Impact of infant formula on growth of bifidobacteria. By S. Musilova[1], V. Rada[1], N. Modrackova[1], and R. Zelenka[2], *[1]Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology ood and Natural Resources, Czech University of Life Sciences, Kamycka 129, Prague 6, 165 21, Czech Republic, [2]Humana GmbH, Herford, Germany*

Introduction

The gut microbiota is established during the first days of life and plays an important role in human health throughout life.(1) These microbiota play a role in the stimulation and development of the immune system, they are involved in metabolism by degrading non-digestible food remnants, producing vitamins (B and K), and participating in short-chain fatty acid metabolism.(2) Bifidobacteria are the predominant group of bacteria in the gut of breastfed and vaginally delivered infants.(3) Most reports found that breast-fed infants have higher number of bifidobacteria, whereas formula-fed infants develop a mixed flora with a lower level of bifidobacteria.(3,4) The colonization of the human gut is a complicated process that is dependent on a number of factors, which include duration of pregnancy, the mother's health, gestational age, antibiotic treatment, hospital hygiene, duration and mode of delivery, and type of feeding.(5,6,7) Therefore, the aim of this study was to determine ability of several bifidobacterial strains to growth in infant formula, human milk and cow milk.

Material and methods

Bifidobacterial strains

The list of used bacterial strains is shown in Table 1. Bifidobacteria were isolated and identified as described in Vlková et al.(8) Bacteria were subcultivated in Wilkins-Chalgren broth supplemented with 5 g/L of soya peptone. Bifidobacteria were stored in Wilkins-Chalgren broth supplemented with soya peptone and glycerol (20% v/v) at 20 °C. All media were purchased from Oxoid (Basingstoke, Hampshire, England).

Growth of bifidobacteria in infant formula, cow milk and human milk

The growth of seven bifidobacterial strains (Table 1) in three different samples of infant formula: AntiColic, Comfort, DG (Humana GmbH, Herford, Germany), one sample of milk powder (BoheMilk, Czech Republic) and one sample of human milk (Institute for the Care of Mother and Child, Prague, Czech Republic) was tested.

Bifidobacteria grown in Wilkins-Chalgren broth supplemented with 5 g/L of soya peptone were centrifuged at 6000 rpm for 5 min. Supernatant was discarded, bacterial cells were flushed with saline and finally resuspended in the saline to prepare bacterial suspension (approx. 10^6 CFU/mL). All samples of milk were prepared according to manufacturer´s instructions and incubated using 96-well microtiter plates. Human milk was treated according

to the Holder method (heat temperature 62.5°C/30 min) which have a minimal impact to lactoferrin, sIgA, and lysozyme.[9]

Each well containing 90 mL of milk (medium) was inoculated with 10 mL of bacterial suspension. Initial concentration of bacteria in the milk was approx. 5 log CFU/mL. The microtiter plates were incubated in anaerobic jar (Anaerobic Plus System, Oxoid, Basingstoke, Hampshire, England) at 37 °C for 24 h. Then, bacterial counts were determined using Wilkins-Chalgren agar modified by the addition of mupirocin (100 mg/L) and acetic acid (1 mL/L) according to Rada and Petr.[10] Reflectoquant equipment (Merck, Darmstadt, Germany) was used for determination pH and lactic acid.

The differences among bifidobacterial counts were evaluated by the multiple range comparison with multiple range tests by Statgraphics Plus version 5.1.

Results and Discussion

Bifidobacterial growth in infant formula was compared with growth of bifidobacteria in milk powder (based on cow milk) as a control and with growth in human milk. There were significant differences between bifidobacterial growth in infant formula and milk samples. The strains of human origin (five strains) grew in all milk samples (Table 1) producing acids (Figure 2 and 3) and reaching colony counts from 6.50 to 9 log CFU/mL. On the contrary, both strains of *B. animalis* were inhibited after incubation in human milk and also in infant formula and exhibited lower viable counts (3-4.5 log CFU/mL) than before incubation (approx. 5 log CFU) without production of acid (Figure 2 and 3). In our previous study, we found out that among wild bifidobacteria from infants, 5 isolates were resistant, 2 moderately susceptible and only 2 susceptible to lysozyme (Table 1). In addition, two lysozyme-resistant strains of human origin grew in human milk, while two lysozyme-sensitive strains of *B. animalis* were inhibited (Table 1). This supports the hypothesis that breast milk lysozyme may act as a selection criterion for dominating bifidobacterial strains in infant intestinal tract as we found out in our previous work.[11]

Our results indicate, that infant formula contained some selection factor, which caused similar as a lysozyme in human milk. If the strain is susceptible to lysozyme may also be susceptible to other factors for example to some components of infant formula (Humana).

Table 1: Bifidobacterial growth in infant formula and in powdered milk (BoheMilk) and in Human milk

	B. animalis subsp. *lactis*1	*B. animalis* subsp. *lactis*2	*B. bifidum* 1	*B. bifidum* 2	*B. bifidum* 3	*B. longum* subsp. *longum*1	*B. longum* subsp. *infantis*2
Origin	Fermented milk product	Fermented milk product	Probiotic capsule	Infant faeces	Infant faeces	Infant faeces	Probiotic capsule
Lysozyme resitante	-	-	+	+	+	+	+
Inoculation dose	4.88 ± 0.00	4.75 ± 0.02	4.59 ± 0.02	4.36 ± 0.00	3.83 ± 0.02	3.36 ± 0.06	2.95 ± 0.05
AntiColic	4.00 ± 0.00	4.00 ± 0.00	7.18 ± 0.02	8.46 ± 0.00	8.18 ± 0.01	6.87 ± 0.11	7.10 ± 0.00
Comfort	4.58 ± 0.52	4.58 ± 0.52	6.51 ± 0.08	8.10 ± 0.00	7.45 ± 0.03	7.24 ± 0.01	7.72 ± 0.08
DG	4.00 ± 0.01	4.02 ± 0.02	6.69 ± 0.09	8.32 ± 0.02	7.74 ± 0.07	6.95 ± 0.05	7.95 ± 0.01
BoheMilk	6.76 ± 0.15	8.04 ± 0.01	9.13 ± 0.08	7.89 ± 0.01	7.73 ± 0.04	6.50 ± 0.24	6.91 ± 0.05
Human Milk	3.00 ± 0.00	4.81 ± 0.03	6.57 ± 0.17	7.19 ± 0.25	7.41 ± 0.00	6.45 ± 0.63	7.81 ± 0.03
Average	4.47 ± 1.31^{a}	5.08 ± 1.58^{a}	7.22 ± 1.02^{bc}	7.99 ± 0.47^{c}	7.70 ± 0.29^{c}	6.80 ± 0.40^{b}	7.50 ± 0.43^{bc}

Data are means ± standard deviation (SD) of three measurements. Values in last raw columns with different superscripts differ ($P < 0.01$). The differences among bifidobacterial counts were evaluated by the multiple range comparison with multiple range tests.

Figure 1: The average of production of lactic acid (mg/L) after incubation in milk samples

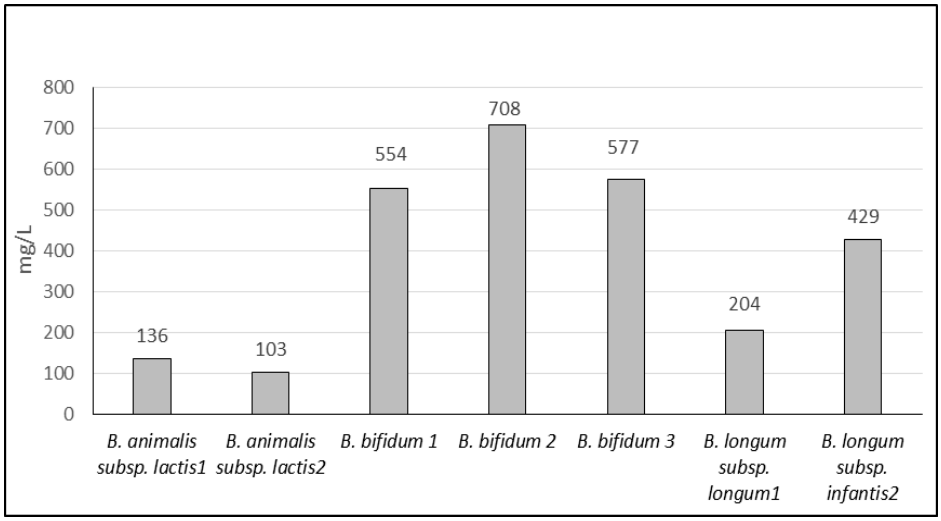
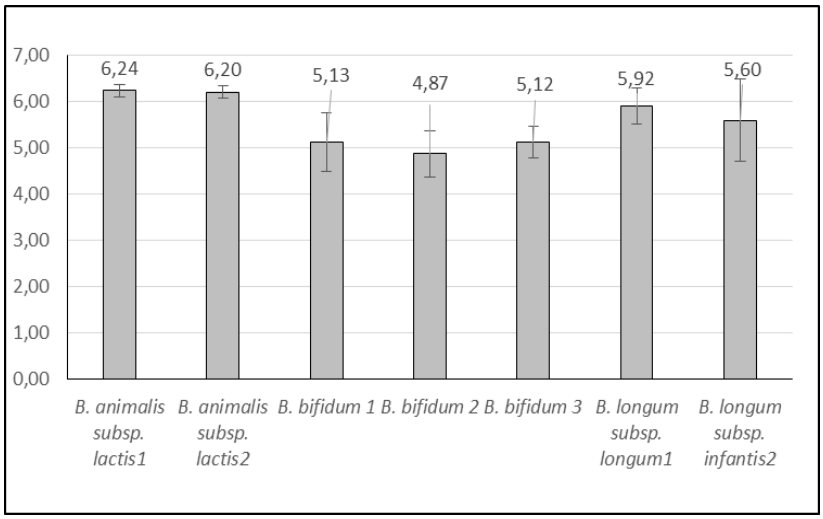


Figure 2: The average of pH after incubation in milk samples



Conclusion

Infant formula contained some selective factors, which inhibited bifidobacterial strains of animal origin similar as a human milk compare with control powdered cow milk. The effect of infant formula on the growth of bacterial strains was the same as the effect of breast milk.

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