INFLUENCE OF CULTURE MEDIUM ON MULTIPLICATION OF LACTIC BACTERIA ISOLATED FROM ORGANIC MILK IN ORDER TO OBTAIN BIOMASS

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ABSTRACT: This paper aims to establish direct and predictive methods (statistical) the possibility of obtaining lactic bacteria *Lactobacillus delbrueckii var. bulgaricus* in higher amounts by using culture medium enriched with carbohydrates in different percentages, for using these in obtaining organic products. The results led to the selection of MRS culture medium enriched with 1% glucose, 2% lactose and 3% maltose as a substrate in order to use multiplication of lactic bacteria isolated from organic milk, collected from organic farms in the area of Sibiu, Romania.

KEYWORDS: *Lactobacillus delbrueckii var. bulgaricus,* organic milk, enriched medium, carbohydrates, statistical analysis

1. INTRODUCTION

Organic products have a large and ever-growing search with the consumer market, in such a way that it is necessary and a reassessment of scientific data which converge to obtain them. Organic milk requires a number of traits which should be to define, but at the same time it is necessary and processing it with a view to obtaining of by-products to meet the requirements imposed by the European legislation in the field (Tita et al., 2012; Iancu et al., 2011). In Romania more and more farms focuses on livestock farming in ecological system and the milk result is processed in order to obtain the traditional yoghurt and cheese (Tita, 2002). For this purpose it is required the use of own lactic cultures, isolated from organic milk, with superior biotechnological properties, which to characterize traditional products obtained, that are influenced by culture and used as animal feed and soil (Harnann et al., 1997; Trachsel et al., 2000).

In this paper we studied the effect of different culture medium on biomass accumulation of lactic bacteria, bacteria isolated from organic milk from three farms in Sibiu, Romania.

Accumulation of biomass can be distinguished by measuring the optical density of a medium inoculated with lactic bacteria in a predetermined period of fermentation (Iancu et al., 2012; Esbensen et al., 1998; Walstra et al., 1999). Most favorable culture medium will lead to superior biomass accumulation, in the sense that cell multiplication is superior to a less nutritious medium, and the products aroma will be influenced by the use of these bacteria (Timmons et al., 2001; Vanam et al., 1994).

2. MATERIALS AND METHODS

2.1. Isolation of strains Lactobacillus delbrueckii var. bulgaricus

As a source for the isolation of strains of *Lactobacillus delbrueckii var. bulgaricus* were used: organic cow's milk coming from the three organic farms denoted by A, B, and C. The milk samples were marked and labeled to identify strains, and decimal dilutions were performed. The last three dilutions were seeded onto solid semiselective medium: MRS medium (peptone 10 g/l, meat extract 10 g/l, glucose 20 g/l, ammonium citrate 2 g/l, Na-acetate 5 g/l , K_2HPO_4 2 g/l, $MgSO_4$ 0.1 g/l, $MnSO_4$ 0.05 g/l, Tween 80 1 ml/l pH 6.2, agar 15 g/l) and medium TS (tryptone 10 g/l, lactose 10 g/l, agar 12 g/l, yeast extract 5 g/l, KH_2PO_4 2 g/l, pH 6.8, agar 15 g/l). Purification of the colonies has been performed by taking those colonies with characteristics in terms of color, shape, size, and reseeding on solid MRS. For identification, the strains were subjected to catalase test and Gram staining. Their storage was performed in MRS liquid medium at -75° C, in the presence of a cryoprotectant (glycerol 25%, v/v). In order to test and improve the biotechnological properties, the isolated strains were multiplied on selective culture medium (1g/1l) whose composition was enriched with different amounts of sugars, according to the scheme below.

2.2. Strains of lactic bacteria

There were selected three strains of bacteria *Lactobacillus delbrueckii var. bulgaricus*

- *Lactobacillus delbrueckii var. bulgaricus* - LDBA 24 - isolated from organic milk from Farm A

- *Lactobacillus delbrueckii var. bulgaricus* - LDBB 16 - isolated from organic milk from Farm B

- *Lactobacillus delbrueckii var. bulgaricus* - LDBC 31 - isolated from organic milk from Farm C

2.3. *Culture media* (Scharlau chemicals, Barcelona, EU)

1. MRS culture medium

2. MRS culture medium with addition of 3% glucose, 2% lactose and 1% maltose

3. MRS culture medium with addition of 3% glucose, 2% lactose and 1% raffinose

4. MRS culture medium with addition of 3% glucose, 2% lactose and 1% melibiose

5. MRS culture medium with addition of 1% glucose, 2% lactose and 3% maltose

6. MRS culture medium with addition of 1% glucose, 2% lactose and 3% raffinose

7. MRS culture medium with addition of 1% glucose, 2% lactose and 3% melibiose

8. MRS culture medium with addition of 1% glucose, 1% lactose and 1% maltose

9. MRS culture medium with addition of 1% glucose, 1% lactose and 1% raffinose

10. MRS culture medium with addition of 1% glucose, 1% lactose and 1% melibiose

2.4. Regime of fermentation

There were established the following parameters:

- temperature fermentation: 37 ° C,
- time: 24 hours

Sartorius fermentor equipped with temperature sensors, pH, optical density (600 nm), carbon dioxide, oxygen.

2.5. Statistical analysis

The results were analyzed using ANOVA statistical program by establishing regression factor.

3. RESULTS AND DISCUSSION

Analyzing Figure 1-3 it is observed that the optical density increases both during fermentation and due to the culture medium that was used. In Figure 1-3 it is noted that MRS culture medium with the addition of 1% glucose, 2% lactose and 3% maltose leads to a maximum accumulation of cells over 24-hour fermentations.
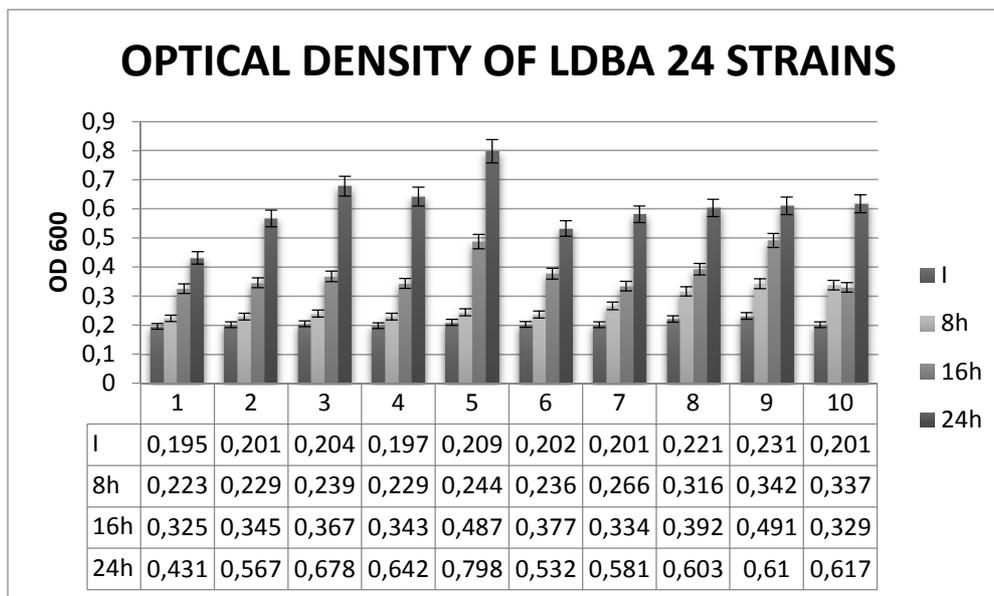

OPTICAL DENSITY OF LDBA 24 STRAINS

	1	2	3	4	5	6	7	8	9	10
I	0,195	0,201	0,204	0,197	0,209	0,202	0,201	0,221	0,231	0,201
8h	0,223	0,229	0,239	0,229	0,244	0,236	0,266	0,316	0,342	0,337
16h	0,325	0,345	0,367	0,343	0,487	0,377	0,334	0,392	0,491	0,329
24h	0,431	0,567	0,678	0,642	0,798	0,532	0,581	0,603	0,61	0,617

Figure 1. Evolution of the optical density measured at 600 nm strain Lactobacillus delbrueckii var. Bulgaricus-LDBA 24 - isolated from organic milk from Farm A
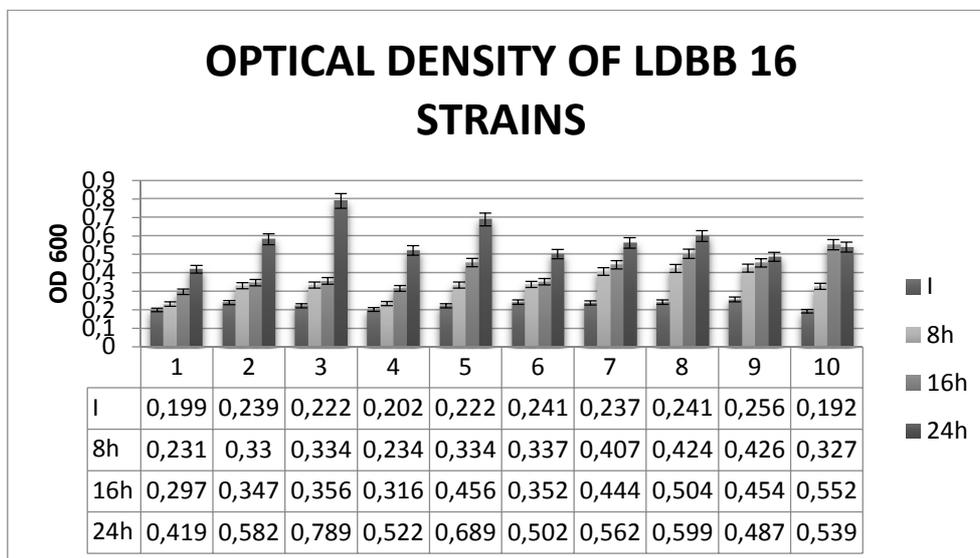
Figure 2. Evolution of the optical density measured at 600 nm strain Lactobacillus delbrueckii var. Bulgaricus-LDBA 24 - isolated from organic milk from Farm B

	1	2	3	4	5	6	7	8	9	10
I	0,199	0,239	0,222	0,202	0,222	0,241	0,237	0,241	0,256	0,192
8h	0,231	0,33	0,334	0,234	0,334	0,337	0,407	0,424	0,426	0,327
16h	0,297	0,347	0,356	0,316	0,456	0,352	0,444	0,504	0,454	0,552
24h	0,419	0,582	0,789	0,522	0,689	0,502	0,562	0,599	0,487	0,539

Figure 3. Evolution of the optical density measured at 600 nm strain Lactobacillus delbrueckii var. Bulgaricus-LDBA 24 - isolated from organic milk from Farm C
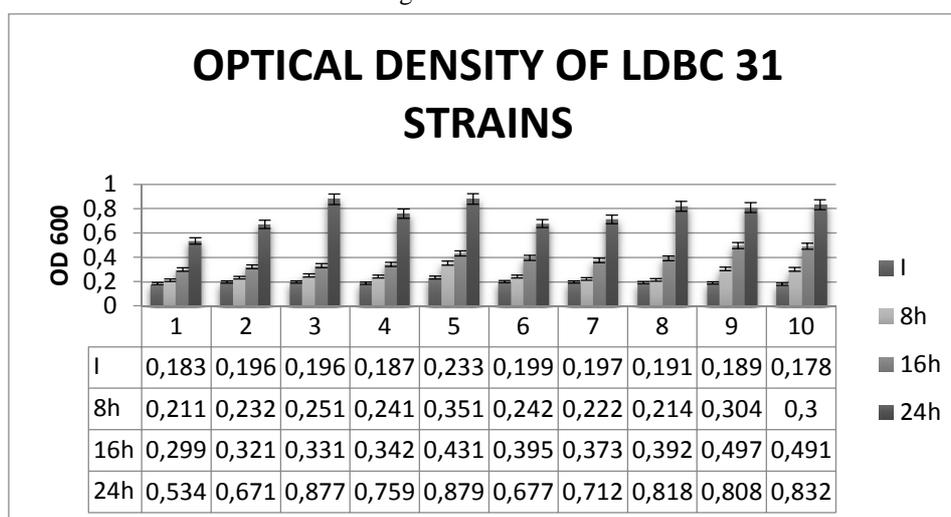
	1	2	3	4	5	6	7	8	9	10
I	0,183	0,196	0,196	0,187	0,233	0,199	0,197	0,191	0,189	0,178
8h	0,211	0,232	0,251	0,241	0,351	0,242	0,222	0,214	0,304	0,3
16h	0,299	0,321	0,331	0,342	0,431	0,395	0,373	0,392	0,497	0,491
24h	0,534	0,671	0,877	0,759	0,879	0,677	0,712	0,818	0,808	0,832

Another propitious culture medium for the accumulation of lactic bacteria is culture medium 3 containing an addition of 3% glucose, 2% lactose and 1% raffinose. It is found that the addition of glucose and maltose leads to a more pronounced multiplication of lactic bacteria considered by this study.

Table 1. The results obtained from statistical point of view

SUMMARY OUTPUT

Regression Statistics

Multiple R	0,780669					
R Square	0,609443					
Adjusted R Square	0,414165					
Standard Error	2,317359					
Observations	10					

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*	
Regression	3	50,27908	16,75969	3,120897	0,10943	
Residual	6	32,22092	5,370153			
Total	9	82,5				

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-2,32373	5,484457	-0,42369	0,686558	-15,7437	11,09625
X Variable 1	-6,35358	17,76193	-0,35771	0,732809	-49,8155	37,10831
X Variable 2	-29,308	10,93363	-2,68054	0,03651	-56,0616	-2,55438
X Variable 3	37,80703	17,45459	2,166023	0,073464	-4,90281	80,51686

RESIDUAL OUTPUT   PROBABILITY OUTPUT

Observation	Predicted Y	Residuals		Percentile	Y	
1	2,84678	-1,84678		5	1	
2	2,385052	-0,38505		15	2	
3	3,401297	-0,4013		25	3	
4	6,994032	-2,99403		35	4	
5	5,645282	-0,64528		45	5	
6	5,178909	0,821091		55	6	
7	4,43235	2,56765		65	7	
8	7,21572	0,78428		75	8	
9	10,07567	-1,07567		85	9	
10	6,824906	3,175094		95	10	

Table 2. Regression Statistics

Regression Statistics

Multiple R	0,780669	$R_{y,x} = \sqrt{\dfrac{\sum_{i=1}^{n}(\hat{y}_i - \overline{y})^2}{\sum_{i=1}^{n}(y_i - \overline{y})^2}} = \sqrt{1 - \dfrac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \overline{y})^2}}$
R Square	0,609443	$R^2 = \dfrac{\Delta_{y/x}^2}{\Delta_y^2} = 1 - \dfrac{\Delta_e^2}{\Delta_y^2} = \dfrac{\sum_{i=1}^{n}(\hat{y}_i - \overline{y})^2}{\sum_{i=1}^{n}(y_i - \overline{y})^2}$
Adjusted R Square	0,414165	$\overline{R}^2 = 1 - \dfrac{\Delta_u^2 / n - k - 1}{\Delta_y^2 / n - 1}$
Standard Error	2,317359	$s_u = \sqrt{\dfrac{\Delta_u^2}{n-2}} = \sqrt{\dfrac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n-2}}$
Observations (n)	10	

ANOVA

Source variation	df	SS	MS =SS/df	F	Significance F
Regression	3 (k)	SSR= $\Delta_x^2 = \sum_{i=1}^{n}(\hat{y}_i - \overline{y})^2 =$ 50,27908	$s_x^2 = \dfrac{\Delta_x^2}{k} =$ 16,75969	Test F=3,120897 F= s_x^2 / s_u^2	0,10943> 0,05
Residual	6 (n-k-1)	SSE= $\Delta_u^2 = \sum_{i=1}^{n}(y_i - \hat{y}_i)^2 =$ 32,22092	$s_u^2 = \dfrac{\Delta_u^2}{n-k-1} =$ 5,370153		

Total	9 (n-1)	$SST = \Delta_y^2 = \sum\limits_{i=1}^{n}(y_i - \bar{y})^2 = 82,5$ $SST = SSR + SSE$	$s_y^2 = \dfrac{\Delta_y^2}{n-1}$		

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	$a_0 = -2,32373$	$s_{a_0} = 5,484457$	-0,42369	0,686558	-0,82493	1,10932
Temps	$a_1 = -6,35358$	$S_{a1} = 17,76193$	-0,35771	0,732809	0,131488	1,01732
	$a_2 = -29,308$	$S_{a2} = 10,93363$	-2,68054	0,03651	-0,52261	0,623911
	$a_3 = 37,80703$	$S_{a3} = 17,45459$	2,166023	0,073464	-0,60223	0,213042

Interpreting results from Table SUMMARY OUTPUT:

R = 0.780669 shows that between the control sample and subsequent samples there is a strong connection.

R^2 = 0.609443 shows that 60.9% of the variation in the number of blank samples is explained by subsequent tests determined.

Standard deviation of error = 5.484457. If this indicator is zero means that all points are on the regression line.

In this table is calculated F test for regression model validation. Since F = 3.120897 and Significance F (significance) is 0.10943 then built alternative regression model is valid and can be used to analyze the dependence between the two variables. Intercept is the free term, therefore the coefficient a_0 is -2.32373. The free term is the point where the explanatory variable (factorial) is 0. Since t_{a_0} = -0.42369 and significance threshold *P-value* is 0.686558>0.05 means that the coefficient is significant in the case of the alternative hypothesis.

a_1 coefficient is -6.35358, which means that the probability the biomass increases every time the concentration of sugars in the medium increases. Since t_{a1} = -0.35771 and significance threshold *P-value* is 0.732809>0.05 means that the coefficient is significant. Confidence interval for this parameter is 0.131488 $\leq \alpha_1 \leq$ 1.01732.

a_2 coefficient is -29.308, which means that the amount of biomass increases depending on the type and concentration of sugars in the culture medium. Because t_{a2} = -2.68054 and significance threshold *P-value* is 0.03651<0.05 means that this coefficient is very significant. Confidence interval for this parameter is -0.52261 $\leq \alpha_1 \leq$ 0.623911.

The coefficient a_3 is 37.80703, which means that it increases the amount of biomass with 37.80703 g / L each time increases the concentration of sugars in the culture medium.

Because t_{a3} = 2.166023 and *P-value* significance threshold is 0.07> 0.05 means that this coefficient is less significant. Confidence interval for this parameter is -0.60223 $\leq \alpha_1 \leq$ 0.213042.

4. CONCLUSIONS

Obtaining lactic bacteria biomass depends on the culture medium used, as well as the type of sugars used for enriching them.

Sugars lead to a higher multiplication of lactic bacteria, but this is directly proportional to the chosen type.

The statistical interpretation leads to the selection of optimum multiplication variant and correct prediction results.

Selected regression coefficients lead to standard error calculation that can be decreased and then lies on the regression line or if they are higher is located at distance of the regression line.

Statistical analysis is necessary in order to provide information on what concerns predictive correlation between selected variables, in our case the culture medium composition and biomass accumulation of lactic bacteria *Lactobacillus delbrueckii var. bulgaricus.*

Isolation of lactic bacteria from organic milk lead to obtaining of products with ecological characteristics and natural.

5. REFERENCES

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