



## SURVIVAL OF *LACTOBACILLUS BULGARICUS* AND *BIFIDOBACTERIUM ANIMALIS* IN YOGHURTS MADE FROM COMMERCIAL STARTER CULTURES DURING REFRIGERATED STORAGE

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### ABSTRACT

All over the world, fermented dairy products have been consumed for nutrition and maintenance of good health for a very long time. This study evaluated the survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Bifidobacterium animalis* ssp. *lactis* BB-12 in yoghurts after the manufacturing during the shelf-life up to 21 days at 4 °C, which is mostly accepted by the consumers. The titratable acidity and pH showed the same patterns of increase or decline after manufacturing and storage of yoghurts. There was a significant difference ( $p < 0.05$ ) in acidity between yoghurts in glass bottle and plastic cup. The increase in numbers of lactobacilli and bifidobacteria and their survival during storage time were dependent on the species and strain of associative yoghurt bacteria (control-only yoghurt lactic acid bacteria and experimental containing except yoghurt culture also *Bifidobacterium animalis* ssp. *lactis* BB-12) and on the packaging material (glass bottle versus plastic cup). It was observed, that counts of bifidobacteria were lower than counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* ( $190$  to  $434 \times 10^7$  at 1d) and slowly increased ( $p < 0.001$ ) at maximum level on day 7 ( $294.3 - 754 \times 10^7$ ) and then slowly declined to  $6.33 \times 10^7$  in glass bottle and  $2.33 \times 10^7$  in plastic cups, respectively. *Lactobacillus delbrueckii* ssp. *bulgaricus* multiplied better in glass bottles than in plastic cups, as observed during experimental period in-group with *Bifidobacterium animalis* ssp. *lactis* BB-12. At the end of the storage period at 4 °C, viable counts of lactobacilli were higher ( $p < 0.001$ ) in glass bottles. All the yoghurts, contained the recommended levels of lactobacilli and bifidobacteria ( $10^7$  cfu.g<sup>-1</sup>) at the end of storage period (21 d).

**Keywords:** survival; yoghurt bacteria; *Bifidobacterium animalis* ssp. *lactis* BB-12; acidity; storage temperature

### INTRODUCTION

The first foods with probiotic bacteria were yogurts, and fermented milks are still the most important food vehicle for the delivery of probiotic bacteria. However, other foods have now appeared which carry probiotic bacteria. Numerous entries in the functional food market are linked to beverages, such as unfermented milk and fruit juices. Cheese is also gaining acceptance in the market. In addition to these commercial products, many research projects have been carried out which propose the addition of probiotics to chocolate, sausages, cereal products, dried products and vegetables. A multitude of food products contain lactic cultures and are subject to enrichment by probiotic bacteria (Hamann and Marth, 1983; Kailasapathy et al., 2008; Lovayová, 2007; Champagne, 2009).

Probiotics are applied as supporting nutritional supplements and the majority of chronic gastrointestinal diseases, where treatment of microbial flora can positively affect the health status and quality of life of these patients.

Considering to the safety of probiotics, exploring their still broader preventive and therapeutic using them early as in childhood, gets them into position became more attractive foods and dietary supplements (FAO/WHO, 2002; Lovayová et al., 2008). At present, most known probiotic organisms are bacteria, belonging to the *Lactobacillus* and *Bifidobacterium* genera.

The viability of lactobacilli and *Bifidobacterium* spp. in yogurts depends on a number of factors such as strain of probiotic bacteria incorporated, the yogurt starter cultures used. It is also fermentation time and storage conditions, pH of the yogurt (post-acidification during storage), sugar concentration (osmotic pressure), milk solids content, availability of nutrients, the presence of hydrogen peroxide, dissolved oxygen content (especially for *Bifidobacterium* spp.), buffering capacity and beta-galactosidase concentration in the yogurt (Dave and Shah, 1998; Shihata and Shah, 2000).

*Lactobacillus delbrueckii* ssp. *bulgaricus* is one of the two bacteria necessary for the production of yoghurts (Kandler and Weiss, 1984; Heller, 2001).

**Shah (2000)** reported that it is important to monitor the survival of probiotic lactobacilli because a number of products contain only a few viable bacteria by the time they reach the market.

Interest in the bifidobacteria started more or less contemporaneously, when Tissier described in the feces of breastfed infants the predominance of bacteria that produced lactic and acetic acid; these bacteria were bifurcated and which he named *Bacillus bifidus* (**Mitsuoka, 1990**), which was later called *Bifidobacterium*.

*Bifidobacterium* BB-12<sup>®</sup> (BB-12<sup>®</sup>) is a catalase-negative, rod-shaped bacterium. It was included in the cell culture bank of Chr. Hansen in 1983. At the time of isolation, BB-12<sup>®</sup> was considered to belong to the species *Bifidobacterium bifidum*. Modern molecular classification techniques reclassified BB-12<sup>®</sup> as *Bifidobacterium animalis* and later to a new species *Bifidobacterium lactis*.

The species *B. lactis* later shown not to fulfill the criteria for a species and was instead included in *Bifidobacterium animalis* as a subspecies. Today, BB-12<sup>®</sup> is classified as *Bifidobacterium animalis* subsp. *lactis*. Despite a change in the name over the years, the strain BB-12<sup>®</sup> has not changed (**Garrigues et al., 2005**).

It is strain that was specially selected by Chr. Hansen for the production of probiotic dairy products. BB-12<sup>®</sup> has been used in infant formula, dietary supplements and fermented milk products worldwide. This strain is technologically well suited, expressing fermentation activity, high aerotolerance, good stability and a high acid and bile tolerance, also as freeze-dried products in dietary supplements. Furthermore, BB-12<sup>®</sup> does not have adverse effects on taste, appearance or on the mouth feel of the food and is able to survive in the probiotic food until consumption (**Garrigues et al., 2010**).

## MATERIAL AND METHODOLOGY

### Sample preparation and yoghurt technology

Control yoghurt (yoghurt culture – *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) and experimental probiotic yoghurt (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Bifidobacterium animalis* ssp. *lactis* BB-12) both of Chr. Hansen, were made from raw cow's milk after pasteurization at 85 °C for 15 seconds and cooling at 45 °C. To improve total solids content in yoghurt at 21%, the skim milk powder was added to the milk and stirred at high speed. After very well mixing, the mixture was heated at the high-pasteurized temperature as described above and kept at this temperature for 20 minutes. Then, the mixture was cooled to 43 ± 2 °C and yoghurt starter culture [2 g.100<sup>-1</sup> (w/w)] and *Bifidobacterium animalis* ssp. *lactis* BB-12 (experimental yoghurt) in the concentration of 107 CFU.g<sup>-1</sup> [1 g.100<sup>-1</sup> (w/w)] were added into the milk. Thereafter, after very well mixing, the mixtures were added into 150 mL cups (both, glass and plastic), sealed, labeled and incubated at the temperature of 43 ± 2 °C for 3.0 – 3.5 hours until titratable acidity of final product reached maximum 60 °SH. Then, the products were cooled in ice water bath and maintained under refrigeration temperature (4 °C) during 1, 7, 14, and 21 days.

The cultures used in this study were in freeze-dried (DVS) form and use according to the manufacturer recommendation.

Analyses of milk and yoghurt samples were done according to the **Commission regulation (EC) No 213/2001**.

### Chemical analysis of raw milk

- For the experiment raw cow's milk free of antibiotic residues was used. Antibiotic residues in milk were determined by Beta star 25 tests, a commercial screening test (Neogen Food Safety, USA) before the yoghurt manufacturing. The antibiotic residue test was performed as described by manufacturer's instruction.
- The basic components of milk samples (milk proteins, fat, lactose, solids-non-fat (SNF), milk density and added water were determined using a LactiCheck ultrasonic milk analyzer (Page & Pedersen International, Ltd., USA). Temperature of the milk samples was 20 ± 1 °C.
- Titratable acidity of milk sample was determined by titration of milk with 0.25 mol.L<sup>-1</sup> NaOH and phenolphthalein as indicator and expressed in degrees of Soxhlet-Henkel (°SH).
- The somatic cell count was determined by the Fossomatic 90 (Denmark). Total bacteria count in milk was detected by the standard plate method using Plate count agar (Oxoid) at 30 °C for 72 hours.

### Chemical analysis of yoghurt

The pH of yoghurts was determined with a digital pH meter (pH 340i/SET). The pH meter was calibrated using reference pH 4.0 and 7.0 buffered solutions as described by manufacturer's instruction. Titratable acidity of yoghurt samples was determined after mixing the yoghurt sample with 10 mL of hot distilled water (~90 °C) according to Soxhlet-Henkel and expressed in Soxhlet-Henkel degrees (°SH). All the analysis was performed in triplicate.

### Microbiological analysis of yoghurt

For enumeration of *Lactobacillus delbrueckii* ssp. *bulgaricus* Lactobacillus MRS agar (Hi Media, India) agar was used. After suspension appropriately and dilution in sterile saline, the ten-fold dilutions were spread into selective medium as described above and incubated at 37 °C for 24 hour anaerobically. Enumeration of *Bifidobacterium animalis* ssp. *lactis* BB-12 was carried out through pour plate technique by using Bifidobacterium agar with L-cysteine hydrochloride (Hi Media, India) and incubated in modified atmosphere at 37 °C for 48 hours (**Fávaro-Trindade and Grosso, 2004**). In cases where no growth was detected, plates were re-incubated at 37 °C for an additional 24 hours. Numbers of bacteria stated for each sample are the means of replicated counts.

Depending on the number and morphological types of colony on a plate, three to five colonies of each type were randomly selected. After purification, isolates were examined for their morphology, Gram staining, and observed under a light microscope (Olympus BX 50, Japan) with a magnification of 1 000 x. For confirmation of bacteria present in yoghurts, one loop of the selected purified bacteria was mixed in a sterile vial containing

porous beads kept in glycerol as cryopreservative and serves as carriers to support microorganisms (Microbank) and stored at -20 °C for MALDI-TOF MS analysis.

MALDI-TOF MS analysis was performed on a Microflex MALDI Biotyper (Bruker Daltonik) according to a standard sample preparation protocol of Bruker Daltonik (Freiwald and Sauer, 2009). MALDI-TOF mass spectra were subjected to numerical analysis (BioTyper 3.1 software, Bruker Daltonik).

### Statistical analysis

For statistical comparison of the results, statistical methods of processing and evaluation of the results were used to compare data processed into the tables and graph (MS Excel 2013). ANOVA parameter test and induction statistics methods un-pair t-test for testing means of related parameters. Correlation phi coefficient was used to assess the dependence of the relationship between the two nominal variables (IBM SPSS statistics 23).

### Scientific hypothesis

The goal of the study, was to analyses a surviving *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Bifidobacterium animalis* ssp. *lactis* BB-12 added for the manufacturing of probiotic yoghurt during the shelf-life up to 21 days which is mostly accepted by the consumers and which were packed into the screw glass bottles and plastic cups.

## RESULTS AND DISCUSSION

Raw milk used for the manufacturing process was acceptable for yoghurt manufacturing process (data not shown).

The pH and titratable acidity changes during yoghurt storage are shown in Figure 1. An overall decline in the pH of all the stored yoghurts occurred during the study. The initial pH (day 1) ranged between 4.53 and 4.79 in plastic cup and glass bottle, respectively. There was a significant difference ( $p < 0.05$ ) in pH between yoghurts in glass bottle and plastic cup during the experimental period. Titratable acidity increased significantly ( $p < 0.05$ ) on day 21 of storage period at 4 °C. Higher lactic acid content was observed in yoghurt in plastic cup (47 °SH vs. 43 °SH on 1 d and 54 °SH vs. 49 °SH in day 21). There were any differences in acidity of yoghurts, both of control and

experimental groups, respectively. These results are in agreement with Tarakci and Erdogan (2003) who reported increased acidity of yoghurt over the storage period. Guler and Mutlu (2005) also observed an increase in titratable acidity during the storage period.

Changes in the viable counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Bifidobacterium animalis* ssp. *lactis* BB-12 during manufacturing and storage period (21d) of yoghurts are listed in Table 1. All lactic acid bacteria used in this study were confirmed by numerical analysis (MALDI-TOF MS) to be *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Bifidobacterium animalis* ssp. *lactis* BB-12.

It was observed, that the initial counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* were  $280.3 \times 10^7$  cfu.g<sup>-1</sup> at day 1 in yoghurts (control) packed into glass bottles and  $283.3 \times 10^7$  cfu.g<sup>-1</sup> in plastic cups, respectively. The count of lactobacilli in experimental group of yoghurt with probiotic strain of *Bifidobacterium animalis* ssp. *lactis* BB-12 was higher both in glass bottle ( $899 \times 10^7$  cfu.g<sup>-1</sup> and plastic cups  $724.3 \times 10^7$  cfu.g<sup>-1</sup>) at 1 d, respectively. This difference could possibly be due to the differences in different pH (4.79 vs. 4.53), respectively. After 1d storage period, the counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* increased in control group of yoghurt samples and reached maximum at 3d period ( $p < 0.001$ ) both for glass bottle and plastic cup. It could be due to the residual activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* during this experimental period. This is in agreement with the rise in titratable acidity and drop in pH for this culture (Figure 1). For next storage periods, counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* showed a sharp decline, which indicated the advantage for the viability of probiotic bacterium *Bifidobacterium animalis* ssp. *lactis* BB-12, used in this experiment (Table 1).

Our results are in agreement with data stated by Cruz et al. (2010), who submitted determination the shelf-life of probiotic flavored yoghurt supplemented with *Bifidobacterium animalis* DN 173010 W.

As shown in Table 1, counts of bifidobacteria were lower than counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* ( $190$  to  $434.7 \times 10^7$  at 1d) and slowly increased ( $p < 0.001$ ) at maximum level on day 7 ( $294.3 - 754 \times 10^6$ ) and then slowly declined to  $6.33 \times 10^7$  in glass bottle and  $2.33 \times 10^7$  in plastic cups, respectively. The similar results were observed also by Dave and Shah (1997), Lovayová and

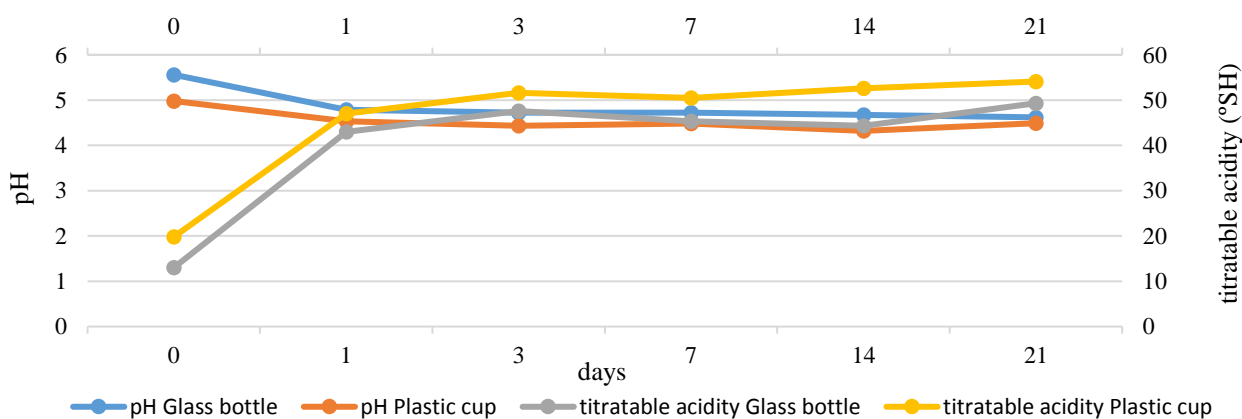


Figure 1 Change in pH and titratable acidity of experimental yoghurt during 21 days.

**Table 1** Survival of *Lactobacillus delbrueckii* spp. *bulgaricus* and *Bifidobacterium animalis* spp. *lactis* BB-12 in yoghurts during storage period at 4 °C.

| Storage period (days) | Control yoghur   |                           | Experimental yoghurt  |                           |  |                           |
|-----------------------|--|---------------------------|---|---------------------------|--|---------------------------|
|                       | <i>Lactobacillus delbrueckii</i> spp. <i>bulgaricus</i> (x 10 <sup>7</sup> cfu.g <sup>-1</sup> ) |                           | <i>Bifidobacterium animalis</i> spp. <i>lactis</i> BB-12 (x 10 <sup>7</sup> cfu.g <sup>-1</sup> ) |                           | <i>Lactobacillus delbrueckii</i> spp. <i>bulgaricus</i> (x 10 <sup>7</sup> cfu.g <sup>-1</sup> ) |                           |
|                       | glass  | plastic cup               | glass   | plastic cup               | glass  | plastic cup               |
| 1                     | 280.3 ±17.62   | 283.3 ±28.01              | 190 ±15.52  | 434.7 ±20.82              | 899 ±25.94 <sup>a</sup>  | 724.3 ±31.09 <sup>a</sup> |
| 3                     | 602 ±168.2 <sup>b</sup>  | 454.7 ±291.4 <sup>b</sup> | 284 ±11 <sup>a</sup>  | 645.3 ±42.39              | 261 ±340.7 <sup>a</sup>  | 160 ±285.1 <sup>a</sup>   |
| 7                     | 113.3 ±10.21 <sup>b</sup>  | 172 ±228.7 <sup>b</sup>   | 294.3 ±9.45 <sup>a</sup>  | 754 ±22.72 <sup>a</sup>   | 408.7 ±19.5 <sup>a</sup>   | 127.3 ±19.6 <sup>a</sup>  |
| 14                    | 44.3 ±4.16 <sup>a</sup>  | 36.33 ±2.52 <sup>a</sup>  | 26 ±30.51 <sup>a</sup>  | 132.7 ±17.47 <sup>a</sup> | 6.33 ±0.58 <sup>a</sup>  | 5.3 ±0.58 <sup>a</sup>    |
| 21                    | 216.7 ±10.2 <sup>a</sup>   | 3.67 ±0.58 <sup>a</sup>   | 6.33 ±0.58 <sup>a</sup>   | 2.33 ±0.58 <sup>b</sup>   | 11.67 ±1.53 <sup>a</sup>   | 7.33 ±0.58 <sup>b</sup>   |

Note: Results are the average of three independent assays. Results are expressed as ± means standard deviations. Unpaired t-tests were done to compare control and experimental group of yoghurts and glass bottle versus plastic cup. The probability (p) of a significant difference between the two values is identified with the following symbols: \* represents  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ . All other comparisons had n.s. in the same row with different superscript lowercase letters are significantly different (a –  $p < 0.001$ , b –  $p < 0.01$ ). Control yoghurt: yoghurt starter culture; experimental yoghurt: yoghurt starter culture and *Bifidobacterium animalis* spp. *lactis* BB-12.

**Burdová (2008).**

In general, from yoghurts manufactured only from yoghurt lactic acid bacteria and in yoghurts in which *Bifidobacterium* tested was included the viable counts of all enumerated bacteria were well above the recommended limit of 107.g<sup>-1</sup> during the storage period of 21 days at 4 °C.

Our results are also comparable with other recent studies (Martin and Chou, 1992; Lankaputhra and Shah, 1996). It seems that multiplication of *Bifidobacterium animalis* ssp. *lactis* BB-12 in experimental groups of yoghurts was due to the presence of *Lactobacillus delbrueckii* ssp. *bulgaricus* in this mixed culture. It is because the free amino acids that are produced by these lactic acid bacteria in yoghurt could have promoted the growth of bifidobacterium, which require free amino acids for its growth and development in yoghurt, respectively (Klaver et al., 1993).

As shown in Table 1, *Lactobacillus delbrueckii* ssp. *bulgaricus* multiplied better in glass bottles than in plastic cups, as observed during experimental period in-group with *Bifidobacterium animalis* ssp. *lactis* BB-12. Also at the end of the storage period at 4 °C, viable counts of lactobacilli were higher ( $p < 0.001$ ) in glass bottles. This is in comparison with the count of *Bifidobacterium animalis* ssp. *lactis* BB-12, those counts were also significantly higher ( $p < 0.001$ ) in yoghurts stored in glass bottle.

These differences could be associated with the limitation of the oxygen permeation in yoghurts filled into screw capped glass bottles, because dissolved oxygen content can have effect on titratable acidity, pH and viable counts of LAB as referred by Dave and Shah (1997). According to these authors, bifidobacteria preferred an environment with dissolved oxygen content and multiplied better in glass bottles than in plastic cups, which is in confirmation with the results of our experimental study.

As reported Burdová and Lovayová (2009) more carefully controlled studies in which energy intake and expenditure are measured needs to be conducted before any conclusions can be drawn regarding the positive effect of cultured dairy foods in humans and on weight gain and feed efficiency in animals. According to Nemcová et al. (2009), bacteria of dairy fermentation mainly of the

*Lactobacillus* genus create, apart from the known substances, many presently unidentified substances that are effective against harmful microorganisms. They have a protective influence in food storage, which can be used clinically. Start cultures are a part of useful microorganisms and their enzymes carry out important biochemical changes during the production process (Kačaniová et al., 2010).

**CONCLUSION**

The presence of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Bifidobacterium animalis* ssp. *lactis* BB-12 was confirmed at each of yoghurt samples packaged in both glass bottles and plastic cups during the completely experimental period of 21 d in total account of more than 107 cfu.g<sup>-1</sup> yoghurts. Although the counts of tested lactobacillus and bifidobacterium were significantly higher in yoghurts packaged in glass bottle, the plastic cups are although suitable for using as packaging material as followed from our experimental results. All manufactured yoghurts had high qualitative properties and contained lactic acid bacteria above recommended limit stated for these bacteria.

**REFERENCES**

Burdová, O., Lovayová, V. 2009. Food safety – Applying the principle “From stable to table” – experience and trends. In *Proceedings Škola – veda – prax I*. Košice : UVL, p. 91-94. ISBN 9788080771430.

Commission Regulation (EC) No 213/2001 of 9 January 2001 laying down detailed rules for the application of Council Regulation (EC) No 1255/1999 as regards methods for the analysis and quality evaluation of milk and milk products and amending Regulations (EC) No 2771/1999 and (EC) No 2799/1999.

Cruz, A. G., Walter, A. H. M., Cadena, R. S., Faria, J. A. F., Bolini, H. M. A., Pinheiro, H. P., Sant’Ana, A. S. 2010. Survival analysis methodology to predict the shelf-life of probiotic flavoured yoghurt. *Food Research International*, vol. 43, no. 5, p. 1444-1448. <https://doi.org/10.1016/j.foodres.2010.04.028>

Dave, R. I., Shah, N. P. 1997. Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter



- cultures. *International Dairy Journal*, vol. 7, no. 1, p. 31-41. [https://doi.org/10.1016/S0958-6946\(96\)00046-5](https://doi.org/10.1016/S0958-6946(96)00046-5)
- Dave, R. I., Shah, N. P. 1998. Ingredient supplementation effects on viability of probiotic bacteria in yogurt. *Journal of Dairy Science*, vol. 81, no. 11, p. 2804-2816. [https://doi.org/10.3168/jds.S0022-0302\(98\)75839-4](https://doi.org/10.3168/jds.S0022-0302(98)75839-4)
- FAO/WHO, 2002. Guidelines for the evaluation of probiotics in food: Report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. (London, Ontario, Canada).
- Fávaro-Trindade, C. S., Grosso, C. R. F. 2004. Stability of free and immobilized *Lactobacillus acidophilus* and *Bifidobacterium lactis* in acidified milk and immobilized *B. lactis* in yoghurt. *Brazilian Journal of Microbiology*, vol. 35, no. 1-2, p. 151-156. <https://doi.org/10.1590/S1517-83822004000100025>
- Freiwald, A., Sauer, S. 2009. Phylogenetic classification and identification of bacteria by mass spectrometry. *Nature Protocols*, vol. 4, no. 5, p. 732-742. <https://doi.org/10.1038/nprot.2009.37>
- Garrigues, C., Stuer-Lauridsen, B., Johansen, E. 2005. Characterisation of *Bifidobacterium animalis* subsp. *lactis* BB-12 and other probiotic bacteria using genomics, transcriptomics and proteomics. *Australian Journal of Dairy Technology*, vol. 60, p. 84-92.
- Garrigues, C., Johansen, E., Pedersen, M. B. 2010. Complete genome sequence of *Bifidobacterium animalis* subsp. *lactis* BB-12, a widely consumed probiotic strain. *Journal of Bacteriology*, vol. 192, no. 9, p. 2467-2468. <https://doi.org/10.1128/JB.00109-10>
- Guler, A.; Mutlu, B. 2005. The effects of different incubation temperatures on the acetaldehyde content and viable bacteria counts of bio-yogurt made from ewe's milk. *International Journal of Dairy Technology*, vol. 58, p. 174-179. <https://doi.org/10.1111/j.1471-0307.2005.00209.x>
- Hamann, W. T., Marth, E. H. 1983. Survival of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in commercial and experimental yogurts. *Journal of Food Protection*, vol. 47, no. 10, p. 781-786. <https://doi.org/10.4315/0362-028X-47.10.781>
- Heller, K. J. 2001. Probiotic bacteria in fermented foods: product characteristics and starter organism. *American Journal of Clinical Nutrition*, vol. 73, no. suppl 2, p. 374-379.
- Champagne, C. P. 2009. Some Technological Challenges in the Addition of Probiotic Bacteria to Foods. In Charalampopoulos, D. and Rastall, R. A. *Prebiotics and Probiotics Science and Technology*. New York, USA : Springer-Verlag, 761 p. ISBN 978-0-387-79057-2. [https://doi.org/10.1007/978-0-387-79058-9\\_19](https://doi.org/10.1007/978-0-387-79058-9_19)
- Kačániová, M., Angelovičová, M., Nováková, I., Pochop, J., Liptajová, D., Kliment, M., Kunová, S. 2010. Bacterial viability in probiotic bars. *Potravinárstvo*, vol. 4, no. 1, p.402-409. [http://www.potravinarstvo.com/dokumenty/mc\\_februar\\_2010/pdf/4/Kacaniova.pdf](http://www.potravinarstvo.com/dokumenty/mc_februar_2010/pdf/4/Kacaniova.pdf)
- Kandler, O., Weiss, N. 1984. Genus *Lactobacillus*. In: Sneath, P. H. A. et al. *Bergey's Manual of Systematics Bacteriology*. Baltimore M. D., USA : Williams and Wilkins, p. 1209-1234. ISBN 978-0-387-21609-6.
- Kailasapathy, K., Harmstorf, I., Phillips, M. 2008. Survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *Lactis* in stirred fruit yoghurts. *LWT*, vol. 41, no. 7, p. 1317-1322. <https://doi.org/10.1016/j.lwt.2007.08.009>
- Klaver, F. A. M., Kingma, F., Weerkamp, A. H. 1993. Growth and survival of bifidobacteria in milk. *Netherlands Milk and Dairy Journal*, vol. 47, p. 151-164.
- Lankaputhra, W. E. V., Shah, N. P. 1996. A simple method for selective enumeration of *Lactobacillus acidophilus* in yogurt supplemented with *L. acidophilus* and *Bifidobacterium* spp. *Milchwissenschaft*, vol. 51, no. 8, p. 446-450.
- Lovayová, V. 2007. Probiotic influenced a gut microflora. In *Proceedings Hygiene alimentorum XXVIII*. Bratislava : Štátna veterinárna a potravinová správa SR, p. 297-298, ISBN 9788080770556.
- Lovayová, V., Majorová, E., Rimárová, K. 2008. Probiotic nutritional supplements and children health. In *Proceedings from seminar of PhD. Students dedicated to academic Boďa: III. volume*. Košice, p. 52-55, ISBN 9788096861828.
- Lovayová, V., Burdová, O. 2008. Assessment of probiotic activities in yoghurt during technological process and storage. *Mliekarstvo*, vol. 39, no. 4, p. 46-49.
- Martin, J. H.; Chou, K. M. 1992. Selection of *Bifidobacteria* for use as dietary adjuncts in cultured dairy foods: 1-tolerance to pH of yogurt. *Cultured Dairy Products Journal*, vol. 27, no. 4, p. 21-26.
- Mitsuoka, T. 1990. *Bifidobacteria* and their role in human health. *Journal of Industrial Microbiology & Biotechnology*, vol. 6, no. 4, p. 263-268. <https://doi.org/10.1007/BF01575871>
- Nemcová, R., Guba, P., Hajdučková, V., Stropfová, V., Lovayová, V. 2009. Viability of probiotic cultures in cheddar cheese. *International Journal of Probiotics and Prebiotics*, vol. 4, no. 2, p. 152-153. [https://doi.org/10.3168/jds.S0022-0302\(00\)74953-8](https://doi.org/10.3168/jds.S0022-0302(00)74953-8)
- Shah, N. P. 2000. Probiotic bacteria: selective enumeration and survival in dairy foods. *Journal of Dairy Science*, vol. 83, no. 4, p. 894-907. [https://doi.org/10.1016/S0958-6946\(00\)00072-8](https://doi.org/10.1016/S0958-6946(00)00072-8)
- Shihata, A., Shah, N. P. 2000. Proteolytic profiles of yogurt and probiotic bacteria. *International Dairy Journal*, vol. 10, no. 5-6, p. 401-408.
- Tarakci, Z., Erdogan, K. 2003. Physical, chemical, microbiological and sensory characteristics of some fruit-flavoured yogurt. *Y.Y.U. Veteriner Dergisi*, vol. 14, no. 2, p. 10-14.

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