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Immobilization Effects of Wheat Bran on Enhanced Viability of Dairy Starters under Acid and Bile Salts Stresses

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Abstract

Background and Objective: Survival of beneficial microorganisms in human gut faces many challenges. Immobilization on dietary fibers not only increases the viability of probiotic cultures, but also improves intestinal microbiota composition and decreases several diseases. Therefore, the objective of this study was to assess effects of wheat bran immobilization on survival of multiple species dairy starters under high acidity and bile salts conditions.

Materials and Methods: Dairy starter association included lactic acid bacteria of *Lactobacillus delbrueckii* RKM 0850, *Lactobacillus paracasei* RKM 0852 and *Lactobacillus. parabuchneri* RKM 0854, acetic acid bacteria of *Acetobacter syzygii* RKM 0855, propionic acid bacteria of *Propionibacterium freudenreichii* subsp. *shermanii* PL and yeast of *Kluyveromyces marxianus* RKM 0853. Physical immobilization of the whole association was carried out using adsorption on 3% wheat bran.

Results and Conclusion: Effects of acid and bile salts stresses on microbial association survival largely depended on the taxonomic affiliation, aeration rate and experiment media. *Kluyveromyces marxianus* RKM 0853 was highly resistant to pH 2.0-3.0 and addition of 0.5-15.0% of bile salts. Furthermore, *Lactobacillus paracasei* RKM 0852 and *Acetobacter syzygii* RKM 0855 were the most resistant microorganisms to bile salts and *Lactobacillus delbrueckii* RKM 0850 and *Lactobacillus parabuchneri* RKM 0854 included the best survival rates at low pH. Wheat bran immobilization promoted survival of the microbial association under stress conditions and prevented loss of viability by more sensitive species. These results are essential for understanding and improvement of stress tolerance in probiotic microorganisms of various taxonomies.

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1. Introduction

Nowadays consumers are increasingly interested in their health and, especially, in foods that are capable of preventing and treating diseases. In this regard, the relevance of obtaining foods with probiotic properties has increased sharply. Probiotic microorganisms demonstrate numerous health beneficial effects producing various biologically active compounds, namely bacteriocins, short-chain fatty acids, vitamins, enzymes, bioactive peptides and exopolysaccharides, exhibiting antimicrobial, antimutagenic, anticarcinogenic and immunomodulatory activity, lowering cholesterol level and maintaining the integrity of the mucous membrane, preventing the adhesion of pathogenic microorganisms [1-4]. It is established that probiotics, which include several strains at once, provided they are balanced, have a more pronounced effect compared to single-component preparations [5,6]. Use of probiotic microorganisms in the production of dairy products is complicated by the problems with survival of these bacteria [7-11]. In technological processes of food production, during storage and passage through the digestive tract, probiotic cultures are subjected to many aggressive influences, which lead to a decrease in their activity, partial

or complete death. The main risk factors for probiotic microorganisms are prolonged exposure to the acidic environment of the stomach, the effect of bile acids, enzymes, antimicrobial components contained in products, and oxygen [12]. The survival rate of various probiotic strains in different parts of the gastrointestinal tract can vary greatly. Some strains quickly die in the stomach, while others are able to pass through the entire intestine in large numbers [13].

Probiotic cultures must either adapt to such a dynamic environment or be protected. In this regard, the development of methods for protecting cells of probiotic cultures and increasing their viability in adverse production and gastrointestinal tract conditions is relevant [14]. One way to maintain activity and viability under aggressive environment of gastrointestinal tract is to immobilize bacterial cells on various carriers. The potential benefit of this strategy is to maintain greater cell viability, providing protection and delivery of cells to the human gastrointestinal tract, despite the high acidity of the stomach [15]. The use of prebiotic additives containing soluble or insoluble dietary fiber for such immobilization, at the same time, favorably affect the intestinal microbiota and helps to improve the condition of a number of diseases [16-20]. According to various investigations, the consumption of dietary fiber has been shown to reduce the risk of coronary heart disease, stroke, hypertension, diabetes, obesity and some gastrointestinal disorders, to improve immune function, serum lipids and glucose levels in blood, to lower blood pressure, promote stool regularity and weight loss [20-26]. Wheat bran possesses antioxidant [27] and immunomodulatory [28] activity and have anti-cancer effect [29,30]. Dietary arabinoxylans of wheat bran impact the gut associated microflora [28] and reduce the plasma total cholesterol and LDL-cholesterol concentrations in hamsters [31]. The wheat bran xylooligosaccharides can serve as a substrate for bifidobacteria growth [32] and protect probiotic cultures in freeze-drying [33]. The purpose of this work was to study the possibility of increasing survival of probiotic dairy starter (*Lactobacillus* (*L.*) *delbrueckii* RKM 0850, *L. paracasei* RKM 0852, *L. parabuchneri* RKM 0854, *Acetobacter* (*A*) *syzygii* RKM 0855, *Propionibacterium (P*.) *freudenreichii* subsp. *shermanii* PL, and *Kluyveromyces (K.) marxianus* RKM 0853) under the action of main intestinal limiting factors - acidic and high bile stresses, by immobilization. Wheat bran was selected for the production of synbiotic beverage due to the highest adhesion of association microorganisms to it in comparison with other possible carriers [34]. Furthermore, wheat bran was previously shown to protect bifidobacteria from bactericidal action of the starter [35]. Moreover, the regular consumption of white bread by the overwhelming majority of the population leads to the most pronounced deficiency of insoluble dietary fibers in comparison with soluble ones, which can be obtained from fruits and vegetables. Of particular interest was to determine the influence of the experiment conditions on the final result.

2. Materials and Methods

2.1 Microorganisms and media

In this study, dairy starters of whey-based beverage, including lactic acid bacteria (LAB) of *L. delbrueckii* RKM 0850, *L. paracasei* RKM 0852 and *L. parabuchneri* RKM 0854, acetic acid bacteria (AAB) of *A. syzygii* RKM 0855, propionic acid bacteria (PAB) of *P. freudenreichii* subsp. *shermanii* PL and lactose-fermenting yeast of *K. marxianus* RKM 0853, were used. The microbial association was prepared in the Laboratory of Food Microbiology, Research and Production Center of Microbiology and Virology, Republic of Kazakhstan and selected based on its antagonistic activity and sensory properties during whey fermentation using wheat bran addition. The major survival media included whey (Amiran, Kazakhstan), physiological saline and MRS (de Man, Rogosa and Sharpe) (TM Media, India).

Wheat bran (Siberian Bran LLC, Russia) was used at 3% (w v^{-1}) in milled (particle size < 0.5 mm) and unmilled (particle size 0.5-3.0 mm) forms. Wheat bran was sterilized with whey. Since AAB of the association (*A. syzygii* RKM 0855) were characterized using the most abundant growth in a mixture of LAB on MRS agar (due to symbiotic interrelationships), this medium was used for the microbial count. *Kluyveromyces marxianus* RKM 0853 was easily differentiable on MRS agar. Enumeration of various species was carried out based on the colony morphology as follows: *K. marxianus* RKM 0853 formed convex colonies often with a prominent center in white or beige-white with 2–5 mm in diameter; *L. delbrueckii* RKM 0850 formed flat, colorless, matte translucent colonies with 1–3 mm in diameter; *L. parabuchneri* RKM 0854 formed bluish-white, convex uneven-edged colonies with 1–3 mm in diameter; *L. paracasei* RKM 0852 formed creamy-white, strongly convex, rounded smooth-edged colonies with 1.0-1.5 mm in diameter; *P. freudenreichii* subsp. *shermanii* PL formed white, convex colonies with 1 mm in diameter; and *A. syzygii* RKM 0855 formed beige, slightly convex gloss colonies with up to 1 mm in diameter. The PAB were counted on ASLA agar with the Propionibacteria Growth Supplement (Titan Biotech, India). A magnifier 3× was used for *A. syzygii* RKM 0855 counts.

2.2 Physical immobilization of the microorganisms on wheat bran

Immobilization of the starters was carried out using physical adsorption of the microbial association on untreated wheat bran. Adsorption occurred nearly 5 min after introducing the starter culture into whey with bran. More than 75% of all microorganisms were successfully

immobilized on wheat bran [34]. This level of adhesion was suggested for the production of food products. Increases in adhesion degrees of the starters in food matrix using chemical procedures are not entirely desirable. Moreover, such procedures could increase costs of the final products.

2.3 Viability at low pH

To assess survival rate in increased acidity of the media, pH of the whey and physiological saline was acidified to 3.0 and 2.0 (Multi-Parameter Analyzer Consort C931, Belgium) using 1 N hydrochloric acid solution and then the two chemicals were sterilized. Control pH was the initial pH of physiological saline (pH 6.2). Test tubes with 10 ml of either whey or physiological saline were used to create predominantly anaerobic conditions, and flasks with 20 ml of the media were used for the experiments with increased aeration by shaking. In general, 1 ml of each bacterium containing 1.5×10^8 CFU ml^{-1} (0.5 McFarland turbidity standard) and 1 ml of *K. marxianus* RKM 0853 containing 2×10^6 CFU ml^{-1} (1 McFarland turbidity standard) were inoculated in 30 ml of whey and incubated at 37 °C for 24 h. The 24-h fermented whey (with or without bran) was introduced into media at 1% (for flasks) or 10% (for tubes) of the media volume. Immediately, the initial quantity of CFUs per milliliter was assessed spreading on MRS agar from 10-fold dilutions in physiological saline. Test tubes were preserved statically and flasks were agitated at 120 rpm for 2 h at 37 °C. Survival of the association micro-organisms was assessed after 1 and 2 h by plating from 10-fold dilutions in physiological saline. Before preparing dilutions, variants were thoroughly agitated and set for 30 s for bran sedimentation. Plates were incubated at 37 °C for 48-72 h.

2.4 Survival in high bile contents

To study viability of the microorganisms in high bile contents, ox bile powder (dried) (TM Media, India) was added into media at 0.5, 1.0, 5.0, 10.0 and 15.0 (% w v^{-1}) concentrations. Physiological saline, whey and MRS were used to study resistance of the association microorganisms to the bile. Cultures were stored at 37 °C for 24 h.

2.5 Microorganism counts and statistical analysis

To calculate CFUs in 1 ml of the media, a series of ten-fold dilutions in physiological saline were prepared and 0.1 ml of each dilution was spread onto three Petri dishes. Plates were incubated at 37 °C for 48 or 72 h. All dilutions with an average number of colonies from 15 to 150 on a plate were used when calculating CFU per milliliter. For digital data reduction and more clarity, number of CFUs per milliliter was transformed into log CFU ml^{-1}.

Statistical analysis of the results was carried out based on the standard method using Student t-test. The standard deviations or standard errors were not indicated to avoid overload with digital data and to facilitate perception. Data with significance levels of $p \leq 0.05$ were considered significant. In comparing data, only statistically significant differences ($p \leq 0.05$) between the average values in samples were reported.

3. Results and Discussion

3.1 Survival under acid stress conditions

When comparing survivals of the bacteria and yeasts in association, *K. marxianus* RKM 0853 included good survival even at pH 2.00 for 2 h, while bacterial growth did not occur when not introducing wheat bran into the media (Table 1). Survival rate of the bacteria at pH 3.0 with no immobilizations was nearly 100% but completely lost after 1 h of exposure at pH 2.0. Immobilization of the starters on wheat bran contributed to the survival of bacteria at pH 2.0. After 1 h, cell viability was almost as much as that of the control (initial value) and decreased only by 2 log after 2 h while the ratio of individual species was reported similar to that of the control. Data showed survival rate of the individual species in physiological saline under acid stress, increased aeration and relatively anaerobic conditions.

The Figure 1 presents data on viability of the association bacteria in aerobic conditions.

Table 1. Survival of the starter microorganisms in acidified whey under anaerobic conditions (log CFU ml^{-1})

Microorganisms	Variant	pH 6.00			pH 3.00			pH 2.00		
		0 h	1 h	2 h	0 h	1 h	2 h	0 h	1 h	2 h
Bacteria	Whey	8.0	7.9	8.5	8.0	7.9	7.7	7.8	-	-
	Whey with bran		8.0	8.3		7.9	8.0		7.6	5.8*
	Whey with ground bran		7.7	8.0		7.9	7.7		7.5	5.5*
Yeast	Whey	5.8	5.8	6.1	5.8	5.5	5.6	5.7	5.8	5.9
	Whey with bran		5.3	6.2		6.2	5.9		6.0	5.5
	Whey with ground bran		5.5	6.2		6.1	6.3		6.2	5.9

- The asterisks mean statistically significant differences at $p \leq 0.05$.
- A dash "-" means the absence of viable cells or their presence in amounts less than 3 log CFU ml^{-1}.

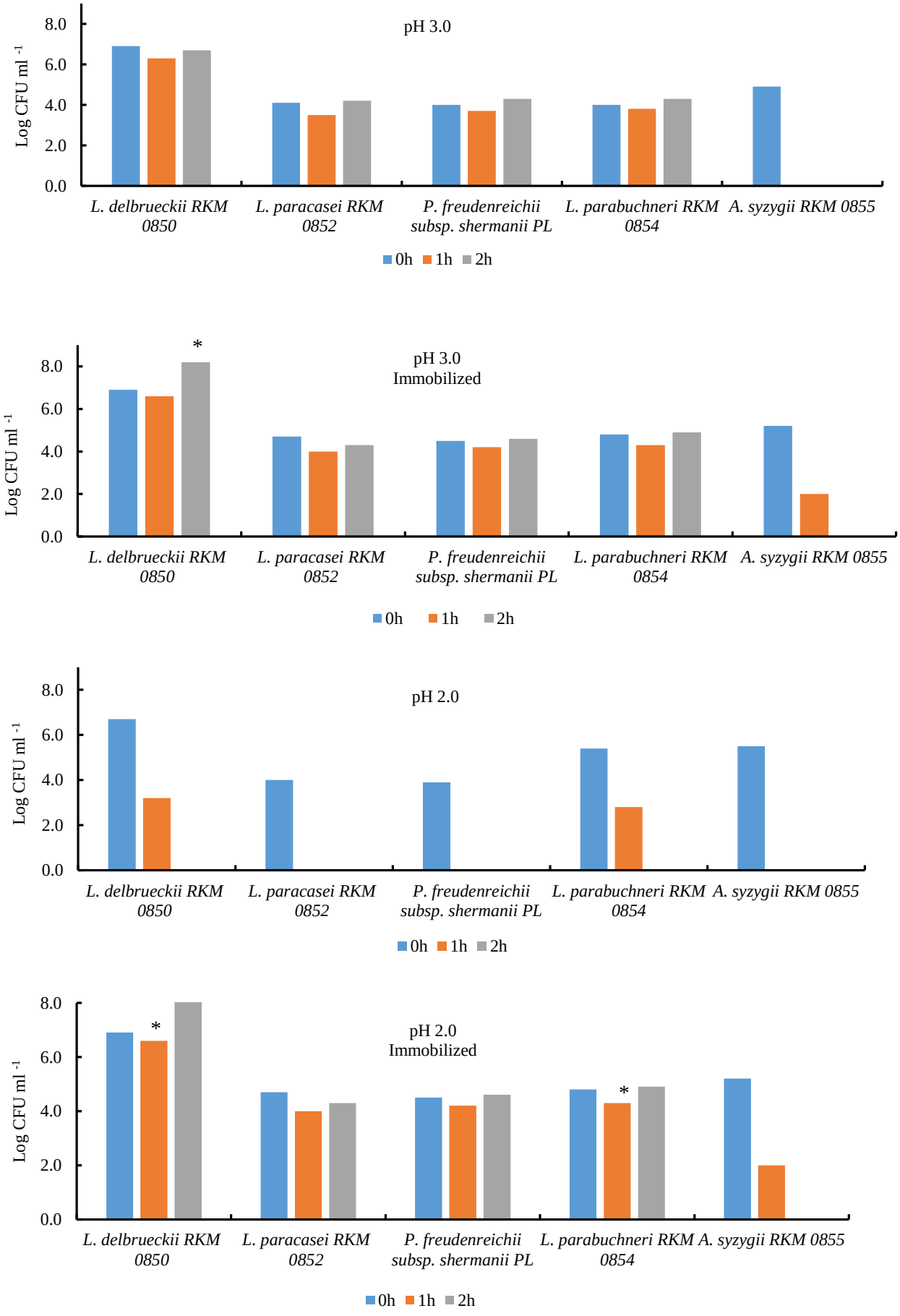

Figure 1. Survival rates of the starter bacteria at low pH in physiological saline under aerobic conditions (The asterisks mean statistically significant differences of free and immobilized cells at $p \leq 0.05$)

Replacement of whey with saline promoted survival rate of the starter microorganisms in variants with no immobilizations and demonstrated their partial survival after 1 h exposure at pH 2.0. This is possibly a consequence of the onset of bacterial growth after transferring into fresh whey and as a result of greater exposure of the cells to stress conditions, compared to resting cells in physiological saline. Data showed various degrees of sensitivity of the association microorganisms to low pH. Therefore, *L. delbrueckii* RKM 0850 and *L. parabuchneri* RKM 0854 were shown to be more resistant to low pH than other species and *A. syzygii* RKM 0855 was the most sensitive strain. Higher (16-20%) initial log CFU ml^{-1} values for *L. paracasei* RKM 0852, *P. freudenreichii* subsp. *shermanii* PL and *L. parabuchneri* RKM 0854 in variants by wheat bran addition indicated positive effects of bran on growth rate and biomass production of these microorganisms. After 1 h incubation in physiological saline with pH of 3.0, log CFU ml^{-1} of PAB and all cultures of LAB, except *L. delbrueckii* RKM 0850, decreased slightly and then increased, reaching the initial value within 2 h. Number of CFUs per milliliter of *L. delbrueckii* RKM 0850 increased by 1.6 log due to the growth of the microorganism. The *A. syzygii* RKM 0855 was not detected upon plating after 1 and 2 h of incubation in saline with pH 3.0. Wheat bran immobilization increased survival of *A. syzygii* RKM 0855 cells, growth of the culture was seen after 1 h, although number of CFU per milliliter was lower than the initial number by 3.4 log. Furthermore, *L. paracasei* RKM 0852, *P. freudenreichii* subsp. *shermanii* PL and *A. syzygii* RKM 0855 included weak survival rates at pH 2.0. The *L. parabuchneri* RKM 0854 and *L. delbrueckii* RKM 0850 demonstrated the highest viabilities at pH 2.0. the log CFU ml^{-1} of these microorganisms respectively decreased by 1.2 and 1.9 log after 1 h and by 2.5 and 2.8 log after 2 h.

Use of immobilization on wheat bran increased resistance of all microorganisms to acid stress. Therefore, species with weak resistance to low pH saved their partial viabilities at pH 2.0 after 1 h, while further resistant species increased number of CFUs per milliliter after 1 h by 1.5 log, compared to that with non-immobilized cultures, being partly viable after 2 h. Since LAB are facultative anaerobic microorganisms and gastrointestinal environment is predominantly anaerobic, dependence of probiotic starter survival on degree of aeration is greatly interested. Unlike previous experiments, wheat bran was not introduced into physiological saline with low acidity in this experiment; however, fermented whey was added to the saline in a ratio of 1:10. For a better comparison of the results, the two major LAB in starter cultures (*L. delbrueckii* RKM 0850 and *L. paracasei* RKM 0852) were used at equal quantities. The microorganisms were characterized by better growths in whey with wheat bran. Therefore, the initial CFUs per milliliter were higher in variants with whey fermented using bran (Table 2). Results showed 100% survival rates of the major starter microorganisms at pH 3.0 for 2 h. After 1 h at pH 2.0 with no immobilizations, CFUs per milliliter decreased by 1.7 log for *L. delbrueckii* RKM 0850 and by 2.7 log for *L. paracasei* RKM 0852. This decrease of survival was much lesser than that in experiments with increased aeration, when *L. paracasei* RKM 0852 was not generally detected after 1 h and CFUs per milliliter of *L. delbrueckii* RKM 0850 decreased by 3.5 log. However, microorganisms grown in whey with no carriers completely lost their viability after 2 h.

Table 2. Survival of *Lactobacillus paracasei* RKM 0852 and *Lactobacillus delbrueckii* RKM 0850 at low pH in physiological saline under anaerobic conditions (log CFU ml^{-1})

Strain	Variant	pH 6.0			pH 3.0		pH 2.0	
		0 h	1 h	2 h	1 h	2 h	1 h	2 h
L. paracasei RKM 0852	Whey	5.9	6.1	6.4	6.1	6.2	3.2*	-
	Whey with bran	6.3	6.0	6.6	6.4	6.2	5. 7*	4.5*
	Whey with ground bran	6.1	6.1	6.6	5.7	6.3	6.1	4.3*
L. delbrueckii RKM 0850	Whey	5.4	5.9	5.9	5.6	5.6	3.7*	-
	Whey with bran	6.3	6.5	6. 5	6.4	6.4	5.4*	3.2*
	Whey with ground bran	6.4	6.3	6.3	6.2	6.4	5.6*	3.5*

- The asterisks mean statistically significant differences at $p \leq 0.05$.
- A dash "-" means the absence of viable cells or their presence in amounts less than 2 log CFU ml^{-1}.

Use of wheat bran as carrier during whey fermentation contributed to the survival of the two microorganisms at pH 2.0. After 1 h, CFUs per milliliter decreased by 0.8 log for *L. delbrueckii* RKM 0850 and by 2.1 log for *L. paracasei* RKM 0852 (the initial values in variant with and without bran). Thus, immobilization on wheat bran contributed to the survival of various LAB cultures to various degrees. Low survival degrees of the association bacteria in absence of immobilizers are most possibly associated with the use of whey in the experiment. It is noteworthy that actively growing cells in the logarithmic growth phase are more susceptible to negative effects [36]. Better survival results

were seen under anaerobic conditions even with no additional supplements of bran in the media.

3.2 Survival in high bile contents

Study of the microbial association survival in media with high bile contents showed high resistances of yeasts to the bile. During aerobic cultivation of the microbial association in whey for 24 h at the highlighted bile concentrations (0.5–15%), number of CFUs per milliliter of *K. marxianus* RKM 0853 did not change. Only *L. paracasei* RKM 0852 was able to aerobically survive in 15% bile conditions with number of CFUs per milliliter decreased by 4.1 logs. Immobilization on ground wheat bran increased survival of the species by 1.4 log CFU ml^{-1}. No statistically significant increases were reported in survival of the starter bacteria, using unmilled wheat bran. The reason possibly included the low frequency of contacts of microorganisms with particles of bran during active agitation, compared to that with more small particles of bran. Use of anaerobic conditions significantly decreased bile resistance of *K. marxianus* RKM 0853. With increases in bile concentration up to 5%, the number of CFU per milliliter of yeast decreased by three logs. Figure 2 presents data on survival of the bacterial starters in MRS media with increased bile contents under static anaerobic conditions.

The most resistant strain to effects of bile salts were *L. paracasei* RKM 0852 and *A. syzygii* RKM 0855. Immobilization of the starters on wheat bran increased the number of viable bacterial cells by 0.5–3.7 logs, depending on the concentrations of bile in media. Survival under stress conditions while changing oxygen availability was earlier shown in other bacteria, yeasts and stress types [37,38]. Use of milled bran did not show significant differences in survival rates, compared to unmilled bran in all experiments, except those with agitations. However, statistically insignificant positive correlations of bacterial counts with unmilled bran and those of yeast counts with milled bran were reported. These correlations were most likely associated with a greater availability of ground bran for the growth of yeasts and hence a larger accumulation of biomass in these variants. When media were agitated, addition of milled bran to the media increased the exposure of their particles to the microbial cells and subsequent immobilization. Under static cultivations, degrees of grinding included no effects on the microbial survival since bran and cells were concentrated in the bottom of the test tubes.

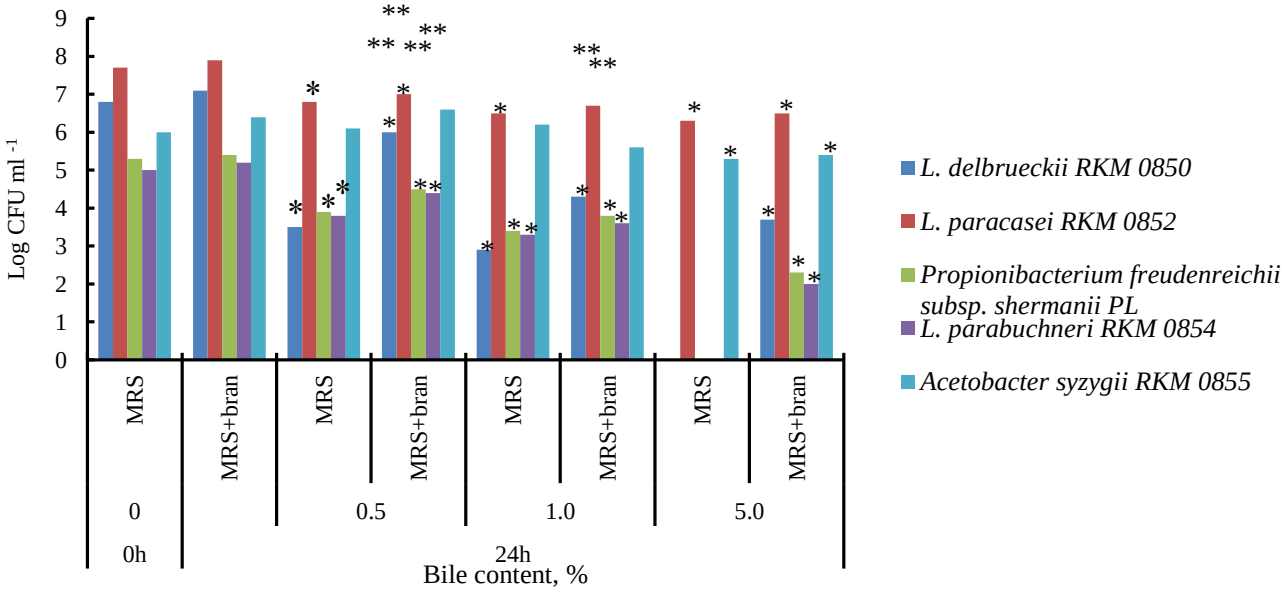

Figure 2. Effects of wheat bran on the survival rates of starter bacteria in high bile contents under anaerobic conditions (The "*" refers to significant decrease in survival compared to 0 h at $p \leq 0.05$; The "**" refers to significant increase in survival of immobilized compared to non-immobilized cells at $p \leq 0.05$)

4. Conclusion

Starter microorganisms were characterized by various survival rates under acid and bile stresses. The highest resistance was shown for yeasts and the lowest for acetic acid bacteria. Survival of bacteria under conditions of gastrointestinal stress varied depending on the species, presence of carrier, availability of nutritious media and aeration degree. The anaerobic treatment was mostly favorable for the survival of bacterial association. Immobilization of dairy starters on wheat bran included significant protective effects at low pH and high bile salt concentrations. This contributed to the conservation of species diversity within the multicomponent starter microorganisms, especially species with weak resistances. Wheat bran immobilization prevented loss of viability of all association microorganisms and improved their survival under stress conditions. Based

on the passing time of dairy foods through the stomach and the fact that acidity of the gastric juice in stomach is 1.5–3.5, the current data reveal good survival rates of synbiotic beverage microorganisms in gastric juices. Immobilization of the starter cells on wheat bran can improve further survivals of the probiotic microorganisms at sufficient quantities. The current results are critical for further analyzing data on survival of the starter microorganisms under stress conditions and preserving microbial diversity of the starters. Moreover, these results can be used in development of next generation probiotics [39] based on microbial taxons identified in healthy people using metagenomic studies of the intestinal microbiota.

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6. Conflict of Interest

The authors declare no conflict of interest.

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اثرات تثبیت سبوس گندم بر افزایش زنده‌مانی آغازگرهای لبنی در شرایط تنش اسیدی و نمک‌های صفراوی

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چکیده

سابقه و هدف: زنده‌مانی ریزاندامگان‌های[1] مفید روده انسان با چالش‌های بسیاری روبه‌روست. تثبیت بر فیبرهای رژیمی نه تنها زنده‌مانی کشت‌های زیست‌یار[2] را افزایش می‌دهد، بلکه ترکیب زیست‌بوم[3] روده‌ای را بهبود می‌بخشد و بروز بسیاری از بیماری‌ها را کاهش می‌دهد. بنابراین، هدف این مطالعه ارزیابی اثرات تثبیت سبوس گندم بر زنده‌مانی گونه‌های متعدد آغازگرهای تحت شرایط اسیدی بالا و نمک صفراوی می‌باشد.

مواد و روش ها: آغازگرهای لبنی شامل باکتری‌های لاکتیک اسید *لاکتوباسیلوس دلبروکی* RKM ۰۸۵۰، *لاکتوباسیلوس پارا‌کازئی* RKM ۰۸۵۲، *لاکتوباسیلوس پارابوچنری* RKM ۰۸۵۴، باکتری‌های استیک اسید *استوباکتر سیزیجی* RKM ۰۸۵۷، باکتری‌های پروپیونیک اسید *پروپیونوباکتریوم فرئودنریچی* زیرگونه *شرمانی* PL و *کلیورومایسس مارکسیانوس* RKM ۰۸۵۳ بودند. تثبیت فیزیکی کل مخلوط[4] با جذب بر ۳٪ سبوس گندم انجام شد.

یافته‌ها و نتیجه‌گیری: اثرات تنش‌های اسیدی و نمک‌های صفراوی بر زنده‌مانی جامعه میکروبی به میزان زیادی به طبقه‌بندی نژادی، میزان هوادهی و محیط کشت آزمایشی دارد. *کلیورومایسس مارکسیانوس* RKM ۰۸۵۳ تا ۳/۰-۲/۰ pH و افزودن ۱۵-۰/۵ درصد نمک‌های صفراوی بسیار مقاوم است. علاوه بر این، *لاکتوباسیلوس پارا‌کازئی* RKM ۰۸۵۲ و *استوباکتر سیزیجی* RKM ۰۸۵۷مقاوم‌ترین ریزاندامگان‌ها به نمک‌های صفراوی بودند و *لاکتوباسیلوس دلبروکی* RKM ۰۸۵۰ و *لاکتوباسیلوس پارابوچنری* RKM ۰۸۵۴بیشترین میزان زنده‌مانی در pH پایین را داشتند. تثبیت سبوس گندم زنده‌مانی جامعه میکروبی تحت شرایط تنش را بهبود بخشید و از از بین رفتن زنده‌مانی توسط گونه‌های حساس‌تر جلوگیری کرد. این نتایج برای درک و بهبود تحمل تنش در ریزاندامگان‌های زیست‌یار مربوط به طبقات نژادی گوناگون ضروری است.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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- تنش صفراوی
- تثبیت
- آغازگر لبنی
- زنده‌مانی میکروبی
- سبوس گندم

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1 Microorganisms
2 Probiotic
3 Microbiota
4 Cocktail