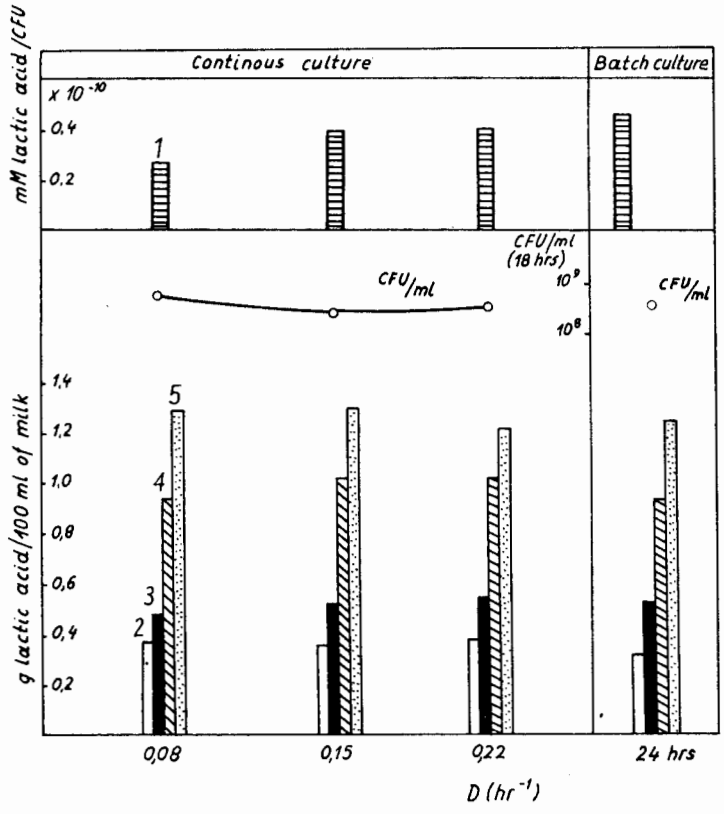

Fig. 6. Lactic acid production activity of *L. casei* 2 in milk; explanations as in Fig. 5

milk (Fig. 5, 6, 7). Only *L. casei* 60/7 gave at D = 0.10 h^{-1} populations with a markedly increased acidifying activity. However, at the same time a greater number of cells growing in milk was observed for that strain (Fig. 7). The specific acidifying activity was congenial for *L. casei* W-10 and *L. casei* 2. It amounted to about $0.3\text{-}0.4\times10^{-10}$ mM of lactic acid per CFU whereas for *L. casei* 60/7 strain, which acidified the milk-medium more strongly, the specific acid-producing activity was increased (0.07-0.08 mM of lactic acid/CFU) but only for the cells collected at dilution rates equal to D = 0.07 and 0.13 h^{-1} (Fig. 7).

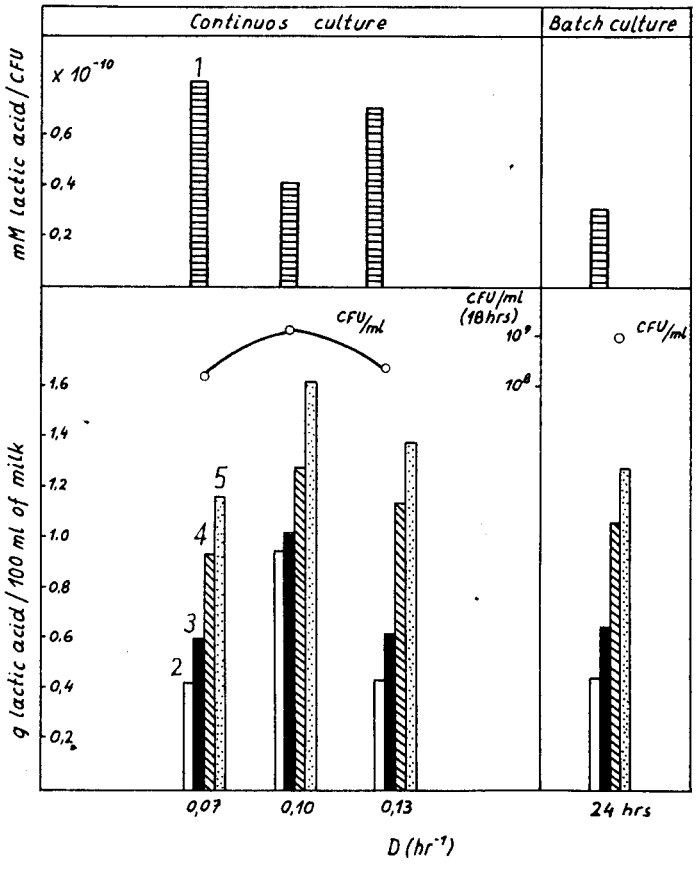

Fig. 7. Lactic acid production activity of *L. casei* 60/7 in milk; explanations as in Fig. 5

DISCUSSION

The application of continuous culture to control of the physiological features of mutants of lactic acid bacteria has been discussed in many papers [2, 3, 4, 6, 10, 11, 16, 18, 19, 24, 25] showing morphological and

physiological stability of the strains under investigation in chosen conditions of continuous work. Additionally the above mentioned works have showed that this method allows for a marked shortening of the time of experiments which is especially important when selecting strains for industrial purposes and also when characterizing new types, particularly mutants. For obtaining maximum biomass production in continuous culture of streptoccoci and lactobacilli having at the same time maximum proteolytic activity the value of pH = 6.1-6.3 was applied as the optimum one for the investigated group of bacteria. With *Str. lactis* strains the above value was used by Lloyd and Pont [12], Pont and Holloway [24], Linklater and Griffin [11]; according to McDonald et all. [16] the strain *Str. lactis* 829 could be cultivated in skim milk supplemented with some substrates in the pH range from 6.0 to 7.5 without any significant changes in the activity of cells, and in lactose — petone medium even at pH ranging from 5.5 to 8.0. The authors, however, to not report what dilution rates would be suitable in that case, and which, in view of our results, are of great importance for the optimalization of continuous conditions for the given purposes. This may be confirmed by the data obtained in the present paper for *Str. diacetilactis* showing that at D = 0.2-0.4 h^{-1} the parental strain had the highest yield of biomass whereas its UV mutant gave a crop of cells by about 100% higher in a wider range of D from 0.3 to 0.6 h^{-1}. The obtained results show that the action of UV rays gives a higher ability of biomass production.

McDonald et al. [16] confirmed the deviations in biomass production ability also in the case of *Str. lactic* populations cultured continuously at D values ranging from 0.2-0.7 h^{-1} but their level of biomass was about 25% lower in comparison with that of *Str. diacetilactis* mutant 20/1 being the subject of our investigations.

Some attention should be paid to the results pointing to a lowering of aroma producing ability of *Str. diacetilactis* populations in conditions of continuous culture. The applied range of D from 0.2 to 0.7 or 1.1 (for 20/1 mutant) caused a decrease of production of acetoine and diacetyl by the mother strain from 67.6 to 28 mg per 100 ml, and in the populations of the mutant from 117 to 6 mg per 100 ml. This phenomenon is probanly due to the nonconvenient phase of growth of *Str. diacetilactis* for producing acetoine and diacetyl in continuous culture. According to many authors the optimal production of these compounds in batch cultivation is connected with 18-24 h culture or longer [20], and thus it is connected with the late stationary growth phase [15, 26, 27].

The phase of growth in which these strains form the products probably prevents the intoxication of the strains by lactic acid. Such products may include acetoine and diacetyl. This assumption is also confirmed by the fact that the mentioned feature is retained in the population. It has been found after inoculating the cells from continuous culture conditions

into milk in which the regular production of the discussed compounds was observed.

Proteolytic activity of the mutants and parental strains in continuous culture was explicitly differentiated in the general profile and in the specificity of digestion of casein fractions. The lack of comparable data in literature on the discussed subject does not allow for a generalization of the obtained results. However taking into account the results reported by Metodieva [17] obtained for *Str. diacetilactis* 239 in continuous culture without pH regulation, it may be stated that with a limited lactose content in the medium, the growth phase in which proteolytic activity is the highest, is determined by D values ranging from 0.2 to 0.6 h^{-1}.

One of the significant conclusions concerns the profile of casein fractions decomposition. This profile did not change in continuous culture conditions at all. the examined dilution rates. *Str. diacetilactis* 239 decomposed β-fraction most strongly, κ-casein to à lesser degree and α_s-casein was digested at the lowest degree. The differences in intensivity of decomposition of the mentioned substrates amounted to 10-20%. The mutant had a 20-40% higher ability of decomposing γ-casein.

The obtained results are undoubtedly a function of the medium composition and the applied conditions of cultivation as in the case of batch culture conditions [1, 8, 9, 22, 23]. Lactobacilli cultivated continuously display a considerably lower increase in biomass than streptococci, especially when compared with the productivity of *Str. diacetilactis* 20/1 mutant. Besides they need the application cultivation conditions correlating with the stationary growth phase of the strain in batch culture. It is worth noting that in the cultivation conditions applied by us the range of dilution rates at which there was a good growth of lactobacilli, was very narrow (D = 0.05 0.13 h^{-1}) At D = 0.20 h^{-1} the growth of *L. casei* decreased by about 60% or there occured a washing out of cells depending on the strain. The differences in the growing ability of these strains are especially striking in comparison with streptococci; since the slowest flow rate for streptococci was the critical one, and caused the washing out of *L. casei* cells.

For strains of lactobacilli a greated differentiation of proteolytic activity has also been proved. Mutant No. 60/7, growing slowly (0.8 g of biomass per litre) in the narrow range of D values from 0.07 to 0.10 h^{-1} had at the same time a higher proteolytic activity as its characteristic feature. No. 2 mutant growing well (1.0, 1.1 g of biomass) [1] in the range of 0.08 to 0.15 h^{-1} revealed a proteolytic activity lower by 75% to 30%. The presented data prove that the yield of biomass is not a decisive factor for the proteolytic activity of strains. This is mainly determined by the cultivation conditions. At a slow flow corresponding to the stationary or advanced stationary phase of growth, the proteolytic activity of strains was higher, whereas their specificity expressed by the

intensity of action on casein fractions remained stable. In case of *Lactobacilli* strains $\varkappa$-casein was digested more intensively, β-fraction not so strongly and α_s-fraction was digested to a least degree with few exceptions. In the rich complex natural medium, *L. casei* strains can grow in a wider range of D from 0.1 to 0.4 h^{-1} [2]. Still better results may be obtained with mixed populations of streptococci and lactobacilli which may be very useful in the dairy industry [2, 9, 19]. The above mentioned data stress both the specificity of the various types of *Lactobacillus* strains as well as the effect of culture conditions on biomass production.

Acid producing activity of the populations isolated from continuous culture and inoculated into milk remained very good at all. the examined dilution rates. It did not undergo any significant fluctuations. The stability of this feature may be confirmed by the results characterizing specific acidifying ability of the populations coming from continuous culture — maximum for all the strains growing with a growth rate close to that of logarithmic phase.

The theoretical conclusions drawn from our investigations may be helpful in regulation of the industrial process for maximum biomass production of lactic acid bacteria with high activity of their proteolytic enzymes.

LITERATURE

1. Elliker P. R., Anderson A. W., Hannesson G.: J. Dairy Sci., 1956, **39**, 1611.
2. Jakubowska J., Oberman H., Libudzisz Z., Metodieva D.: Ferment. Technol. Today. Proc. IVth Internat. Ferment. Symp., March 19-25, 1972 et. G. Teruti, Soc. Ferment. Technol. Japan.
3. Keen A. R.: J. Dairy Res., 1972, **39**, 133.
4. Keen A. R.: J. Dairy Res., 1972, **39**, 151.
5. Kramitz L. O., Kaplan N. O.: Methods in Enzymology. New York, Ac. Press., 1957, **3**, 277.
6. Lewis P. M.: J. Appl. Bacteriol., 1967, **30**, 406.
7. Libudzisz Z., Metodieva D.: Roczn. Technol. i Chemii Żywn., 1970, **19**, 133.
8. Libudzisz Z.: Roczniki I.P.M., 1970, **12**, 87.
9. Libudzisz Z.: Acta Aliment. Pol., 1975, **1**, 247.
10. Linklater P. M., Griffin C. J.: J. Dairy Res., 1971, **38**, 127.
11. Linklater P. M., Griffin C. J.: Austr. J. Dairy Technol., 1971, 26, 22.
12. Lloyd G. T., Pont E. G.: XVIII Int. Dairy Congr., 1970, 1E. 126.
13. Lloyd G. T., Pont E. G.: J. Dairy Res., 1973, **40**, 149.
14. Lloyd G. T., Pont E. G.: J. Dairy Res., 1973, **40**, 157.
15. Matuszewski T., Pijanowski E., Supińska J.: Roczn. Nauk. Roln. i Leśn., 1936, **34**, 1.
16. McDonald I. J., Reiter B., Rogers P. L.: Can. J. Microbiol., 1973, **19**, 1285.
17. Metodieva D.: Ph. D. thesis Inst. Technol. Fermentacji i Mikrobiologii, Politechnika Łódzka, 1971.
18. Oberman H.: Zesz. Nauk P.Ł. Chemia Spoż., 1968, **16**, 5.
19. Oberman H., Libudzisz Z.: Acta Microbiol. Pol. ser. B., 1973, **5**, 151.

20. Oberman H., Piątkiewicz A., Kasperkiewicz-Jamroz T., Kosiorowska J.: Sprawozdanie z pracy nauk. bad. wykonanej w Inst. Technol. Ferment. i Mikrobiol. P. Ł., 1974.
21. Ohimya K., Sato Y.: Agr. Biol. Chem., 1967, **31**, 1318.
22. Ohimya K., Sato Y.: Agr. Biol. Chem., 1969, **33**, 662.
23. Ohimya K., Sato Y.: Agr. Biol. Chem., 1969, **33**, 669.
24. Pont E. G., Holloway G. J.: Austr. J. Dairy Technol., 1968, **23**, 22.
25. Petterson H. E.: Appl. Microbiol., 1975, **29**, 437.
26. Seitz E. W., Sandine W. E., Elliker P. R., Day E. A.: Canad. J. Microbiol., 1963, **9**, 431.
27. Speckman R. A., Collins E. B.: J. Bacteriol., 1968, **95**, 74.
28. Tiwari M. P., Madan Lal., Nambudripad V. K. N., Laxminarayana H: Indian J. Dairy Sci., 1972, **25**, 263.
29. Tysarowski W.: Biochemia praktyczna PZWL, Warszawa 1968.

Manuscript received: July, 1977.
Authors addresses: Gdańska 166, 90-537 Łódź.

H. Oberman. Z. Libudzisz

FIZJOLOGICZNA AKTYWNOŚĆ *STR. DIACETILACTIS* I *LACT. CASEI* W SYSTEMIE HODOWLI CIĄGŁEJ

Instytut Technologii Fermentacji i Mikrobiologii, Politechnika, Łódź

Streszczenie

W hodowli ciągłej paciorkowców i pałeczek fermentacji mlekowej w podłożach laktozowo-peptonowych badano wpływ szybkości rozcieńczania (D) w celu uzyskania maksymalnej produkcji biomasy o wysokiej aktywności fizjologicznej.

Uzyskane wyniki wykazały, że dla *Str. diacetilactis* optymalna produkcja biomasy jest osiągana przy $D = 0{,}20$ do $D = 0{,}65$ godz.$^{-1}$. Produkcja związków aromatu zwiększała się wraz z obniżaniem szybkości rozcieńczania, jednakże populacje izolowane przy różnych wartościach D zachowywały normalną aktywność aromatyzującą, co stwierdzono po przeszczepieniu bakterii do mleka.

Kultury *L. casei* wykazywały duże zróżnicowanie międzyszczepowe we wszystkich porównywanych cechach fizjologicznych. Najwyższy przyrost biomasy uzyskiwano w wąskim zakresie D od 0,07 do 0,13 godz.$^{-1}$. Populacje paciorkowców i pałeczek uzyskane w hodowli ciągłej cechowały się różną aktywnością proteolityczną, zależnie od szczepu i stosowanej szybkości rozcieńczania. Wszystkie szczepy zachowały jednak niezmienioną specyficzność trawienia frakcji kazeinowych α_s, β, $\varkappa$ i γ.

Specyficzna aktywność kwasząca populacji z hodowli ciągłej po przesianiu do mleka była zbliżona przy szybkościach rozcieńczania optymalnych dla produkcji biomasy.