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Selection of *Lactobacillus* Strains for Functional Whey-Based Beverage Production

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Abstract: The aim of this study was selection of appropriate *Lactobacillus* strains for production of functional whey-based beverage. Sixteen strains of different species of *Lactobacillus* genera were studied and their abilities to carry out fast production of the beverage with satisfactory fermentation parameters were assessed. Preliminary screening was performed by evaluating volumetric productivity of tested strains after 24 h of the fermentation. Further selection was based on determining beverage production parameters such as fermentation time, titratable acidity, cell number and sensory characteristics. The strains showed remarkable differences in cell number and sensory properties, which were particularly useful in the selection. Based on the obtained results, the strains *Lb. casei* ssp. *casei* ATCC 27139 and *Lb. johnsonii* NRRL B-2178 are good candidates for the beverage production. These strains are rather similar in terms of beverage production ability, but strain *Lb. johnsonii* NRRL B-2178 is a top candidate for functional whey based beverage production. This strain attained titratable acidity of 9.2 °SH after 10 h of fermentation, appropriate odor and cell number of 6.8 log (CFU mL^{-1}).

Key words: Whey, functional beverages, probiotics, *Lactobacillus*, fermentation.

1. Introduction

Whey which is generated in the process of cheese production is a major by-product of the dairy industry. The excess whey, with failure to processing, is becoming a very big pollutant because of high chemical oxygen demand (COD) (57-75 g L^{-1}) [1] and biological oxygen demand (BOD_5) (35-40 g L^{-1}) [2, 3] values, what is completely at odds with the potential that such material posses. Discharging of the excess whey is an unforgivable loss of nutritionally valuable raw material. The global production of whey is increasing over the years and has reached the level of 178 million ton in 2010. Long-term predictions estimate that the global whey production will grow more than 2% per year until 2020 [4], which means that its utilization must be increased. In recent years, the production and export of the whey in Serbia is almost nonexistent. Serbian export of whey has value of 10 thousands dollars, in contrast to the import which has significant value and ranges from 1.9 to 4.7 million dollars [5].

There are many possibilities for whey utilization. In recent years, much attention has been focused on manufacturing of high value products such as lactic acid, ethanol, microbial proteins, β-D-galactosidase and vitamins [6]. Great demands in terms of technological equipment and creating acceptable technological solutions make these processes still very expensive to implement, so most factories are limited to simpler processes of whey utilization [7]. The production of functional whey based beverages, by fermentation with lactic acid bacteria (LAB), is economically the most favorable way of whey processing. In this manner, within a single

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technological process all whey potentials can be exploited, since whey represents a high biological value material [8]. The role of LAB in functional whey based beverages is to assist in: (1) the preservation of the milk by the generation of lactic acid and possibly antimicrobial compounds; (2) the production of flavor compounds (e.g., acetaldehyde in yoghurt and cheese) and other metabolites (e.g., extracellular polysaccharides) that will provide a product with the organoleptic properties desired by the consumer; (3) improving the nutritional value of food, as in, for example, the release of free amino acids or the synthesis of vitamins; and (4) the provision of special therapeutic or prophylactic properties against cancer [9-12] and control of serum cholesterol levels [13]. Commonly used species of LAB in production of functional whey based beverages are *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *S. thermophilus* [14], *Lactobacillus reuteri*, *Bifidobacterium bifidum* [15], *Lactobacillus rhamnosus*, *Propionibacterium freudenreichii* ssp. *shermanii* [16], *Lactobacillus casei* [17], *Lactobacillus plantarum*, *Lactobacillus helveticus*, *Enterococcus faecium* [18], *Bifidobacterium animalis* ssp. *lactis* [19] and *Lactobacillus paracasei* [20]. Large number of strains belonging to these species are not explored enough for the production of functional beverages and no information given could be used for the production of functional beverages. Most of the research made regarding fermented whey drink formulations was based on the use of probiotic [21], yogurt starters [22, 23] or kefir grains in reconstituted whey or whey and milk mixtures [24].

The aim of this study was the selection of appropriate *Lactobacillus* strains for the production of functional or probiotic whey-based beverage.

2. Materials and Methods

2.1 Microorganisms and Media

Sixteen strains belonging to the different species of *Lactobacillus* genera were studied. The strains used in this work (Table 1) were obtained from the collection of Department of Biochemical Engineering and Biotechnology, Faculty of Technology and Metallurgy, Belgrade.

Stock cultures of lactic acid bacteria (LAB) were stored at -20 °C in 3 mL vials containing De Man, Rogosa, Sharpe (MRS) broth and 50% (v/v) glycerol as a cryoprotective agent. For the preparation of laboratory cultures, a drop of stock culture was transferred in 3 mL of the MRS broth and incubated for 18 h under anaerobic conditions at the optimal growth temperature (30 or 37 °C). All working strains were pre-cultured twice in MRS broth prior to experimental use.

2.2 Whey Fermentation

Sweet whey powder (Lenic Laboratories, Belgrade, Serbia), with following composition: proteins 12.11%, lipids 1.0%, and carbohydrates 69.62%, was reconstituted in water to contain 8% of dry matter. A volume of 100 mL of the reconstituted whey with pH value 6.2 was poured into sterile glass bottles of 200 mL. Samples were pasteurized at 60 °C for 60 min, cooled at fermentation temperature and immediately inoculated by adding 2% (v/v) of 18 h-old testing cultures grown in MRS broth. All fermentations were

Table 1 Volumetric productivities (*P*) obtained after 24 h of whey fermentation with different strains of *Lactobacillus* sp..

Strain No.	Strains of *Lactobacillus* sp.	*P* (g L^{-1} h^{-1})
1	*Lb. gasseri* NRRL B-14168	0.124
2	*Lb. gasseri* NRRL B-4240	0.059
3	*Lb. casei* ssp.*casei* ATCC 27139	0.262
4	*Lb. casei* ssp. *casei* NRRL B-441	0.244
5	*Lb. helveticus* ATCC 15009	0.358
6	*Lb. helveticus* NRRL B-734	0.088
7	*Lb. reuteri* ATCC 23272	0.123
8	*Lb. paracasei* ssp. *paracasei* NRRL B-4564	0.083
9	*Lb. rhamnosus* TM1	0.239
10	*Lb. rhamnosus* ATCC 7469	0.280
11	*Lb. acidophilus antibiophilus*	0.262
12	*Lb. acidophilus* ATCC 4356	0.267
13	*Lb. delbrueckii* ssp. *lactis* NRRL B-1942	0.097
14	*Lb. delbrueckii* ssp. *lactis* NRRL B-4525	0.341
15	*Lb. johnsonii* NRRL B-2178	0.207
16	*Lb. delbrueckii* ssp. *bulgaricus* ATCC 11842	0.171

performed at 37 °C, statically. In the experiment of preliminary screening, the samples were incubated for 24 h. Subsequently, the volumetric productivity (*P*) was determined. In the experiment of evaluation of beverage production performances, fermentation was carried out until pH = 4.6 was attained. During the incubation time, the samples were withdrawn every 1 h for determination of pH value. By the time when pH = 4.6 was reached, fermentation was stopped by quick cooling. Beverage production performances of tested strains were evaluated by determining the basic fermentation parameters such as fermentation time (h), titratable acidity (°SH), cell number (log (CFU mL^{-1})) and sensory characteristics.

2.3 Analytical Methods

The volumetric productivity and titratable acidity were determined on the basis of produced lactic acid, by titration of 20 mL of whey samples with 0.1 M NaOH to pH = 8.2 in the presence of phenolphthalein as the indicator [25]. Titratable acidity was expressed in Soxhlet-Henkel degrees (°SH), while the volumetric productivity was expressed in g L^{-1} h^{-1}. These parameters were calculated by the following Eq.:

$$^{\circ}SH = a \times f_{NaOH} \times 2$$

°SH = 0.0225 % of lactic acid;

g L^{-1} h^{-1} $_{(lactic\ acid)}$ = °SH × 0.225/24;

a = mL 0.1 M NaOH;

f = titration correction factor of 0.1 M NaOH.

Cell number was determined by pour plate counting method on MRS agar and expressed in log (CFU mL^{-1}) [26]. For determination of sensory characteristics, a panel group of five sensory analysts were evaluating odor with 1-5 grades according to fallowing scale: 1-on sauerkraut, 2-on sourdough, 3-on whey, 4-on mild yogurt, 5-on yogurt [27].

3. Results and Discussion

3.1 Preliminary Screening Based on Fermentation Activity of Tested Strains

The fermentation activity of 16 tested strains was investigated. The fermentation activity was evaluated by determining the volumetric productivity of tested strains. The results are presented in Table 1. As shown in Table 1, the majority of strains (62.5%) showed an acceptable level of fermentation activity. The highest productivity of 0.358 g L^{-1} h^{-1} was obtained in fermentation by the strain *Lb. helveticus* ATCC 15009, while the lowest productivity of 0.059 g L^{-1} h^{-1} was obtained by the strain *Lb. gasseri* NRRL B-4240.

Based on the obtained results, one strain of each species was selected for further testing of the production performances. The preliminary selected strains were *Lb. gasseri* NRRL B-14168, *Lb. casei* ssp. *casei* ATCC 27139, *Lb. helveticus* ATCC 15009, *Lb. rhamnosus* ATCC 7469, *Lb. acidophilus* ATCC-4356, *Lb. delbrueckii* ssp. *lactis* NRRL B-4525 and *Lb. johnsonii* NRRL B-2178.

3.2 Selection Based on Beverage Production Performances

After the preliminary screening, the beverage production performances were investigated. Seven pre-selected strains were characterized on the basis of fermentation parameters which were important in the production of functional beverages.

Time of fermentation was one of the most important parameters which could substantially decrease the beverage production costs and thus valorize whey from the cheese production. In addition, dairy products should contain ≥ 6 log (CFU mL^{-1}) of viable probiotic bacteria to induce beneficial effects on health which is a prerequisite for regular consumption [28, 29]. Since whey itself has not an acceptable taste, the sensory characteristics are an essential parameter for inclusion of produced beverage in a human consumption. Fig. 1 presents the fermentation time achieved during the fermentation of whey with pre-selected strains. Fig. 2 presents the cell number achieved after the fermentation of whey with pre-selected strains.

According to the literature fast strains decreased milk pH to about 4.6 in 8 h [30]. In terms of the fermentation time, LAB significantly longer adapts and

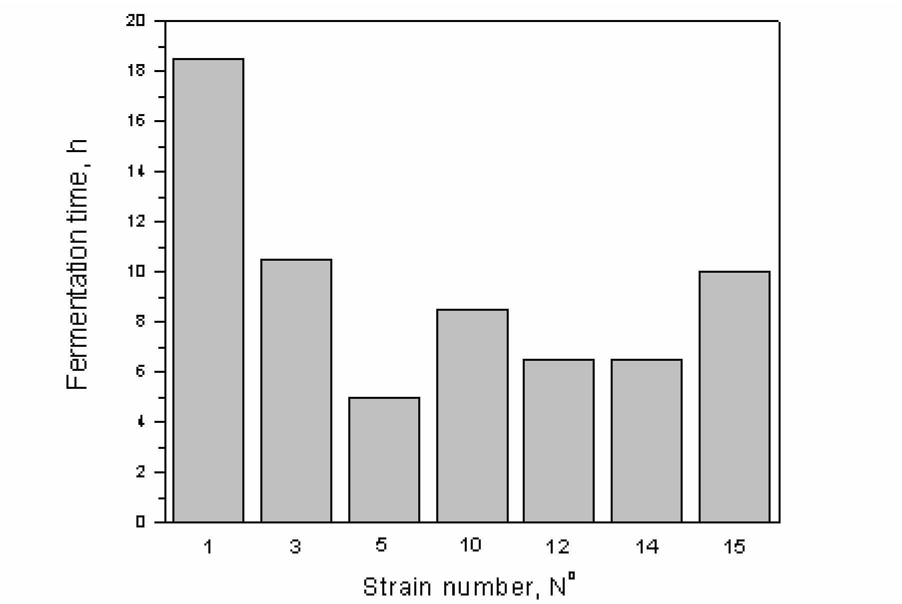

Fig. 1 Fermentation time achieved during the fermentation of whey with pre-selected strains. Strain number, No is presented in Table 1.

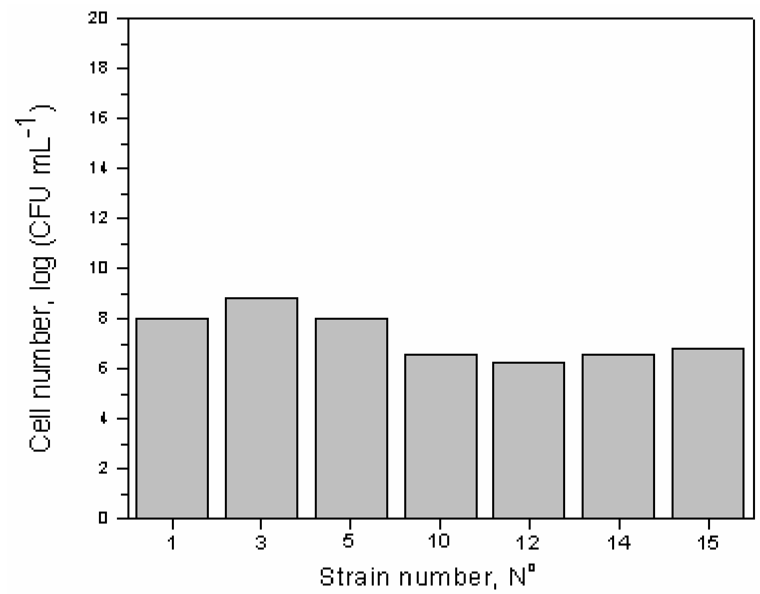

Fig. 2 Cell number achieved after the fermentation of whey with pre-selected strains. Strain number, No is presented in Table 1.

grows in whey. The result is a longer fermentation time, 15 h or more [31-33]. Normally fermentation time for monocultures is 11-17 h [34, 35], while for the mixed culture is about 6-8 h [16, 31].

As shown in Fig. 1 and Fig. 2, short fermentation time (5-6.5 h) was obtained with strains *Lb. helveticus* ATCC 15009, *Lb. acidophilus* ATCC-4356 and *Lb. delbrueckii* ssp. *lactis* NRRL B-4525, but only *Lb. helveticus* ATCC 15009 achieved high cell number of 8.0 log (CFU mL^{-1}). Strain *Lb. rhamnosus* ATCC 7469 achieved quite short fermentation time (8.5 h) but a cell number of 6.6 log (CFU mL^{-1}). On the other hand, strains *Lb. casei* ssp. *casei* ATCC 27139 and *Lb. johnsonii* NRRL B-2178 had quite longer fermentation time (10.5 and 10.0 h, respectively) and achieved cell numbers of 8.8 log (CFU mL^{-1}) and 6.8 log (CFU mL^{-1}), respectively. Strain *Lb. gasseri* NRRL B-14168 had too long fermentation time of 18 h. It is interesting to note that the strain *Lb. delbrueckii* ssp. *lactis* NRRL B-4525 which exhibited the best volumetric productivity and short time of fermentation did not achieve the highest cell number at the end of fermentation, which is consisted with the literature [36]. This could be due to the differences in lactic acid production abilities and growth characteristics. Strains *Lb. casei* ssp. *casei* ATCC 27139, *Lb. helveticus* ATCC 15009 and *Lb. johnsonii* NRRL B-2178 were selected as the most promising. These strains were further characterized based on the sensory characteristics and titratable acidity and results are presented in Figs. 3 and 4.

As shown in Fig. 3 and Fig. 4, the strain *Lb. helveticus* ATCC 15009 exhibited very low grade for odor which was like sauerkraut. This is probably a consequence of unpleasant odor substances produced by this strain [37]. This strain was therefore rejected as a possible producer of the beverages. Strains *Lb. casei* ssp. *casei* ATCC 27139 and *Lb. johnsonii* NRRL B-2178 achieved higher grades (4 and 5, respectively) for odour than the strain *Lb. helveticus* ATCC 15009.

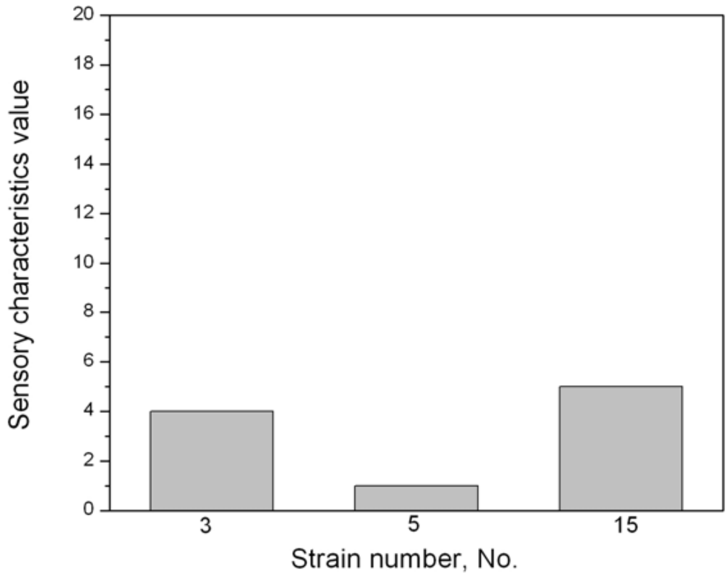

Fig. 3 Sensory characteristics value achieved after the fermentation of whey with three selected strains. Strain number, No is presented in Table 1.

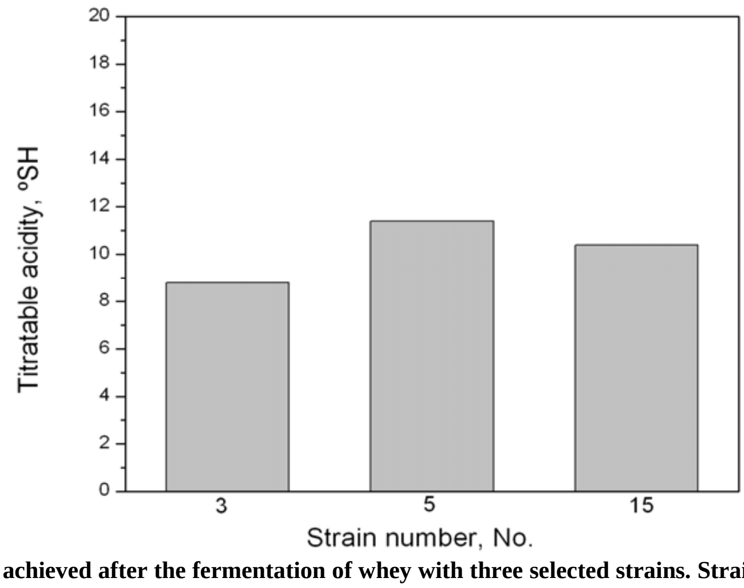

Fig. 4 Titratable acidity achieved after the fermentation of whey with three selected strains. Strain number, No is presented in Table 1.

As shown in Fig. 4, these two strains achieved titratable acidity of 8.8 and 9.2 °SH, respectively and they are rather similar in terms of beverage production ability. The strain *Lb. johnsonii* NRRL B-2178 is a top candidate for production of functional whey based beverage due to much stronger odor like yogurth which was evaluated with grade 5. It is also interesting to note that the strain *Lb. johnsonii* NRRL B-2178 has probiotic characteristics and shows high viability in the presence of whey proteins during the storage [38], which is essential for the beverage shelf-life. In addition, all strains produced residue-free beverages with the sour-salty taste beacuse of high amount of present lactic acid.

4. Conclusions

Fermentation of whey by LAB could be an interesting alternative for the production of functional beverages. Our study showed that the strains *Lb. casei* ssp. *casei* ATCC 27139 and *Lb. johnsonii* NRRL B-2178 are good candidates for the beverage production. The strain *Lb. johnsonii* NRRL B-2178, out of 16 tested strains, is a top candidate for the functional whey based beverage production. This strain attained titratable acidity of 9.2 °SH after 10 h of fermentation, appropriate odor and cell number of 6.8 log (CFU mL^{-1}). The research will go on to employ this strain or eventually the combination of this strain with other strains in optimization the beverage production, especially in terms of sensory characteristics.

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