STUDIES CONCERNING THE OBTAINING OF BIOMASS FROM *LACTOBACILLUS PARACASEI SSP. PARACASEI* USING CORN EXTRACT AS NITROGEN SOURCE

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Abstract

The modern zootechnics characterised by keeping the animals in unnatural conditions (high density, industrial feeding, chicken separation and stress) is not favourable for the animals. It results production problems with high economic risk by decreasing the zootechnic performances (low weight gain and high consumption index) and health alterations (intestinal disorders, diarrhea, infections). Thus, the purpose of this study consists in proving that the Lactobacillus paracasei ssp. paracasei BS6 strain has a similar productivity to the MRS medium. So as to perform the tests, a medium containing 2% glucose, 50% corn extract – 1% d.s., 1% $Ca(OH)_2$ was used. This medium was chosen because corn extract is a natural raw material often used in the biosynthesis processes in drug industry. Corn extract is a subproduct resulted from corn processing for starch and sugar obtaining. It represents one of the most complex nutritive substrata for microorganisms development and a well-balanced source of nitrogen, carbon, sulfur and mineral salts. Three batch fermentations were realised. Glucose consumption, lactic acid accumulation, viability, maximum growth speed (μ_{max}), duplication time (T_D), productivity (P) were determined. The results analysis proved that the development of Lactobacillus paracasei ssp. paracasei strain is similar to that of the MRS medium.

Key words: *Lactobacillus paracasei*, glucose, productivity, fermentation

INTRODUCTION

It is considered at present that the prebiotics can be used as preparations containing monocultures or polycultures of viable bacteria or other microorganisms, selected from the animal intestinal flora or from other accepted methods. Introduced as fodder additives in animals' food, they lead to the improvement of the general health, stimulating the digestive processes and the productive performance of farm animals. The role of the microorganisms within the digestive tube of the animals is very important in order to maintain the general health and productive performance of mongastric animals. [1]

The prebiotic microorganisms which can be found in the preparations for farm animals multiply in the animals' rumen, thus blocking the proliferation of the undesired pathogen strains. The prebiotic microorganisms attach to the intestinal mucous membrane by multiplying blocking the pathogen microorganisms. Generally, the prebiotic products intended to animals contain *Lactobacillus* and *Enterococcus* microorganisms. These strains produce organic acids (mainly, lactic acid) that determine the reduction of pH within the digestive system. Besides the organic acids, many of these strains also produce antimicrobial substances. Thus, they determine the emergence of a medium that is unfavorable to the multiplication of the pathogen strains. [1, 2, 3]

The aim of this research is represented by obtaining biomass from *Lactobacillus paracasei ssp. paracasei* in batch system, using a medium containing corn extract as an azote source. The aim was to obtain productivity similar to that accomplished on MRS, the standard medium. [2]

MATERIAL AND METHOD

The biological material and the media. In order to develop the experiments, the *Lactobacillus paracasei ssp. paracasei* BS 6 strain was used from the collection of the Faculty of Biotechnologies, USAMV Bucharest. The strain is kept in the freezer at -82^{0}C, in a protective environment containing 20% glicerol.

A New Brunswick batch bioreactor is used in order to obtain biomass. The medium used contains glucose 2%, corn extract – 50% s.u. 1%, $Ca(OH)_2$ 1%. The medium as well as the fermentation recipient are sterilized at 115^{0}C, for 20 minutes in an autoclave Raypa The inoculation of the bioreactor is made in proportion to 10% culture obtained in MRS medium. The conditions of fermentation are: 37^{0}C, pH 5.5, stir 100 rpm once at every 12 hours of fermentation in order to homogenize the medium for 15 minutes. [4]

The determination of lactic acid production. It was accomplished by NaOH 0.1N *titration*. For the determination, it is taken into account the fact that 1 ml NaOH 0.1N corresponds to 0.009008 g of lactic acid.

The determination of the glucose quantity. The Standard Test for glucose measuring with a toluidine, accomplished by the National Institute of Research and Development ICCF Bucharest was used for this determination.

The determination of the kinetic parameters. The determination of the value of maximum specific speed for cellular growth μ_{max}. The value of μ from the equation for the time interval between two tests of a culture was calculated. The maximum value found represents the maximum specific speed μ_{max}, for the respective culture. The determination of the *time for cellular concentration doubling (T_D):* was calculated according to the formula*:* $T_D = \frac{\ln 2}{\mu}$. *The determination of cellular productivity:* Cellular productivity represents the mass of dried cells obtained per unit of volume of culture, in a time unit: $P = D \times X$, in batch system $P = \mu \times X$ (P = the cellular productivity, $g \times l^{-1} \times h^{-1}$, D = rate of dilution, h^{-1}, X = cellular concentration, $g \times l^{-1}$, μ = speed of cellular growth).

RESULTS AND DISCUSSIONS

The first stage of research is represented by the determination of the evolution of *Lactobacillus paracasei ssp. paracasei* BS 6 strains under laboratory conditions when using MRS medium. In this case, glass bottles of 250 ml each, with screwed cork were used to keep anaerobiotics.

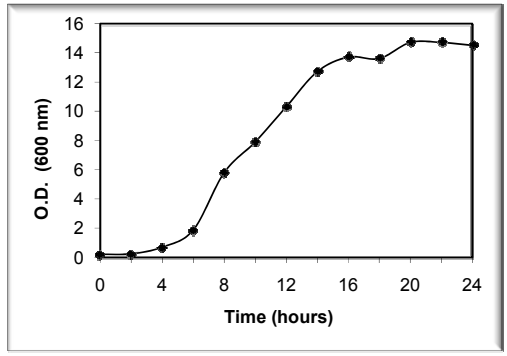

Figure 1. The profile of the curve of *Lactobacillus paracasei* strains' growth *on* MRS medium

Table 1.
The average evolution of the functions X(t) and μ(t) in the case of *Lactobacillus paracasei* CMGB16 culture on MRS medium

Sample	**1**	**2**	**3**	**4**	**5**	**6**
Timp (hours)	4	8	12	16	20	24
X, g×l^{-1}	0,7	5,8	10,3	13,7	14,7	14,5
lnX	-	1,75	2,33	2,61	2,68	2,67
lnX – ln(X-1)	-	-	0,58	0,28	0,07	-
μ, h^{-1}	-	-	0,145	0,07	0,0175	-

μ_{medium} = 0,0775 h^{-}1; μ_{max} = 0,145 h^{-1}; $T_D = \frac{\ln 2}{\mu} = \frac{0,69}{0,145}$ = 4,75 hours;

P = 0,145 h^{-1}×12 g/l =1,74 h^{-1}×g×l^{-1}

It can be noticed from the diagram (**Figure 1**) that a short lag phase lasts only 2 hours, after which the strain enter the logarithmical phase of growth that lasts until the 20th hour of fermentation. Until 16 hours of fermentation, the curve has an ascending phase, after which the strain gradually enters the phase preceding the stationary phase. The stationary phase lasts for approximately 2 hours. After 22 hours, the microorganism enters the decline phase.

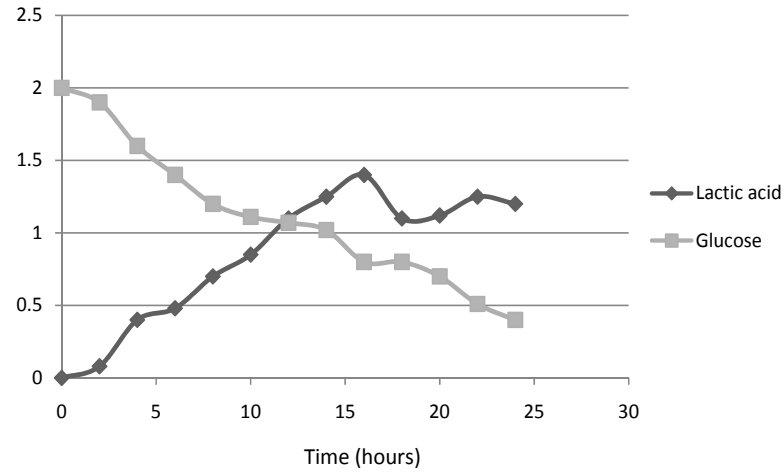

Figure 2. The accumulation of the lactic acid and glucose consumption for the strain of *Lactobacillus paracasei* on MRS medium

The accumulation de lactic acid follows the phases of growth curve. During the first part of the logarithmical phase of growth, the accumulation of lactic acid is also more important, having an accelerated rhythm. The accumulation of the lactic acid is reduced in the second half of this phase. In the first hours of the stationary phase, a synthesis also occurs, and the concentration increases with small values. Once the decline phase begins, a slight reduction of the quantity of lactic acid also occurs. The consumption of glucose aims at a descendant curve, generally characterized by a constant linear decrease. The greatest consumption occurs during the first 16 hours, in the first half of the logarithmical phase of growth, due to the necessary used for the multiplication of the microorganism. Even during the stationary phase, the value of glucose decreases, with values smaller and smaller.

Further on, the evolution of the microorganism on the medium containing corn extract was established, at the level of bioreactor, in the batch system. The evolution of *Lactobacillus paracasei* strains on this medium is presented in **Figure 3**.

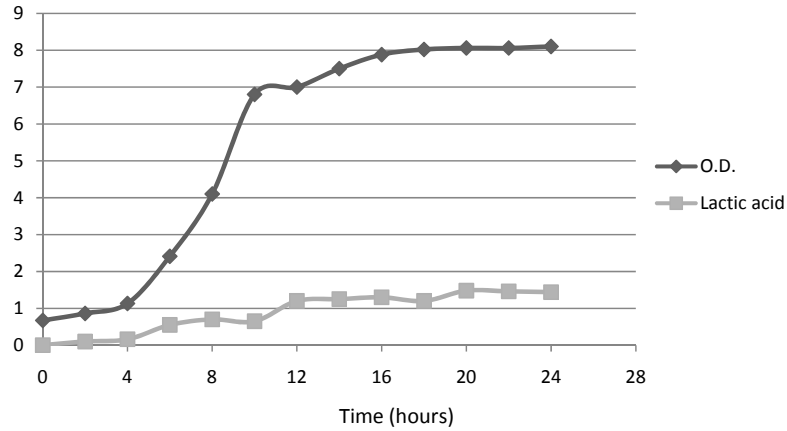

Figure 3. The evolution of the curve of growth and the accumulation de lactic acid during the culture in batch system – fermentation 1

Table 3.
The average evolution of the functions X(t) and μ(t) in the case of *Lactobacillus paracasei* culture in the bioreactor – fermentation 1

Sample	**1**	**2**	**3**	**4**	**5**	**6**
Time (hours)	4	8	12	16	20	24
X, g×l^{-1}	1,13	4,1	7	7,88	8,06	8,1
lnX	0,12	1,41	1,94	2,06	2,08	2,09
lnX – ln(X-1)	-	1,29	0,53	0,12	0,02	0,01
μ, h^{-1}	-	0,32	0,13	0,03	0,005	0,0025

μ_{medium} = 0,0975 h^{-}1; μ_{max} = 0,32 h^{-1}; $T_D = \frac{\ln 2}{\mu} = \frac{0,69}{0,32}$ = 2,15 hours;

P = 0,32 h^{-1} × 8 g/l = 2,56 h^{-1}×g×l^{-1}

As compared to the results at the level of the laboratory, the logarithmical phase of growth is bigger, ending after 20 hours of fermentation. The greatest speed of growth occurs in the interval of 4 – 12 hours, after which it gradually decreases. The accumulation of the lactic acid someway aims at the profile of the curve of growth. Once the strains enter the stationary phase, the synthesis gradually decreases, with values smaller than 0.02%.

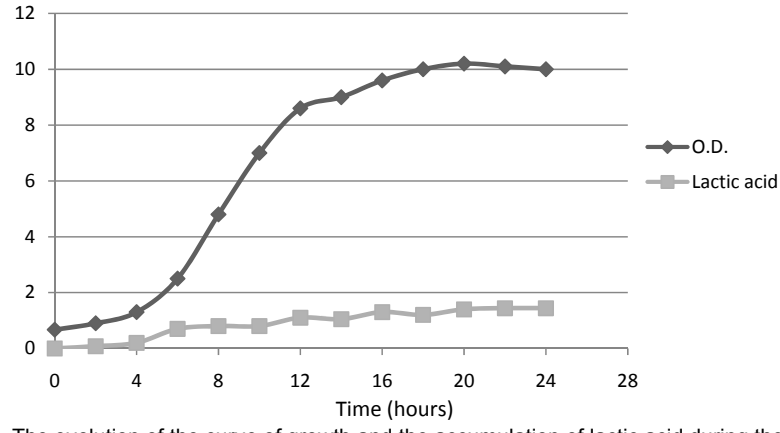

Figure 4. The evolution of the curve of growth and the accumulation of lactic acid during the culture in batch system -fermentation 2

Table 4.
The average evolution of the X(t) and μ(t) functions in the case of *Lactobacillus paracasei* culture in the bioreactor – fermentation 2

Sample	**1**	**2**	**3**	**4**	**5**	**6**
Time (hours)	4	8	12	16	20	24
X, g×l^{-1}	1,3	4,8	8,6	9,6	10,2	10
lnX	0,26	1,56	2,15	2,26	2,32	2,3
lnX – ln(X-1)	-	1,3	0,59	0,11	0,06	-
μ, h^{-1}	-	0,325	0,147	0,027	0,015	

μ_{medium} = 0,1285 h^{-}1; μ_{max} = 0,325 h^{-1}; $T_D = \frac{\ln 2}{\mu} = \frac{0,69}{0,325}$ = 2,12 hours;

P = 0,325 h^{-1} × 8 g/l = 2,6 h^{-1}×g×l^{-1}

As compared to the results at the level of fermentation 1, the logarithmical phase of growth also ceases after 20 hours of fermentation. The greatest speed of growth occurs in the interval of 2 – 12 hours, after which it gradually decreases. The accumulation of the lactic acid also aims in this case at the profile of the growth curve. Once the strains enter the stationary phase, the synthesis ceases, without the reduction of the total quantity accumulated.

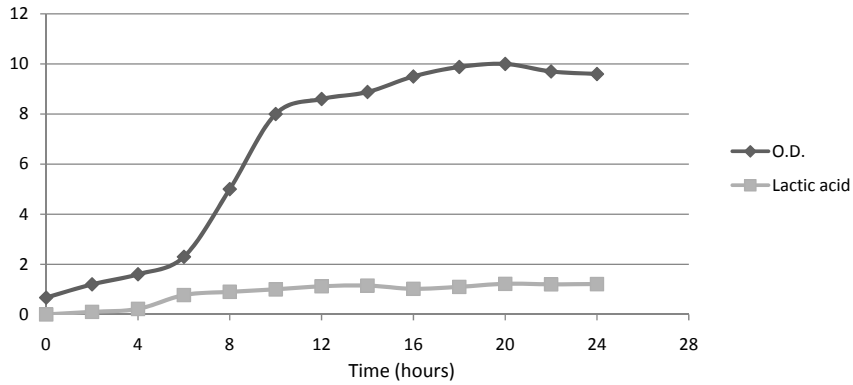

Figure 5. The evolution of the growth curve and the accumulation of lactic acid during culture in batch system – fermentation 3

Table 4.
The average evolution of the functions X(t) and μ(t) in the case of *Lactobacillus paracasei* culture in the bioreactor – fermentation 3

Sample	**1**	**2**	**3**	**4**	**5**	**6**
Time (hours)	4	8	12	16	20	24
X, g×l^{-1}	1,6	5	8,6	9,5	10	9,6
lnX	0,47	1,6	2,15	2,25	2,3	2,26
lnX – ln(X-1)	-	1,13	0,55	0,1	0,05	-
μ, h^{-1}	-	0,282	0,13	0,0025	0,012	

μ_{medium} = 0,106 h^{-}1; μ_{max} = 0,282 h^{-1}; $T_D = \frac{\ln 2}{\mu} = \frac{0,69}{0,282}$ = 2,44 hours;

P = 0,282 h^{-1} × 8 g/l = 2,25 h^{-1}×g×l^{-1}

The third fermentation, in batch system, confirms the previous data regarding the profile of the growth curve. Similarly to the first two fermentations, it can be also noticed that the logarithmical phase of growth ceases after 20 hours of fermentation. The accumulation of lactic acid is constant during the logarithmical phase of growth and it maintains during those 24 hours of fermentation.

The result of the studies made with *Lactobacillus paracasei ssp. paracasei* BS 6 strain is the procurement of a significant quantity of probiotic biomass. The usage of corn extract leads to a productivity with 30% greater unlike the case of MRS medium. The biomass obtained is recovered by means of centrifuging and it is dried by lyophilization. After lyophilization, a white – creamy powder, with a characteristic smell appears. The viability titre of the lyophilied biomass is 6×10^8 CFU/gram.

CONCLUSIONS

The evolution of the strain for *Lactobacillus paracasei ssp. paracasei* BS 6 strain was established on MRS media and on the medium used for the batch system culture that contains corn extract. Optical density and the production of lactic acid were determined for each medium, and the maximum speed and average growth, μ, as well as cellular productivity, P were established.

Three fermentations were developed in order to check the results. The medium used for the culture in batch system at the bioreactor is economic due to the usage of glucose and corn extract. The average productivity on this medium is 2.47 $h^{-1}\times g\times l^{-1}$. The time for the medium doubling, T_D, is 2.23 h. All these values show that this medium with corn extract is ideal for the culture of strains in the bioreactor and for obtaining some great quantities of viable microbial biomass.

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