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Edited by
Radoje V. PANTOVIC
and
Zoran S. MARKOVIC

EcoIst '13

Hotel "Jezero", Bor Lake, Bor, SERBIA
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TECHNICAL FACULTY BOR

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DEVELOPMENT STARTER CULTURES FOR PRODUCTION FUNCTIONAL BEVERAGES FROM COW'S WHEY

Maja Vukasinovic-Sekulic[1*], Lj. Mojovic[1], M. Rakin[1], M. Bulatovic[1], Z. Tasic[2]

[1]University of Belgrade, Faculty of Technology and Metallurgy,
Karnegijeva 4, Belgrade, SERBIA
[2]University of Belgrade,Technical Faculty, Vojske Jugoslavije 12, Bor, SERBIA

[*]*vukasinovic@tmf.bg.ac.rs*

ABSTRACT

Organoleptic characteristics of fermented dairy beverages largely depend on the proper selection of bacteria used for the construction of starter cultures and purpose of this study was to evaluate activity of eight two-component starter cultures in cow's whey. During fermentation, some degreeof symbiotic relationship betweenthe correspondingspecies of the genus*Lactobacillus* or *Bifidobacterium bifidum* and*Streptococcus salivarius* ssp. *thermophilus* was noticed, as fermentation time has been significantly reduced, in comparison with single strains. All fermented whey beverages had satisfactory organoleptic characteristics and total bacterial count wasgreaterthan 106cfu/ml, so they fulfil criteriato call themprobioticbeverages.

Key words: starter culture, probiotic, lactic acid bacteria, cow's whey, functional beverage.

INTRODUCTION

During the last decade, consumer's interest in functional foods significantly increased as a result of greater awareness of importance of inclusion healthy food in the diet. For today's consumers are not only important to extend life span, but also tomakesuch a life healthier. This is supported by the fact that cost of medical care is rising every day and functional foods appeared as a cheaper and effective means to protect health and well-being without relying on expensive pharmaceuticals products.

Whey is the major by-product of the cheese and casein industry which has not been sufficiently present in the human diet, despite the fact that it has wide range of functional attributes significant for nutritional purposes. In comparison with casein, egg and soy proteins, whey proteins are a rich source of the essential amino acids which are thought to play a important role as anti-oxidants (methionine, cysteine) and metabolic regulators (leucine, isoleucine, and valine) in protein, glucose and lipid metabolism. Due to the latter effect and fact that it does not have a lot of calories, whey is recommended in a diet of obese persons as its regular consumption may have a role in weight control. In addition, whey proteins (α-lactalbumin, β-lactoglobulin, lactoferrin, lactoperoxidase, and bovine serum albumin) have antimicrobial and antiviral actions, immune system

stimulation, anticarcinogenic activity and other metabolic features important for promotion of health and prevention of diseases. Beside proteins, whey is rich sources of minerals (copper, zink, iron, iodine) and water soluble vitamins (B5, B2, C, B6) which could also improve physiological functions in the body [1].

Lactic acid fermentation is the simples and safest means to transform large volume of whey into functional beverages with desirable sensory profiles. This way could be an interesting alternative for whey utilisation, since with fermentation, dairy factory could avoid expensive technological process, such as ultrafiltration and drying, which are mainly used during preparation whey powder or whey protein concentrate powder. Also for production of functional whey beverages dairy industry can use the same equipments as for the production yogurt or other fermented dairy drinks which allow utilisation of whey almost immediately after cheese production [2,3,4].

Many different species of lactic acid bacteria are successfully used as a starter cultures in the productionof variousfermented products and till now unwanted side-effects related with consummation of this food have never been documented. Presence of lactic acid bacteria in food can greatly improve the safety, shelf life, nutritional value, flavour and overall quality of the fermented products [5]. In recent years there has been a growing interest for production functional food which contains certain species of lactic acid bacteria and bifidobacteria with probiotic characteristics. Regular use of probiotics in the diet reduce signs and symptoms of lactose intolerance, contribution prevention and treatment of certain diarrhoeal diseases, stabilisation of gut mucosal barrier, reduction of bacterial enzyme activities and stimulation of the immune system [6].

Considering the potential of whey has, the aim of this study was to develop new starter cultures and to evaluate their growth and activity (fermentation time, titratable acidity (^{0}SH) and organoleptic characteristics) in reconstituted sweet cow's whey. Based on these resultswill be select starter cultures for production of a functional whey beverages.

MATERIALS AND METHODS

PREPARATION OF RECONSTITUTED COW'S WHEY FOR THE FERMENTATION PROCESS

Sweet whey powder with following chemical composition (lactose 69,62 g/100g, proteins 12,11 g/100g, lipids 1g/100g) was used for preparation reconstituted whey. Whey powder was dissolved in sterile distilled water under aseptic conditions to approximately 6,5% (w/w) of total solid. After reconstitution, flasks with whey were pasteurized in a water bath (60 °C for 1 hour) with constantmixing for bettertransfer of heat.After pasteurization whey is rapidly cooled to a temperature of 42^{o}C, which was chosen for fermentation process.

MICROBIAL CULTURES USED FOR DEVELOPMENT STARTER CULTURES

Seven strains from the genus *Lactobacillus* (*Lb. delbrueckii* ssp. *lactis* NRRL B-4525, *Lb. acidophilus* ATCC 4356, *Lb. helveticus* ATCC 15009, *Lb. reuteri* ATCC 23272, *Lb. rhamnosus* ATCC 7469, *Lb. delbrueckii* ssp. *bulgaricus* ATCC 11842, *Lb. johnsonii* NRRL B-2178) and one strain of *Bif. bifidum* NRRL B-41410 and *St. salivarius* ssp. *thermophilus* CNRZ S3 (389) were chosen for construction starter cultures. Cultures were stored at -20 ^{0}C in MRS or M17 broth with 20% (v/v) glycerol and prior to experimental use strains were activated twice in MRS broth (*Lactobacillus* strains and *Bif. bifidum*) or M17 broth with lactose (*St. salivarius* ssp. *thermophilus*). All strains were incubated anaerobically overnight at the optimal temperature for growth (37 ^{0}C, no longer than 18h).

ACIDIFICATION ACTIVITY OF INDIVIDUAL STRAINS IN RECONSTITUTED WHEY

Before construction of starter cultures, acidification activity of all strains was tested in reconstituted cow's whey. Flask with 50ml of reconstituted whey was inoculated with 1% (vol/vol) of individual strain and incubated at 42^{0}C for 24h. Evaluation of acidification activity was performed on fermented whey samples after 6 and 24h of fermentation, measurement pH and titratable acidity (^{0}SH).

STARTER CULTURES USED FOR PRODUCTION FERMENTED WHEY BEVERAGES

Composition of eight two-component starter cultures is presented in a table 1.

Table 1. Composition of starter cultures used for production fermented whey beverages

Starter cultures	Composition of starter cultures
S1	*Lb. helveticus* ATCC 15009, *St. salivarius* ssp. *thermophilus* CNRZ S3 (389) (2:1)
S2	*Lb. delbrueckii* ssp. *lactis* NRRL B-4525, *St. salivarius* ssp. *thermophilus* CNRZ S3 (389) (2:1)
S3	*Lb. delbrueckii* ssp. *bulgaricus* ATCC 11842, *St. salivarius* ssp. *thermophilus* CNRZ S3 (389) (2:1)
S4	*Lb. acidophilus* ATCC 4356, *St. salivarius* ssp. *thermophilus* CNRZ S3 (389) (2:1)
S5	*Lb. rhamnosus* ATCC 7469, *St. salivarius* ssp. *thermophilus* CNRZ S3 (389) (2:1)
S6	*Bif. bifidum* NRRL B-41410, *St. salivarius* ssp. *thermophilus* CNRZ S3 (389) (2:1)
S7	*Lb. johnsonii* NRRL B-2178, *St. salivarius* ssp. *thermophilus* CNRZ S3 (389) (2:1)
S8	*Lb. reuteri* ATCC 23272,*St. salivarius* ssp. *thermophilus* CNRZ S3 (389) (2:1)

Characteristics of eight developed starter cultures were compared with activity of commercial freeze dried starter culture (Lactoferm ABY-6) containing *St. salivarius* ssp. *thermophilus* 80%, *Lb. acidophilus* 13%, *Bif. bifidum* 6% and *Lb. delbrueckii* ssp. *bulgaricus* 1% (Biochem srl- Centro Ricerche Biochimiche). Inoculum was prepared by rehydrating 1 g of freeze-dried ABY-6 starter culture in 100 mL whey and then

reactivated at 42 °C for 30 min. After reactivation, 2% (v/v) of inoculum was added in reconstituted cow's whey sample.

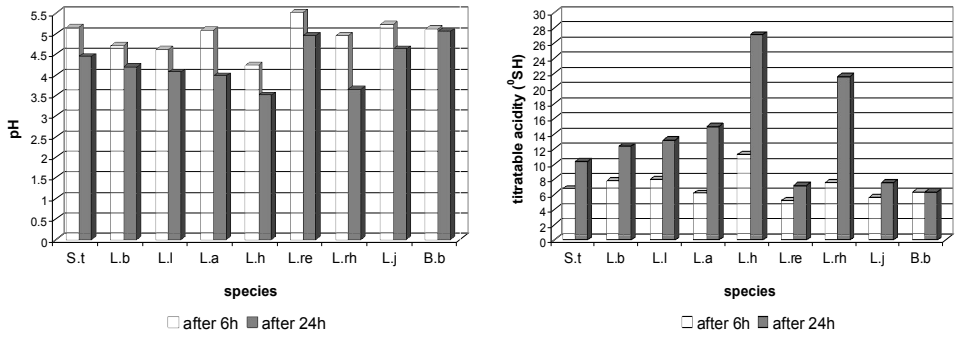
PRODUCTION OF FERMENTED WHEY BEVERAGES

Erlenmeyer flasks with 200ml of pasteurized cow's whey (temperature 42^{o}C) were inoculated with 2% (v/v) two-componentstarter cultures presented in table 1 and previously activated ABY-6 starter culture. After inoculations, the contents of erlenmeyer flasks were thoroughly mixed and incubated at 42^{o}C in a circulatingwater bath, until pH of around 4,6 was reached. At the end of incubation, the content of the erlenmeyer flasks was rapidly cooled in ice water to stop further whey fermentation. Cooled samples were analyzed for titratable acidity (^{0}SH) and the viable count of bacteria was determined using plate count method and results were expressed as cfu/ml. The count of lactobacilli and *Bif. bifidum* was estimated on MRS agar plates after microaerophilic incubation 3 days at 37 ^{0}C. Microaerophilic conditions were obtained by a one more layer of MRS agar over the MRS agar inoculated with bacteria. The count of *St. salivarius* ssp. *thermophilus* was determined on M17 agar plates with lactose after incubation 48h at 37 ^{0}C. After completing analysis, the samples were stored at 4^{o}C and after 24 h were evaluated organolepticlly (appearance, taste, odor, body and texture).

RESULTS AND DISCUSSION

Characteristics of fermented products largely depend on proper selection starter cultures used for fermentation process. Acidification activity of strains is one of critical technological aptitudes which can have influence on selection strains for developing starter cultures [5]. Unfortunately, not all strains of lactic acid bacteria and bifidobacteria are capable to grow fast in media such as milk and whey. This is especially characteristic for probiotic strains that inhabit the intestinal tract of humans and animals, which usually have longer adaptation time and grow slowly in milk and whey, that results in a longer fermentation time required to reach a pH of 4,6 [7,8,9,10]. For these reasons in this study before developing starter cultures, the acidification activity of nine selected species of lactic acid bacteria and bifidobacteria was evaluated in reconstituted cow's whey prepared to have 6,5% of dry matter, similar to the liquid cow's whey obtained after cheese production. The pH value of reconstituted cow's whey at the beginning of the fermentation was around 6,15 while the titratable acidity was 3,48 ^{0}SH. Fermentation process was carried out at 42^{o}C, temperature usually used for a production fermented dairy beverages (yogurt, sour milk, acidophil beverages).

Changes in pH and titrabile acidity values of whey samples after 6 and 24h of fermentation are presented in Figure 1. The quickest pH decrease was noticed in sample with the *Lb. helveticus* ATCC 15009, while *Lb. reuteri* ATCC 23272 had theslowest decrease of pH value (Fig. 1a).



a) b)

Figure 1. Acidification activity in reconstituted cow's whey a) changes of pH values of whey samples after 6 and 24h of fermentation; b) changes of titratable acidity (°SH) values of whey samples after 6 and 24h of fermentation

Abbreviations: **S.t.**- *St. salivarius* ssp. *thermophilus* CNRZ S3 (389); **L.b.**- *Lb. delbrueckii* ssp. *bulgaricus* ATCC 11842; **L.l.**- *Lb. delbrueckii* ssp. *lactis* NRRL B-4525 ; **L.a.**- *Lb. acidophilus* ATCC 4356; **L.h**.- *Lb. helveticus* ATCC 15009; **L.re**- *Lb. reuteri* ATCC 23272; **L.rh**.- *Lb. rhamnosus* ATCC 7469; **L.j**.- *Lb. johnsonii* NRRL B-2178; **B.b.**- *Bif. bifidum* NRRL B-41410;

From the study group, particularly distinctive are strains *Lb. helveticus* ATCC 15009, *Lb. delbrueckii* ssp. *lactis* NRRL B-4525 and *Lb. delbrueckii* ssp. *bulgaricus* ATCC 11842 which after 6h of fermentation decrease pH value below 4,7. Other strains (*St. salivarius* ssp. *thermophilus* CNRZ S3 (389), *Lb. acidophilus* ATCC 4356, *Lb. rhamnosus* ATCC 7469, *Lb. johnsonii* NRRL B-2178, *Bif. bifidum* NRRL B-41410), with the exception *Lb. reuteri* ATCC 23272, had the pH value after 6h of fermentation around 5,0. Trend of decreasing pH value continued between 6 and 24 h of fermentation and most samples at the end of fermentation process had pH value lower than 4,5. Again, sample inoculated with strain *Lb. helveticus* ATCC 15009 had the lowest pH values after 24 h of fermentation.

The highest titratable acidity after 6 h and 24h of fermentation was observed in sample inoculated with strain *Lb. helveticus* ATCC 15009 (°SH =11,3 and 27,1, respectively), which was expected, since the same sample had the lowest pH value at same period of time (Fig 1b). For the most other tested strains, the main increase in titratable acidity was observed between the 6th and 24th hours. Exception are strains *Lb. reuteri* ATCC 23272, *Lb. johnsonii* NRRL B-2178, *Bif. bifidum* NRRL B-41410 which in this period slightly increase titratable acidity. This species can be found as a normal microflora in intestinal tract of humans and animals and their strains usually show slow activity in substrate such as milk and whey since this media does not contain all the essential amino acids and vitamins necessary for growth this group of bacteria [6,7]. So strain *Bif. bifidum* Bb-12 need more than 28h in a milk, and around 20 h in whey, to reach pH around 4,5 which is slightly faster activity than results obtain in this studies for strain *Bif. bifidum* NRRL B-41410 [7].

Based on the results obtained for acidification activity of single cultures, eight two-component starter cultures are formed, to include one strain from genus *Lactobacillus* or *Bif. bifidum* and one strain of *St. salivarius* ssp. *thermophilus* in a rate 2:1. During the fermentation process following characteristicswere monitored: fermentation time to reach pH 4,6, titratable acidity and viable cell count of bacteria on MRS and M17 agar.Samples were incubated until pH of about 4,6 is reached, since from the literature is known that lower pH values can adversely affect the stability of the obtained fermented beverages during cold storage [7.8,9]. Characteristics of eight two-component starter cultures are presented in Figure 2.

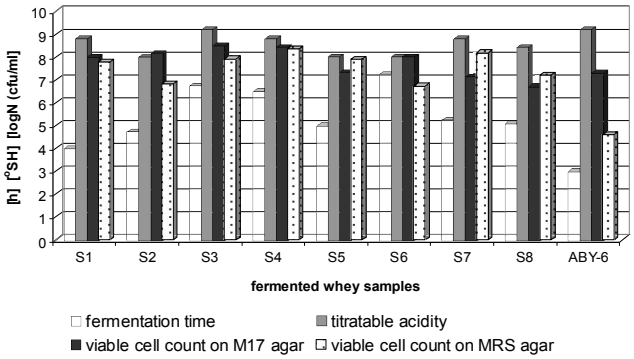


Figure 2. Characteristics of eight two-component starter cultures during fermentation in in reconstituted cow's whey

The duration of fermentation in reconstituted cow's whey ranged from 4 hours till 7,25 hours, while the titratable acidity was in the range 8.0 to 9.2 ^{o}SH which corresponds to the value achieved with culture ABY-6. (Figure 2). The shortest fermentation time is achieved with starter cultures S1 and S2, containing species *Lb. helveticus/St. salivarius* ssp. *thermophilus* or *Lb. delbrueckii* ssp. *lactis/St. salivarius* ssp. *thermophilus* respectively, which also as a single cultures achieved good acidification activity in the same kind of whey after 6 hours of fermentation (Figure 1). In comparison with activity of commercial starter culture ABY-6, fermentation time was slightlylonger with cultures S1 and S2 (3 hours, 4 hours and 4,75 hours, respectively). The longest fermentation time (7,25 hours) is achieved with starter culture S6, which beside *St. salivarius* ssp. *thermophillus* contains *Bif. bifidum* NRRL B-41410, which achieved slow activity as a single strain. In all examined starter cultures it was noticed some degreeof symbiotic relationship betweenthe correspondingspecies of the genus*Lactobacillus* or *Bif. bifidum* and*St. salivarius* ssp. *thermophilus* as fermentation time was significantly shortenedcompared with the results obtained for their single acidification activity. After fermentation, all fermented whey beverages had total count of viable cell more than 10^6 cfu/ml, with different prevalenceof strainsin the population. It was noticed that some fermented whey beverages (S2, S3, S6, ABY-6) had higher viable cell count of *St. salivarius* ssp. *thermophilus*, while other (S1, S4) had almost equally cell count of *St. salivarius* ssp. *thermophilus* and lactobacilli or the cell count of lactobacilli was higher

(S5, S7, S8). Particular, the very low proportion of strains from *Lactobacillus* sp. and *Bif. bifidum* species were found in fermented whey beverage from ABY-6 culture (around 10^4 cfu/ml). All fermented whey beverages had an excellent appearance and distinctive pleasant taste and odor, depending of starter cultures used

CONCLUSION

The results obtained in this study show that the whey isa suitable substratefor the growthof lactic acid bacteriaandthe production of functional beverages Developed starter cultures in this study have potential to be used instead of commercial starter culture ABY-6, since fermented whey beverages have satisfactory organoleptic characteristics. Further research will provide better information about stability of produced beverages during cold storage.

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