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ASSESSMENT OF CELL DENSITY AND ACIDIFYING PROPERTIES VARIATION OF FORTY-TWO *LACTOBACILLUS* STRAINS ISOLATED FROM “LUKANKA” SAUSAGE

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ABSTRACT

Forty-two *Lactobacillus* strains isolated from „Lukanka“ sausage were examined on the level of growth monitored by optical density within 72 hours spread on twelve time classes. The range of the OD comprises recorded values within the range of minimum (0) and maximum (2.31). Standard deviation of the observed OD varies between. The pH level of the cultures with recorded OD₆₀₀ comprises values between minimum and maximum (3.60 to 6.00) with standard deviation (0.84). We applied a linear regression models in the study for statistical analysis of the variation of the recorded traits considered with the categories of the measured fixed effects of strain and hour. The GLM estimates of the recorded ODs and the induced acidity have high significance of the variances (*F*-statistics, *P* < 0.001). The differences between the averages of the categories within the strains and hours give us information about the possible variation dependences at following significance.

Keywords: General Linear Model (GLM), *Lactobacillus*,

Introduction

The quality of many raw meat fermented products, such as dry sausages, depends to a great extend of the microflora participating in the ripening. One of the most important roles of *Lactobacillus* strains in this process is their acidifying properties for the conservation of the final product, depending as well of the total cell number, as of the acidifying properties of the particular strains. The aim of the study is the measurement of the acidifying properties of twenty-two *Lactobacillus* strains, isolated from “Lukanka” sausage and previously described (2).

Materials and Methods

Bacterial strains and cultivation conditions

All *Lactobacillus* strains were grown on MRS liquid broth at 30°C for 16–18 hours if not mentioned otherwise.

Growth kinetics and pH measurement

Single colony from each strain was inoculated in 3 ml MRS liquid broth, and allowed to grow overnight at 30°C. For the growth kinetics experiments 15 ml liquid cultures in MRS broth in three repeats from each *Lactobacillus* strain were prepared by inoculating 0.1% volume of the overnight cultures. They were allowed to develop at 30°C for 72 hours. Meanwhile the optical density at 600 nm (OD₆₀₀) was measured after vigorous vortexing for 2-3 seconds in *Ultrospec 1000E* spectrophotometer (*GE Healthcare*) of each culture every three hours for the first 24 hours, and after that every 12 hours till the 72nd hour. Three independent measurements of the OD₆₀₀ of each culture were performed. After each OD measurement, the pH in the samples was also recorded.

Statistical analysis

The statistical analysis of the experimental data was performed under the general linear model and background

0	0,00	6,00	0	0,00	6,00	0	0,00	6,00	0	0,00	6,00	0	0,00	6,00	0	0,00	6,00
3	0,32	5,70	3	0,10	5,90	3	0,03	5,88	3	0,13	5,89	3	0,04	5,83	3	0,02	5,90
6	0,70	5,19	6	0,27	5,88	6	0,15	5,81	6	0,34	5,87	6	0,17	5,83	6	0,06	5,88
9	1,23	4,51	9	0,47	5,87	9	0,53	5,73	9	0,68	5,85	9	0,45	5,43	9	0,43	5,88
12	1,65	4,14	12	0,68	5,63	12	0,94	5,59	12	0,98	5,81	12	0,87	5,22	12	0,87	5,24
15	1,87	3,96	15	0,98	5,30	15	1,56	4,54	15	1,30	5,53	15	1,45	5,07	15	1,33	5,13
18	1,99	3,92	18	1,30	4,69	18	1,98	4,31	18	1,70	5,06	18	1,80	4,98	18	1,74	4,85
21	2,09	3,87	21	1,69	4,28	21	2,15	4,15	21	2,00	4,48	21	2,00	4,00	21	2,08	4,72
24	2,11	3,80	24	2,00	4,05	24	2,27	4,04	24	2,17	4,25	24	2,10	3,93	24	2,25	4,65
36	2,09	3,77	36	2,10	3,80	36	2,29	3,83	36	2,28	3,86	36	2,20	3,87	36	2,30	3,99
48	2,04	3,77	48	2,15	3,78	48	2,22	3,83	48	2,24	3,86	48	2,18	3,80	48	2,23	3,90
60	2,00	3,77	60	2,10	3,72	60	2,14	3,83	60	2,18	3,83	60	2,13	3,77	60	2,14	3,85
72	1,98	3,77	72	2,00	3,72	72	1,98	3,83	72	2,00	3,83	72	2,00	3,77	72	2,00	3,80

The variation of the both observed traits, OD₆₀₀ and pH, are influenced significantly by all included factors – strains and time (F-statistics, P < 0.001). Following the distribution of the included strains, the observed acidity measured by the pH level also is very significant for all the categories. Concerning the LS-estimates of the OD₆₀₀ most significant influences aside with most extreme negative values were given by strains S2, S4, S5, S6, S20, S21, S24 and S31 while extreme positive values were given by strains S11, S18, S37 and S38 (Table 2).

TABLE 2

Assessment of the influence of strains on OD₆₀₀ by linear contrasts from the mean

Strain vs Mean	Contrast estimate	Significance
S1	-0.123	0.001 ***
S2	-0.186	0.000 ***
S3	-0.099	0.005 ***
S4	-0.138	0.000 ***
S5	-0.140	0.000 ***
S6	-0.025	0.477 n.s.
S7	-0.188	0.000 ***
S8	-0.023	0.522 n.s.
S9	0.092	0.009 ***
S10	-0.042	0.240 n.s.
S11	0.171	0.00 ***
S12	0.093	0.009 **
S13	0.021	0.543 n.s.
S14	-0.090	0.010 **
S15	-0.052	0.140 n.s.
S16	-0.079	0.025 *
S18	0.118	0.001 ***
S19	-0.063	0.073 n.s.
S20	-0.124	0.000 ***
S21	-0.135	0.000 ***
S22	0.085	0.015 *
S23	0.084	0.016 *
S24	-0.126	0.00 ***
S25	0.082	0.019 *
S26	0.023	0.514 n.s.
S27	-0.061	0.087 n.s.
S28	0.094	0.010 **
S29	0.087	0.014 **
S30	0.021	0.548 n.s.
S31	-0.142	0.000 ***
S32	-0.025	0.484 n.s.
S33	-0.069	0.049 *
S34	0.045	0.215 n.s.
S35	0.071	0.043 *

S36	0.021	0.554 n.s.
S37	0.228	0.000 ***
S38	0.125	0.000 ***
S39	0.094	0.009 **
S40	0.039	0.260 n.s.
S41	-0.012	0.738 n.s.
S42	-0.071	0.043 *
S43	0.080	0.020 *

*** - P<0.001; ** - P<0.01; * - P<0.05; n.s. - non significant

Table 3 represents the influence of the time categories to the OD. The highest negative extreme values are up to the 15th hour while the second gap of the experimental time gives positive highly significant differences for the total mean. These results correlate completely with a typical bacterial growth.

TABLE 3

Assessment of the influence of time on OD₆₀₀ by linear contrasts from the mean

№	Hours	LS-estimate	Significance
1	0	-1.198	0.000 ***
2	3	-1.114	0.000 ***
3	6	-0.960	0.000 ***
4	9	-0.718	0.000 ***
5	12	-0.408	0.000 ***
6	15	-0.050	0.000 ***
7	18	0.299	0.000 ***
8	21	0.574	0.000 ***
9	24	0.752	0.000 ***
10	36	0.792	0.000 ***
11	48	0.757	0.000 ***
12	60	0.691	0.000 ***
13	72	0.624	0.000 ***

*** - P<0.001; ** - P<0.01; * - P<0.05; n.s. - non significant

The total acidifying properties of the examined strains vary significantly in all strains, and all over the measured time. Although some of the strains could be differentiated by highly extreme and significant differences (S4, S20, S24, S37 and S38) (Table 2 and Table 3).

The total variation of the traits recorded in the experiment (OD and pH) due to the used strains and the applied time intervals of measurements is highly significant following the

F-statistics estimates between the variances according to the applied general linear models ($P < 0.001$) (Table 4 and Table 5).

TABLE 4

Variation of OD_{600} due to the included factors and co-variable

Source of variation	Degrees of freedom (DF)	MS	F-statistics	Significance
Strains	42	0.142	8.747	***
Time	13	30.675	1891.261	***
pH (co-variable)	1	0.409	25.229	***

TABLE 5

Variation of pH as a function of strains and time

Source of variation	Degrees of freedom (DF)	MS	F-statistics	Significance
Strains	42	335.531	1824.897	$P < 0.001$
Time	13	168.757	934.682	$P < 0.001$

TABLE 6

Descriptive statistics of the studied traits OD_{600} and pH

Trait	Min	Max	Mean value	Standard deviation	Coefficient of determination
OD_{600}	0.00	2.31	1.31	0.84	64.0 %
pH	3.60	6.00	4.80	0.89	18.5 %

The applied model shows for the observed OD 64% coefficient of determination of the model while the coefficient of determination of the acidifying properties is 16.5%. This means that the differences between the mean values of the OD and the standard deviation are smaller than the differences between the standard deviation of the mean values of the measured pH. Table 6 shows the spread of the measured minimum and maximum parameters as well the mean values and the standard deviation of both observed traits. This figure was again confirmed the higher variability between strains as regards both measured characters than the time categories used uniformly in the experiment.

Conclusions

We Strains S37 and S38 show better growth in respect of the significant differences observed traits. The applied model for statistical analysis emphasises the high variability of the OD reached and acidifying properties of the *Lactobacillus* strains

isolated from "Lukanka" sausage.

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