Growth of bifidobacteria in mammalian milk

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ABSTRACT: Microbial colonization of the mammalian intestine begins at birth, when from a sterile state a newborn infant is exposed to an external environment rich in various bacterial species. An important group of intestinal bacteria comprises bifidobacteria. Bifidobacteria represent major intestinal microbiota during the breast-feeding period. Animal milk contains all crucial nutrients for babies' intestinal microflora. The aim of our work was to test the influence of different mammalian milk on the growth of bifidobacteria. The growth of seven strains of bifidobacteria in human milk, the colostrum of swine, cow's milk, sheep's milk, and rabbit's milk was tested. Good growth accompanied by the production of lactic acid was observed not only in human milk, but also in the other kinds of milk in all three strains of *Bifidobacterium bifidum* of different origin. Human milk selectively supported the production of lactic acid of human bifidobacterial isolates, especially the *Bifidobacterium bifidum* species. The promotion of bifidobacteria by milk is species-specific. Human milk contains a key factor for the growth of specific species or strains of human-origin bifidobacteria compared to other kinds of milk. In contrast, some components (maybe lysozyme) of human milk inhibited the growth of *Bifidobacterium animalis*. Animal-origin strains of bifidobacteria were not able to significantly grow even in milk of animal origin, with the exception of *B. animalis* subsp. *lactis* 1,2, which slightly grew in sheep's milk.

Keywords: human milk; colostrum of swine; cow's milk; sheep's milk; rabbit's milk; lysozyme

In normal conditions the mammalian gastrointestinal tract at birth is sterile. Since the birth, microorganisms coming from the environment start colonizing the intestinal tract of the young. The development of intestinal microflora depends on numerous factors, such as the type of birth, environmental contamination, sanitary conditions, and the geographical distribution of bacterial species (Mackie et al., 1999; Orrhage and Nord, 1999; Mountzouris and Gibson, 2003). The dominating group of bacteria in the intestinal tract of breast-fed newborns comprises bifidobacteria. Mammalian milk promotes the development of favourable intestinal bacteria that can protect the intestinal tract from the proliferation of pathogenic bacteria. Mammalian milk is a heterogeneous complex of biological substances such as saccharides, mainly oligosaccharides, amino acids, essential nutrients,

vitamins, and minerals, all of which promoting the development of gastrointestinal microflora in the newborn during the first few days of its life. There are multiple variances between human and other kinds of mammalian milk. Human milk is richer than cow's milk especially in the content of prebiotic oligosaccharides, minerals, vitamins, and antimicrobial compounds. Proteins found in human milk (lactoferrin, haptocorrin, immunoglobulins, lactoperoxidase, and lysozyme) protect the infants against infectious diseases (Lönnerdal, 2003). For instance, the levels of lysozyme naturally present in the milk of dairy animals are 1600–3000 times lower than those in human milk (up to 400 mg/l) (Chandan et al., 1968). Lysozyme is an enzyme which catalyzes the cleavage of the glycosidic linkage in peptidoglycan, the main component of the cell walls of bacteria (Phillips, 1966). It also contributes to the stimulation of beneficial gut microflora and maturation of the intestinal tract (Lönnerdal, 2003).

Other milk components that make human milk unique are prebiotic oligosaccharides, the third largest solid group of milk components after lactose and lipids. Each kind of mammalian milk possesses different oligosaccharide contents and structures. Colostrum of humans and mature human milk contains 22~24 g/l and 12~13 g/l of oligosaccharides, respectively. Human milk oligosaccharides have a far more complicated structure (Newburg and Neubauer, 1995; Kunz et al., 2000) than oligosacharides in other mammalian milk; for instance, bovine milk normally contains 1–2 g/l of free saccharides other than lactose but larger amounts occur in the colostrum of cattle (Davis et al., 1983). The role of individual oligosaccharides in mammalian milk has not been fully investigated yet, particularly in the case of non-human oligosaccharides. Besides their bifidogenic effect, they are also perceived as the soluble receptor analogs that inhibit the attachment of pathogenic bacteria, viruses, and bacterial toxins to the mucosa of the colon of newborns (Newburg et al., 2005).

In our previous study, the growth of different species of bifidobacteria in human milk was tested and significant differences were discovered (Rockova et al., 2011). The aim of the present work was to test the influence of different kinds of animal milk on the growth of bifidobacteria. In our previous research, the resistance of bifidobacteria to lysozyme was tested and, therefore, the content of lysozyme in animal and human milk was tested, too.

MATERIAL AND METHODS

Bacterial strains

The list of bifidobacterial strains used is shown in Table 1. Three strains (2 *B. bifidum* and 1 *B. longum*) were isolated from infant faeces. Two strains of *B. animalis* were isolated from fermented milk products. The remaining two strains were isolated from commercial probiotic products. All strains were isolated and identified using biochemical tests and PCR, as described by Vlková et al. (2005). The subcultivation of bifidobacteria was performed in Wilkins-Chalgren broth supplemented with 5 g/l of soya peptone. Strains were stored in Wilkins-Chalgren broth supplemented with soya peptone and glycerol (20% v/v) at –20°C.

Growth of bifidobacteria in milk

The human milk sample was obtained from the Institute for the Care of Mother and Child (Prague, Czech Republic). The donor mother had delivered her child three months previously. The animal kinds of milk (Holstein breed) were sourced from the Experimental Cowshed Station of the Czech University of Life Sciences Prague. Milk samples were pasteurized (62.5°C for 30 min), and then cooled in a water bath in order to kill pathogenic microorganisms but with minimal loss to the product's intrinsic resistance factors and biologically active compounds (Packard, 1982; Ewaschuk et al., 2011).

The bifidobacteria in the milk samples were cultivated and enumerated as described by Rada et al. (2010). Briefly: bifidobacteria grown at 37°C for 24 h in Wilkins–Chalgren broth (Oxoid, Basingstoke, UK) were centrifuged (6000 *g* for 5 min), the supernatant was discarded, bacterial cells were flushed with saline and then finally re-suspended in the saline to be prepared for bacterial suspension (approximately 10^7 CFU/ml). The microtiter plate (Gama Group, Trhové Sviny, Czech Republic) contained 100 µl of milk sample, and each hole was inoculated with bifidobacterial suspension containing approximately 10^7 CFU/ml. After incubation, the pH and lactic acid were determined by Reflektoquant equipment (Merck, Darmstadt, Germany). Following cultivation, bifidobacteria were evaluated using TPY agar modified by adding mupirocin (100 mg/l) and acetic acid (1 ml/l), according to Rada and Petr (2000).

Determination of lysozyme in milk samples

The human milk samples were collected from 35 mothers and obtained from the Institute for the Care of Mother and Child (Prague, Czech Republic). The donors were mothers 10 ± 4 weeks after delivery. The samples of the macaque milk (*Macaca mulatta*) came from the Biotest (Konárovice, Czech Republic). Other animal milks were obtained from Experimental Cowshed Station of the Czech University of Life Sciences Prague. All milk samples were frozen immediately after collection and stored at –30°C until used. Samples were thawed at 40°C in a water bath and homogenized immediately before analysis. The lysozyme content in human milk was determined using the ELISA (Enzyme-Linked Immunosorbent Assay) Kit (Biomedical Technologies Inc., Stoughton, USA). For analysis, each sample was diluted at 1 : 12 000. Briefly: ELISA is an indirect method that uses interaction with a specific antigen antibody complex to form an antigen-antibody. The sandwich ELISA assay for human lysozyme content of monoclonal antibody specific for lysozyme is bound to polystyrene wells, after incubation with samples; the plate is then washed and incubated with a second human lysozyme specific antibody and subsequently detected using Horseradish Peroxidase conjugate of Donkey anti-goat (sheep) IgG. The concentration of human lysozyme is proportional to colour intensity. Colour (absorbance) was measured by the Multifunctional Micro Plate Reader (TECAN, Durham, USA) at 450 nm. Exact levels were obtained from a standard curve using purified human lysozyme.

The lysozyme content in all samples of milk was also determined using the agar plate (lyso-plate) technique (Osserman and Lawlor, 1966).

Statistical analyses

STATGRAPHICS Centurion XV.II software (Version 15.2.05, 2007) was used to perform analyses of variance with Duncan's multiple range tests at a confidence level of 95% for the differences among bifidobacterial counts and pH values for all experimental groups. The significance of the contents of lysozyme in milks was tested by a Student's *t*-test.

RESULTS AND DISCUSSION

The growth of bacteria in milk is shown in Table 1 and the values of pH and lactic acid in Figures 1 and 2.

Growth in human milk

If the strains actually grew, they produced lactic acid and acetic acid in the approximate proportion 3 : 2, because the bifidobacterial pathway yields 2.5 ATP molecules from 1 mol of fermented glucose, as well as 1.5 mol of acetate and 1 mol of

Table 1. Growth of bifidobacteria (log CFU/ml) cultivated in mammalian milk samples at 37°C for 24 h under anaerobic conditions

Strains	Strains origin	Human milk	Colostrum of swine	Cow's milk	Sheep's milk	Rabbit's milk
Initial concentrations (log CFU/ml)		5.94 ± 0.46	6.00 ± 0.68	7.38 ± 0.25	8.16 ± 0.68	7.50 ± 0.20
B. animalis subsp. *lactis* 1	fermented milk product	4.53 ± 0.71[a]	5.48 ± 0.05[a]	7.06 ± 0.40[b]	8.07 ± 0.20[b]	8.67 ± 0.11[d]
B. animalis subsp. *lactis* 2	fermented milk product	4.61 ± 0.12[a]	6.83 ± 0.18[cd]	8.21 ± 0.01[cd]	8.50 ± 0.13[c]	8.55 ± 0.02[cd]
B. bifidum 1	probiotic capsule	8.70 ± 0.09[b]	7.18 ± 0.06[d]	6.78 ± 0.03[b]	7.45 ± 0.08[a]	9.36 ± 0.04[f]
B. bifidum 2	infant faeces	8.62 ± 0.00[c]	6.69 ± 0.44[c]	8.08 ± 0.17[c]	7.27 ± 0.10[a]	9.12 ± 0.20[e]
B. bifidum 3	infant faeces	8.21 ± 0.01[d]	7.17 ± 0.20[d]	8.23 ± 0.01[cd]	8.59 ± 0.03[c]	7.62 ± 0.07[b]
B. longum 1	infant faeces	6.58 ± 0.03[de]	5.80 ± 0.27[ab]	8.41 ± 0.04[d]	7.88 ± 0.33[b]	8.42 ± 0.01[c]
B. longum 2	probiotic capsule	7.70 ± 0.10[e]	6.00 ± 0.19[b]	6.38 ± 0.02[a]	8.91 ± 0.09[d]	6.49 ± 0.03[a]

values in columns with different superscripts differ ($P < 0.05$); differences among bifidobacterial counts were evaluated by the multiple range comparison with multiple range tests

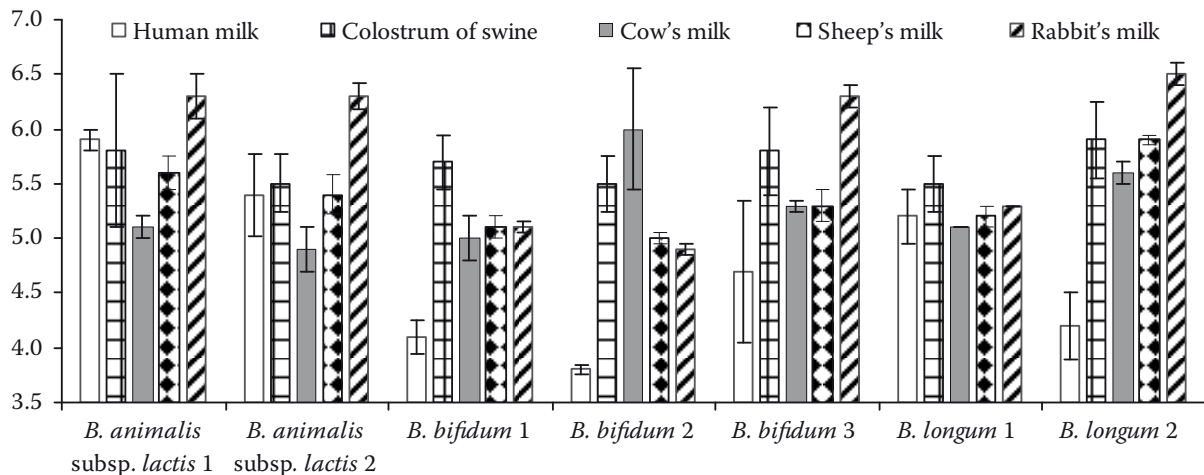data are means ± standard deviation (SD) of three measurements

Figure 1. pH values in mammalian samples of milk cultured under anaerobic conditions at 37°C for 24 h

lactate (Palframan et al., 2003). Luxuriant growth of bifidobacteria in milk samples resulted in producing lactic acid (> 1000 mg/l) and a final pH of around 4. All bifidobacterial strain species of *B. bifidum* and *B. longum* 2 were able to grow in the human milk sample tested, producing 1672.5 mg of lactic acid on average (pH 4.2 ± 0.37) and reaching colony counts from 6 to 8 log CFU/ml. One strain of human origin *B. longum* 1 was not able to grow in human milk, producing a minimal quantity of lactate (260 mg/l, pH 5.2). The colony counts of this strain were the same before and after incubation.

Both bifidobacterial strains originating from animal milk (*B. animalis*) were inhibited by human milk. The colony counts of this strain decreased from 6 to 4.57 ± 0.42 log CFU/ml, and concentrations of lactate were only 195 ± 35.36 mg/l (pH = 5.65 ±0.35). Both strains of *B. animalis* were susceptible to lysozyme.

Growth in the colostrum of swine

Bifidobacterial strains were not able to grow in the colostrum of swine, but also no strains were inhibited by this sample of milk. Colony counts were 6.41 ± 0.74 log CFU/ml and values of lactate 641.7 ± 254.2 mg/l (pH 5.7 ± 0.17). The exception was the strain *B. bifidum* 2 of human origin, reaching 6.69 log CFU/ml and producing 1630 mg/l of lactate (pH 5.5).

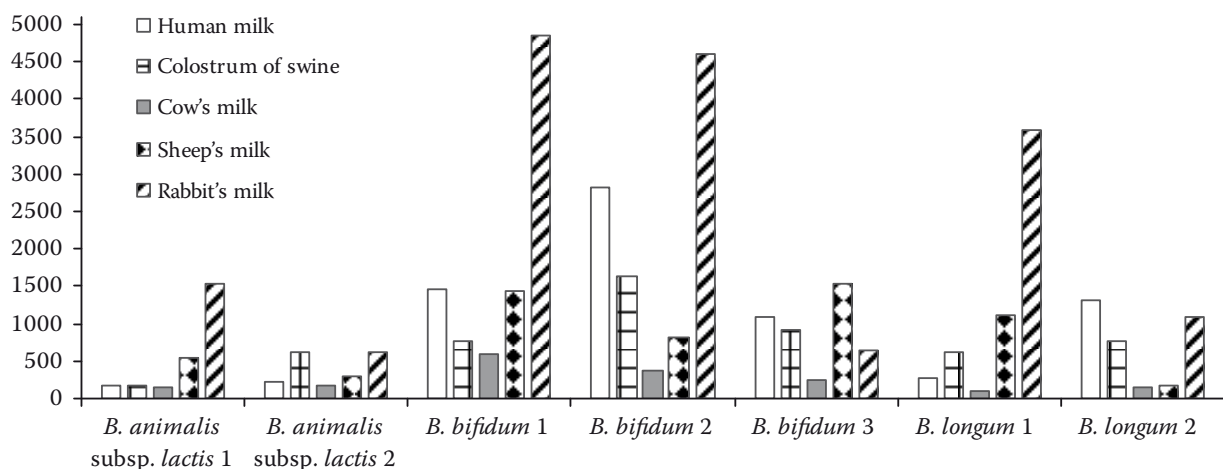
Figure 2. Lactate content (mg/l) in mammalian samples of milk cultured under anaerobic conditions at 37°C for 24 h

Growth in cow's and goat's milk

Limited growth of bifidobacterial strains was observed in cow's and goat's milk. The counts of colonies were almost the same before and after incubation (initial concentration approximately 7.82 log CFU/ml); the final concentration after incubation of bacteria in milk was approximately 7.84 log CFU/ml. In addition, bifidobacteria produced approximately 250 mg/l of lactate (pH > 5.5). These values demonstrate that strains were not able to grow in milk. Only two strains of human origin *B. bifidum* 1, 2 exhibited limited growth in goat's milk, producing 1235 ± 0.0 mg/l of lactic acid with pH 5.0 ± 0.14.

Growth in rabbit's milk

Rabbit's milk stimulates the growth of some bifidobacterial strains (Table 1, Figures 1 and 2). The animal strains *B. animalis* 1, 2 were not inhibited by this sample of milk, but also it did not support their growth. The small volume of this sample did not enable the authors to measure lactate. Only three strains of human origin *B. bifidum* 1, 2 and *B. longum* 2 had a limited growth in rabbit's milk (Table 1, Figures 1 and 2); counts of the colony increased from 7.82 to 8.32 ± 1.59 log CFU/ml with pH 5.5 ± 0.87.

Although there are many differences in the composition of mammalian milks and numerous differences in the complexity and composition of milk saccharides, all kinds of milk share in common the fact that they are produced in mammary glands and their function is to support (promote) the development of any suckling young. Furthermore, the milk of almost all mammals contains lactose, either in a free form or at the reducing ends of oligosaccharides and it also contains a unique mixture of oligosaccharides (Urashima et al., 2001). Some oligosaccharides are species specific; for example, the colostrum of bovines contains ten sialyl oligosaccharides (Kuhn and Gauhe, 1965; Schneir and Rafelson, 1966; Veh et al., 1981), in which two kinds of sialic acid, Neu5Ac and Neu5Gc, are found, in contrast to human milk or human colostrum oligosaccharides, which contain only Neu5Ac (Urashima et al., 2001). Brinkman-Van den Linden et al. (2000) discovered that Neu5Gc is present in all mammals except for humans because of specific hominid mutation in CMP-sialic acid hydroxylase.

Bifidobacteria are fastidious organisms that require specific growth factors such as free amino acids and peptides (Cheng and Nagasawa, 1985). Human milk is considered a suitable medium for the growth of bifidobacteria because of its optimal composition of oligosaccharides and peptides. Only human milk fully supported the growth of some bifidobacterial strains of *B. bifidum* 1, 2, 3 and *B. longum* 2. Both animal strains *B. animalis* 1, 2 and one strain of human origin, *B. longum* 1, were significantly inhibited by human milk. While some kinds of milk seem to exhibit a nutrient deficiency for the growth, others distinctly promote the growth of some bifidobacterial strains. Only specific bifidobacterial strains, such as *B. bifidum* 1, 2 and *B. longum* 2, exhibited limited growth in animal kinds of milk.

Determination of lysozyme in milk samples

For determining lysozyme in human milk, the ELISA method and microbiological lyso-plate technique were used. There were significant differences in determinations of lysozyme as recorded by the ELISA kit and lyso-plate technique (Table 2). The values which the authors measured by the ELISA method were 2 times higher than values determined by the lyso-plate technique. The concentrations of lysozyme ranged 1–131.83 μg/ml and 17.43–184.02 μg/ml, as determined by the lyso-plate technique and ELISA method, respectively. The authors found out that the ELISA method is a specific procedure for determining the lysozyme in samples. This method probably detected all the lysozyme in human milk, including molecules without antibacterial activity. In contrast, the lyso-plate technique is a method able to determine only biologically active lysozyme. To be honest, it should be noted, that the lyso-plate method can also be susceptible to other antimicrobial components in milk. Chicken egg white is currently the major commercial source of lysozyme. Lysozyme from chicken egg whites is only 60% identical to human lysozyme. The fact that antibodies opposing human and chicken egg white lysozyme do not exhibit cross-interference points to significant structural differences.

Bifidobacteria are Gram-positive bacteria, which are more susceptible to lysozyme (antimicrobial enzyme EC 3.2.1.17) than Gram-negative bacteria (Lönnerdal, 2003). Lysozyme is single chain protein of 123 amino acids found in many human

Table 2. Content of lysozyme in mammalian milk samples

Milk	Count of samples	Lysozyme (µg/ml)	
		lyso-plate technique	ELISA
Human milk	35	27.91 ± 32.50*	66.69 ± 40.04*
Macaque rhesus's milk	10	20.94 ± 24.11	nt
Colostrum of swine	2	15.85[†]	nt
Cow's milk	8	< 1	nt
Sheep's milk	3	< 1	nt
Colostrum of goat	1	19.96	nt
Rabbit's milk	5	10.07 ± 6.40	nt

data are means ± standard deviation (SD); nt = not tested

*superscripts differ ($P < 0.002$)

[†]mean from two determinations

cells and tissues, human milk, and egg white (Field 2005; Paramasivam et al., 2006). It hydrolyses β (1,4) linkage between *N*-acetylglucosamine and *N*-acetylmuramic acid in peptidoglycan of the bacterial cell wall. This non-specific immunological component is found in human milk at a concentration 3000 times higher than in milk from other animal species (Clare et al., 2003). Cow's and goat's milks possess very low levels of lysozyme (0.16 and 0.23 mg/l) relative to human milk (400 µg/ml) (Chandan et al., 1968; Clare et al., 2003), as confirmed by our results in Table 2.

It could be concluded that only human milk was able to support the growth of specific species or strains of human origin bifidobacteria. Animal kinds of milk seem to be an insufficient environment for the growth of bifidobacteria, including strains of animal origin, despite the high concentration of lactose and other nutrients in their content. In contrast, non-human mammalian milk appears to be quite suitable for the survival of bifidobacteria. Hence, for example, cow's milk can be used for conserving industrial bifidobacterial strains and milk-based food can contain live probiotic bacteria, including bifidobacteria.

REFERENCES

Brinkman-Van den Linden E.C.M., Sjoberg E.R., Raj Juneja L., Crocker P.R., Varki N., Varki A. (2000): Loss of *N*-glycolylneuraminic acid in human evolution. Implications for sialic acid recognition by siglecs. The Journal of Biological Chemistry, 275, 8633–8640.

Chandan R.C., Parry R.M., Shahani K.M. (1968): Lysozyme, lipase, and ribonuclease in milk of various species. Journal of Dairy Science, 51, 606–607.

Cheng C.C., Nagasawa T. (1985): Purification and some properties of an aminopeptidase from *Bifidobacterium breve*. Japanese Journal of Zootechnical Science, 56, 257–266.

Clare D.A., Catignani G.L., Swaisgood H.E. (2003): Biodefense properties of milk: the role of antimicrobial proteins and peptides. Current Pharmaceutical Design, 9, 1239–1255.

Davis D.T., Holt C., Christie W.W. (1983): The composition of milk. In: Mepham T.B. (ed.): Biochemistry of Lactation. Elsevier, Amsterdam-New York, 71-117.

Ewaschuk J.B., Unger S., O'Connor D.L., Stone D., Harvey S., Clandinin M.T., Field C.J. (2011): Effect of pasteurization on selected immune components of donated human breast milk. Journal of Perinatology, 31, 593–598.

Field C.J. (2005): The immunological components of human milk and their effect on immune development in infants. Journal of Nutrition, 135, 1–4.

Kuhn R., Gauhe A. (1965): Bestimmung der Bindungsstelle von Sialinsureresten in Oligosacchariden mit Hilfe von Perjodat. Chemische Berichte, 98, 395–413.

Kunz C., Rudloff S., Baier W., Klein N., Strobel S. (2000): Oligosaccharides in human milk: structural, functional, and metabolic aspects. Annual Review of Nutrition, 20, 699–722.

Lönnerdal B. (2003): Nutritional and physiological significance of human milk proteins. The American Journal of Clinical Nutrition, 77, 1537–1543.

Mackie R.I., Sghir A., Gaskins H.R. (1999): Developmental microbial ecology of the neonatal gastrointestinal tract. The American Journal of Clinical Nutrition, 69, 1035–1045.

Czech J. Anim. Sci., 58, 2013 (3): 99–105

Original Paper

Mountzouris K.C., Gibson G.R. (2003): Colonization of the gastrointestinal tract. Annales Nestle, 61, 43–54.

Newburg D.S., Neubauer S.M. (1995): Carbohydrates in milks: analysis, quantities and significance. In: Jensen R.G. (ed.): Handbook of Milk Composition. Academic Press, San Diego, USA, 273–249.

Newburg D.S., Ruiz-Palacios G.M., Morrow A.L.: (2005): Human milk glycans protect infants against enteric pathogens. Annual Review of Nutrition, 25, 37–58.

Orrhage K., Nord C.E. (1999): Factors controlling the bacterial colonization of the intestine in breast-fed infants. Acta Pediatrica, 430, 47–57.

Osserman E.F., Lawlor D.P. (1966): Serum and urinary lysozyme (muramidase) in monocytic and monomyelocytic leukemia. The Journal of Experimental Medicine, 124, 921–952.

Packard V.S. (1982): Human milk: extraction, processing, and storage. In: Stewart G.F., Schweigert B.S., Hawthorn J. (eds): Human Milk and Infant Formula. Academic Press, New York, USA, 176–186.

Palframan R.J., Gibson G.R., Rastall R.A. (2003): Carbohydrate preferences of *Bifidobacterium* species isolated from the human gut. Current Issues in Intestinal Microbiology, 4, 71–75.

Paramasivam K., Michie C., Opara E., Jewell A.P. (2006): Human breast milk immunology: a review. International Journal of Fertility, 51, 208–217.

Phillips D.C. (1966): The 3-dimensional structure of an enzyme molecule. Scientific American, 215, 78–90.

Rada V., Petr J. (2000): A new selective medium for the isolation of glucose nonfermenting bifidobacteria from hen caeca. Journal of Microbiological Methods, 43, 127–132.

Rada V., Šplíchal I., Ročková Š., Grmanová M., Vlková E. (2010): Susceptibility of bifidobacteria to lysozyme as a possible selection criterion for probiotic bifidobacterial strains. Biotechnology Letters, 32, 451–455.

Rockova S., Nevoral J., Rada V., Marsik P., Sklenar J., Hinkova A., Vlkova E., Marounek M. (2011): Factors affecting the growth of bifidobacteria in human milk. International Dairy Journal, 21, 504–508.

Schneir M.L., Rafelson M.E. (1966): Isolation and characterization of two structural isomers of *N*-acetylneuraminyllactose from bovine colostrum. Biochimica et Biophysica Acta, 130, 1–11.

Urashima T., Saito T., Nakamura T., Messer M. (2001): Oligosaccharides of milk and colostrum in non-human mammals. Glycoconjugate Journal, 18, 357–371.

Urashima T., Asakuma S., Messer M. (2007): Milk oligosaccharides. In: Kamerling J.P.Y. (ed.): Comprehensive Glycoscience. Elsevier, Amsterdam, the Netherlands, 695–724.

Veh R.W., Michalski J.C., Corfield A.P., Sander-Wewer M., Gies D., Schauer R. (1981): New chromatographic system for the rapid analysis and preparation of colostrum sialyloligosaccharides. Journal of Chromatography, 212, 313–322.

Vlková E., Nevoral J., Jencikova B., Kopecčný J., Godefrooij J., Trojanová I., Rada V. (2005): Detection of infant faecal bifidobacteria by enzymatic methods. Journal of Microbiological Methods, 60, 365–373.

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