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## Probiotic bacteria in fermented dairy products

### *Probiotische Kulturen in fermentierten Milchprodukten*

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### Summary

Positive effects or health benefits by the regular consumption of probiotic products are often described. *Lactobacillus* and *Bifidobacteria* spp. are the most frequently used bacteria species in fermented milk products, which are particular directly declared by the manufacturer or summarized as “probiotic” only. The gastric acid-, bile- or enzyme resistance and a consumption of adequate amounts are necessary to develop probiotic effects. In Germany, the BfR (Federal Institute for Risk Assessment) recommends a regularly, daily intake of  $10^8$  until  $10^9$  cfu/g of probiotic cultures and notes that for measurable bacterial productivity more than  $10^6$  cfu/g or ml product are required. However, legal determinations of minimal counts in probiotic products and explicit specifications for the declaration of the probiotic cultures do not exist. The aim of the present study, was to enumerate and identify probiotic bacteria in commercially available 37 probiotic fermented milk products at the end of shelf life. Average concentrations of more than  $1.0 \times 10^6$  cfu/g were detected in 36 samples. In one sample the declared amount of “10 milliards/150 g” ( $\log_{10}/150$  g) was not reached. The identified species were in accordance with the declaration in 36 samples. However, in one product *Lactobacillus rhamnosus* could be confirmed whereas *Lactobacillus acidophilus* was declared.

**Keywords:** probiotic dairy products, *Lactobacillus*, bifidobacteria, probiotics

### Zusammenfassung

Positive Effekte auf die Gesundheit des Menschen durch den regelmäßigen Verzehr von probiotischen Erzeugnissen sind zahlreich belegt. In Milchprodukten handelt es sich vielfach um Bakterien der Gattung *Lactobacillus* und *Bifidobacterium*, die zum Teil konkret ausgezeichnet oder nur unter dem Begriff „probiotisch“ deklariert sind. Um probiotische Wirkungen entfalten zu können, ist eine gewisse Magensaft-, Gallensaft- bzw. Enzymresistenz und die regelmäßige Aufnahme entsprechender Mengen erforderlich. Nach Empfehlungen des BfR (Bundesinstitut für Risikobewertung) sollten sie für messbare Stoffwechselleistungen im Produkt in einer Konzentration von mehr als  $10^6$  KbE/ml bzw. g vorliegen und eine regelmäßige, am besten tägliche Dosis von  $10^8$  bis  $10^9$  probiotischen Kulturen ist erforderlich (Anonymus 1999). Eine rechtlich verbindliche Festlegung der Mindestkeimzahlen in probiotischen Milchprodukten und eindeutige Vorschriften für die Deklaration der eingesetzten Kulturen existieren jedoch nicht. In die vorliegende Studie wurden daher 37 probiotische Milcherzeugnisse einbezogen, um zu prüfen, ob und in welcher Höhe die vom Hersteller deklarierten Kulturen am Ende des Mindesthaltbarkeitsdatums nachzuweisen sind. In 36 Proben wurden Konzentrationen von über  $1,0 \times 10^6$  KbE/g bestimmt. In einem Milcherzeugnis wurde die ausgewiesene Anzahl von 10 Milliarden/150 g nicht erreicht. Die nachgewiesenen Mikroorganismen waren in 36 Produkten identisch mit der Deklaration. In einem Produkt konnte *Lactobacillus rhamnosus* anstatt des vom Hersteller ausgelobten *Lactobacillus acidophilus*-Stammes detektiert werden.

**Schlüsselwörter:** probiotische Milchprodukte, *Lactobacillus*, Bifidobacterien, Probiotika

## Introduction

Probiotic fermented milk products are in the focus of public interests in the last years (Hayn et al., 2005). This fact relies on the scientifically proven positive effects by the therapy of chronic inflammatory intestinal (Lorea Baroja et al., 2007), diarrheal diseases (Van Niel et al., 2002; D'Souza et al., 2002), stimulation of the immune system (Klein and Jahreis, 2004), prevention of atopic dermatitis (Kalliomäki et al., 2001) or the cholesterol reduction by hypercholesterolemia (Ataie-Jafari et al., 2009). Further, the intensity of diarrhea or the duration of disease in children with rotavirus infection could be minimized by a minimal daily probiotic intake of  $1.0 \times 10^{10-11}$  cfu/g (Van Niel et al., 2002). A consumer perception of Annunziata and Vecchio (2013) confirmed the importance of health claims in gaining consumer acceptance of functional foods. For the consumer it is more about the benefit (health) than about the precise way in which it is delivered (Van Trijp and Van der Lans, 2007).

In contrast, studies exist which show no additive positive effects towards conventional products (Fabian and Elmadfa, 2007; Meyer et al., 2006). Furthermore, the identification of bacterial species, apart from the quantitative examination, is essential because all effects are strain specific and the possibility to combine different bacterial properties in "multi-species-probiotics" can enhance these effects (Vasiljevic and Shah, 2008). Ideally, they operate synergistic and reach allergy prevention (Timmerman et al., 2008). The culture type and concentration used in the products are necessary to ensure probiotic effects, also at the end of shelf life (Ravula and Shah, 1998). However, no minimal counts are regulated by the German legislation so far. The working group on probiotic micro-organisms in food at the former Federal Institute for Consumer Health Protection and Veterinary Medicine (now BfR) recommended a minimal effective dosage of  $10^8$  to  $10^9$  cfu per serving and noted that counts above  $>1.0 \times 10^6$  cfu/ml or g in probiotic products are required to measure productivity of bacterial metabolisms (Anonymus, 1999).

The aim of the present study was to enumerate and identify probiotic bacteria in commercially available 37 probiotic fermented milk products at the end of shelf life and to compare the results with the manufactures data on the package.

## Material and methods

37 commercially available products, which are further described in Table 2, were investigated. The cultures, including members of the genera *Lactobacillus* [(*L.*), *L. acidophilus*, *L. johnsonii*, *L. casei*] and *Bifidobacterium* [(*B.*) (*B. animalis*)], were declared in detail in 36 products (10 x as single, 13 x as stock cultures) and in all other cases, awarded as "probiotics" only. The quantitative determination of the probiotic cultures was initiated at the end of the given shelf life. To specify the type or genus level (eg. bifidobacteria) cultural standard ISO test methods were used, i. e. ISO/DIS 20128 for *L. acidophilus*, ISO/DIS 29981/IDF 220 for bifidobacteria and ISO 15214 for mesophilic lactic acid bacterial/*L. casei*. For the determination of *L. johnsonii* a

modified version, according to standard protocols of the MLUA Oranienburg were applied using HHD medium (medium for detection of hetero- and homofermentative differentiation of lactic acid bacteria, Biolife 401529). Probiotic properties are strain specific and can be confirmed by cost-intensive DNA studies. Therefore, corresponding isolates were confirmed by means of biochemical test system (API® 50 CH Bio Merieux), and/or 16S rDNA sequencing (table 1).

**TABLE 1:** Primers applied for 16SrDNA-Sequencing and RAPD-PCR.

primer	sequence	reference
amplification of a 899 bp fragment		
Primer 27f	5'AGA GTT TGA TCM TGG CTC AG 3'	
Primer 926R	5' CCG TCA ATT CCT TTR AGT TT 3'	Stackebrandt and Goodfellow (1991)
amplification of a 1041 bp fragment		
Primer 16S-FW	5' GAA GAG TTT GAT CAT GGC TCA G 3'	Weisburg et al. (1991)
Primer 16S-Rev	5' ACG ACA GCC ATG CAG CAC CT 3'	
RAPD-PCR		
Random sequence primer	5' AGT CAG CCA C 3'	Tynkkynen et al. (1999)

The 16S rDNA sequencing by Taq DNA polymerase and PCR buffer ([pH 8.3]) were obtained from Qiagen (Hilden, Germany). The amount of Taq DNA polymerase used was 2.0 U in a total reaction volume of 50 µl. The concentration of each primer was 0.5 mM, and that of each deoxynucleotide was 200 mM. The amount of template used was 1 µl of an appropriate dilution of the extracted DNA. A Mastercycler personal (Eppendorf) was used for the PCR cycling. Initial denaturation was carried out at 94 °C for 5 min, followed by a thermocycling program with 34 amplification cycles (annealing for 60 s at 53 °C; extension for 90 s at 72 °C; and denaturation for 60 s at 94 °C) and final extension for 10 min at 72 °C. Reaction mixtures were subsequently cooled to 4 °C. The PCR products were controlled by agarose gel electrophoresis with 1.5 % agarose in 0.53 Tris-borate-EDTA (TBE) buffer [pH 8.0]) and analyzed by sequencing (Sequence Laboratories Göttingen GmbH).

Specific differentiation of *L. acidophilus* and *L. johnsonii* was achieved by RAPD-PCR (Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction) regarded to Tynkkynen et al. (1999) (table 1). The RAPD genotyping by template DNA for RAPD analysis was extracted from cells from subculture of lactobacilli isolates on MRS agar plate. A single colony was harvested and transferred to Eppendorf tubes containing 100 ml of Tris-EDTA (TE) buffer (pH 8.0). RAPD analysis was performed in a 50-ml reaction volume consisting of 200 mM deoxynucleoside triphosphate (Qiagen) a 0.4 mM concentration of random sequence primer (table 1), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 4 mM MgCl<sub>2</sub>, 2.5 U of Taq DNA polymerase (Qiagen), and 5 µl of template. The temperature profile in the Mastercycler was 35 cycles as follows: 94 °C for 1 min, 32 °C for 2 min, and 72 °C for 2 min. The initial denaturation was performed at 94 °C for 5 min, and the final extension was done at 72 °C for 5 min. Amplification products were analyzed electrophoretically in 1 % agarose gels containing ethidium bromide.

## Results

In table 2 all strains, their declaration and the counted amounts of probiotic cultures at the end of the shelf life of the investigated products are summarized.

TABLE 2: Declaration and concentration of the declared species.

no	declaration	advertised cultures/ declared numbers of bacteria	culture	bacterial count cfu/ml/g
1	yogurtdrink	probiotic	LAB L. a. B. a. s. l.	1.0x10 <sup>7</sup> 1.5x10 <sup>7</sup> 6.3x10 <sup>6</sup>
2	yogurtdrink	probiotic	LAB L. a. B. a. s. l.	1.1x10 <sup>7</sup> 3.5x10 <sup>7</sup> 6.3x10 <sup>6</sup>
3	acidophilic milk	L. a.	L. a.	1.5x10 <sup>6</sup>
4	acidophilic milk	L. a.	L. a.	1.0x10 <sup>6</sup>
20	yogurtdrink	LA-5 BB-12 L. casei/more than "500 millions probiotic cultures/bottle (500g)" (log 8.7 probiotic cultures/500g)	L. a. B. a. s. l.	2.8x10 <sup>6</sup> 2.9x10 <sup>6</sup>
21	yogurtdrink	LA-5 BB-12/more than "500 millions probiotic cultures/bottle (500g)" (log 8.7 probiotic cultures/500g)	L. a. B. a. s. l.	1.5x10 <sup>6</sup> 3.4x10 <sup>6</sup>
22	yogurtdrink	LA-5 BB-12/more than "500 millions probiotic cultures/bottle (500g)" (log 8.7 probiotic cultures/500g)	L. a. B. a. s. l.	3.9x10 <sup>6</sup> 2.3x10 <sup>6</sup>
23	yogurtdrink	LA-5 BB-12/more than "500 millions probiotic cultures/bottle (500g)" (log 8.7 probiotic cultures/500g)	L. a. B. a. s. l.	1.8x10 <sup>6</sup> 3.0x10 <sup>6</sup>
37	fermented drink	L. casei Shirota/With "milliards" ( $\geq \log 9$ ) active L. c. shirota	LAB L. casei	1.1x10 <sup>8</sup>
6	probiotic drink	L. casei LA-5 BB-12	LAB L. a. B. a. s. l.	2.0x10 <sup>7</sup> 1.4x10 <sup>7</sup> 9.4x10 <sup>6</sup>
7	probiotic drink	L. casei LA-5 BB-12	LAB L. a. B. a. s. l.	7.7x10 <sup>6</sup> 1.2x10 <sup>7</sup> 8.2x10 <sup>6</sup>
14	probiotic drink	L. casei LA-5 BB-12	LAB L. casei L. a. B. a. s. l.	4.2x10 <sup>6</sup> 1.0x10 <sup>6</sup> 6.4x10 <sup>6</sup>
19	probiotic drink	L. casei	LAB L. casei	1.1x10 <sup>7</sup>
24	probiotic drink	L. casei	LAB L. casei	2.5x10 <sup>7</sup>
25	probiotic drink	L. casei	LAB L. casei	3.4x10 <sup>7</sup>
26	probiotic drink	L. casei	LAB L. casei	2.8x10 <sup>7</sup>
27	probiotic drink	L. casei	LAB L. casei	2.2x10 <sup>7</sup>
28	probiotic drink	L. casei	LAB L. casei	1.6x10 <sup>6</sup>
32	probiotic drink	L. casei	LAB L. casei	2.3x10 <sup>7</sup>
38	probiotic yogurt	Lactobacillus LC1/contains at least 1 "milliards LC1/100g" (log 9 LC1/100g)	L. johnsonii	7.7x10 <sup>7</sup>
8	probiotic yogurt	LA-5 BB-12	L. a. B. a. s. l.	1.4x10 <sup>7</sup> 4.4x10 <sup>6</sup>
9	probiotic yogurt	LA-5 BB-12	L. a. B. a. s. l.	4.3x10 <sup>6</sup> 4.3x10 <sup>6</sup>
10	probiotic yogurt	LA-5 BB-12	L. a. B. a. s. l.	4.0x10 <sup>6</sup> 4.4x10 <sup>6</sup>
11	probiotic fruityogurt	LA-5 BB-12	L. a. B. a. s. l.	2.1x10 <sup>6</sup> 4.4x10 <sup>6</sup>
12	probiotic yogurt	L. casei/"10 milliards/cup (150 g)" (log 10/cup (150g))	L. casei	3.0x10 <sup>7</sup>
13	probiotic yogurt	probiotic	LAB L. casei	4.1x10 <sup>7</sup>
15	probiotic yogurt	Efficilis-cultures (L. casei)	LAB L. casei	5.3x10 <sup>7</sup>
16	probiotic fruityogurt	Efficilis-cultures (L. casei)	LAB L. casei	3.3x10 <sup>7</sup>
17	probiotic fruityogurt	Efficilis-cultures (L. casei)	LAB L. casei	2.2x10 <sup>7</sup>
18	probiotic yogurt	Efficilis-cultures (L. casei)	LAB L. casei	8.6x10 <sup>6</sup>
29	probiotic fruityogurt	L. casei	LAB L. casei	6.3x10 <sup>6</sup>
30	probiotic yogurt	L. casei	LAB L. casei	1.0x10 <sup>6</sup>
31	probiotic yogurt	probiotic	LAB L. casei	3.4x10 <sup>7</sup>
33	probiotic yogurt	LA-5 BB-12 L. casei L. actisens/"125 millions probiotic cultures/cup (125 g)" (log 8,1/cup (125g))	L. a. B. a. s. l.	1.5x10 <sup>7</sup> 1.3x10 <sup>7</sup>
34	probiotic yogurt	BB-12	B. a. s. l.	2.3x10 <sup>7</sup>
36	probiotic yogurt	Bifidus-Kultur ActiRegularis/4 milliards per 100 g	B. a. s. l.	1.5x10 <sup>7</sup>
5	probiotic yogurt	L. a. BB	L. rham. BB	1.7x10 <sup>7</sup> 5.3x10 <sup>7</sup>
35	milk mixed product	L. casei defensiv more than "10 milliards/bottle (100 g)" (log 10/bottle (100g))	L. casei	2.4x10 <sup>8</sup>

L. a. = L. acidophilus; L. rham. = L. rhamnosus; BB-12 = Bifidobacterium 12; B. a. s. l. = Bifidobacterium animalis subspecies lactis; LAB = lactic acid bacteria / total plate count

In 36 samples the declared cultures could be confirmed. Sample no. 5 contained *L. rhamnosus* instead of the advertised *L. acidophilus*. In all probiotic products high numbers of cultures with more than  $1.0 \times 10^6$  cfu/g were detectable. The declaration of one probiotic yoghurt announced more than “10 milliards/150 g” ( $\log_{10}/150$  g), but an amount of  $4.5 \times 10^9$  cfu/150 g was only detectable.

## Discussion

High amounts of probiotics in fermented food shortly after manufacturing are often documented (Kruis and Iburg, 2006). Oliveira et al. (2001) detected in all fermented milk products with different supplementations (whey, casein and milk proteins) more than  $2.2 \times 10^7$  cfu/ml (*L. acidophilus* and *L. rhamnosus*) after 7 days storage at 4 °C. Goat milk, fermented with a combination of *L. acidophilus* and *B. animalis* showed 20 h after adding bacteria  $7.1 \times 10^8$  cfu/g and  $6.3 \times 10^7$  cfu/g, respectively. After the decrease on the first storage day, the counts remained essentially constant (*L. acidophilus*  $7.9 \times 10^7$  cfu/g; *B. animalis*  $3.1 \times 10^7$  cfu/g) during storage of 10 days by refrigerating temperature of 5–7 °C (Kongo et al. 2006).

Karna et al. (2007) tested five (three samples of each product) commercially available probiotic dairy products immediately after buying and they contained, except of three, at least  $1.0 \times 10^6$  cfu/g on average, whereas variations between  $1.6 \times 10^2$  cfu/g and  $2.3 \times 10^8$  cfu/g occurred.

However, our results show that also at the end of the shelf life adequate concentrations of efficacious probiotic strains are available. This is confirmed by a number of studies. Similar data demonstrated Shin et al. (2000) where concentrations of more than  $1.0 \times 10^6$  bifidobacteria spp./ml/g were measured in milk and yogurt from retail outlets at all four points of time (three, two and one week prior and past their expiration date). Kailasapathy et al. (2008) confirmed those results by counting *L. acidophilus* and *B. animalis* ssp. *lactis* ( $1.0 \times 10^6$  cfu/g until  $1.0 \times 10^7$  cfu/g) in fruit yoghurts at the end of a 35-day shelf life.

Many studies proofed a slight decrease of certain strains during storage of the probiotic products.

The Saxonian Investigation Center (LUA, 2006) tested probiotic dairy products and found in only three out of 23 samples amounts less than  $1.0 \times 10^5$  cfu/g (two samples less than  $1.0 \times 10^5$  cfu/g for *Lactobacillus* spp.; one sample less than  $<1.0 \times 10^5$  Bifidobacteria spp.).

*In-vitro* tests of acid tolerance (after incubation for 105 min at 37 °C in 0.2 mol HCl-KCl/L buffer, pH 2.0, plus 0.1 % peptone) confirm that the survival of probiotic bifidobacteria (n=3) with concentrations above  $1.0 \times 10^5$  cfu/g correlates with the amounts *in vivo* (yogurt) (Tuomola et al., 2001). However, significant differences were detected between *Bifidobacteria* spp. in produced probiotic yogurts during a 28 day-storage; whereas *B. animalis* showed a constant level around  $1.07 \times 10^7$  cfu/g, a reduction of 99.0 % for *B. longum* was measured with a final amount of  $2.0 \times 10^5$  cfu/g (Akalin et al., 2004).

Nighswonger et al. (1996) added probiotic bacteria ( $1.0 \times 10^7$  cfu/g) to yoghurt and cultured buttermilk shortly after fermentation. *L. casei* was stable whereas *L. acidophilus* showed differences among the strains; some were reduced to  $2.0 \times 10^5$  cfu/g at the end of 28 days of refrigerated storage at 7 °C. Similar findings were made by Hekmat et al. (2009) concerning the survival of probiotic yogurt

cultures; whereas *L. reuteri* decreased significantly to less than  $1.0 \times 10^1$  cfu/g, *L. rhamnosus* remained stable at  $4.0 \times 10^7$  cfu/g at day 28 in products containing no prebiotic agents. One investigated product did not contain the advertised amount of probiotics similar to our findings for one yoghurt (no. 12). This was also obtained by Coeuret et al. (2004), who tested several commercially available products i. e. fermented milk. Four samples contained the declared amount of  $1.0 \times 10^6$  up to  $1.0 \times 10^9$  cfu/g, in five samples the numbers were below the declared content as deep as  $1.5 \times 10^4$  cfu/g and no viable *Lactobacillus* spp. were found in one product.

In bio-yoghurts in 9 out of 10 samples more than  $1.0 \times 10^6$  cfu/g were shortly after production detectable, at the end of the best before date in five yogurts only (Jayamanne and Adams, 2006). Gueimonde et al. (2004) confirmed those results testing ten commercial fermented milks on the account of probiotic bacteria during the storage of 30 days. *Lactobacillus* spp. were in all samples in concentrations of at least  $1.0 \times 10^5$  cfu/g, *Bifidobacteria* spp. were generally in lower numbers and decreased below this level in two products. Wang et al. (2012) developed a probiotic goats' milk yoghurt containing *L. acidophilus*, *L. casei* and *Bifidobacterium* spp. *L. casei* remained stable, bifidobacteria showed a gradual decline during the storage of 12 weeks but was still above the level of  $1.0 \times 10^6$  cfu/g but there were no viable counts of *L. acidophilus* by the fourth week.

Dave and Shah et al. (1997a) investigated the survival of probiotic cultures in yogurt starting at fermentation up to a shelf life of 35 days. In 5-days intervals the amount of the single strains was determined. Amounts less than  $1.0 \times 10^5$  cfu/g were found in one of the four commercially available starter cultures. The rapid decreasing of *L. acidophilus* seems to be caused by hydrogenperoxide produced by *L. delbrueckii* ssp. *bulgaricus* which confirms earlier data from Gilliland and Speck (1977). They described the negative effect of hydrogenperoxide produced by non-probiotic bacteria on *L. acidophilus*. Ahlfeld (2008) also showed that strains of *L. acidophilus* decreased constantly in milk mix products, whereas *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, *Bifidobacterium* sp. and *S. thermophilus* remained stable ( $\log_{10} \text{BCI} \leq 0,7$  lg cfu/ml).

Some technological procedures such as microencapsulation of lactic acid bacteria (Kailasapathy, 2006; Jähne et al., 2013; Shoji et al., 2013), level of starter culture (Dave and Shah, 1997b) using ruptured starter bacteria (Shah and Lankaputhra, 1997), using two step fermentation (Lankaputhra and Shah, 1997) or several supplementations such as cysteine (Dave and Shah, 1997c), ascorbic acid (Dave and Shah, 1997d), fructooligosaccharids (Akalin et al., 2004) or inulin (Donkor et al., 2007) should guarantee a better survival of probiotic bacteria during yogurt manufacturing. Even fibers from fruit by-products enhance probiotic viability and reached in all treatments between  $1.0 \times 10^6$  cfu/g and  $1.0 \times 10^9$  cfu/g until 28 d (Esprito Santo et al., 2012).

In contrast, bifidobacteria were not affected by hydrogenperoxide and increasing acetic acid amounts seem to stimulate their growth or the ability to survive (Dave and Shah 1997a). However, the sensitivity of bifidobacteria towards acidity was also described by El-Dieb et al. (2012) and Donkor et al. (2006), and the acetic acid generation during the fermentation resulted in lower counts of bifidobacteria.

Concerning the labeling we could show that nearly all probiotics were correctly declared. Only one product did not contain the advertised *L. acidophilus*, but *L. rhamno-*

*sus*. In the study of Karna et al. (2007) all probiotic bacteria, which are claimed to be present in the five tested dairy products, could also be detected, but in two products also *Actinomyces israelii* was identified additionally, which might have been introduced from the processing plant environment.

In contrast, five of 11 organic yoghurts tested by Hamilton-Miller et al. (1999) described the types of bacteria they contained correctly. Ibrahim and Carr (2006) found in none of five investigated probiotic yogurts the declared *Bifidobacteria* spp. after four weeks of refrigerated storage at 4 °C.

However, in our investigation, it seemed difficult to identify the probiotic strains correctly. Besides some ecological features, they show nearly identical phenotypical properties. Strain characterization using traditional phenotypic identification methods often provides variable results and may lead to false identifications. In addition, due to the further development and integration of molecular biological methods in taxonomy, the systematic classification of lactic acid bacteria is subjected to constant changes (Klein et al., 1998). Commonly used probiotic strains such as the “Shirota“-strain (Yakult), “La 1“-strain and the “GG“-strain (Gorbach & Goldin) were renamed according to the application of molecular biological methods. All these strains were described as *L. acidophilus*. Actually the Shirota-strain is classified as *L. casei* and the “GG“- strain tested as *L. rhamnosus*. The “La 1“-strain associates with *L. johnsonii* (Reuter et al., 2002). The systematic rearrangements in practice usually require long time to prevail. A synonymous use in the taxonomic designation/declaration is often the result, which could be an explanation for the deviation in our findings.

The results show, that in all 37 commercially available probiotic fermented milk products high numbers of cultures with more than  $1.0 \times 10^9$  cfu/g were detected at the end of the best-before date. Furthermore, in 36 samples the declared cultures could be confirmed and only one product did not contain the advertised *L. acidophilus*.

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