British Journal of Nutrition (2002), **87**, 405–420
DOI: 10.1079/BJN2002563

Review article

Intestinal microflora of human infants and current trends for its nutritional modulation

Konstantinos C. Mountzouris[1,2], Anne L. McCartney[1] and Glenn R. Gibson[1]*

[1]*Food Microbial Sciences Unit, School of Food Biosciences, The University of Reading, Whiteknights, PO Box 226, Reading RG6 6AP, UK*

[2]*Axiou 60, Thrakomakedones, 13676 Attiki, Greece*

(Received 3 July 2001 – Revised 19 December 2001 – Accepted 15 January 2002)

Diet, among other environmental and genetic factors, is currently recognised to have an important role in health and disease. There is increasing evidence that the human colonic microbiota can contribute positively towards host nutrition and health. As such, dietary modulation has been proposed as important for improved gut health, especially during the highly sensitive stage of infancy. Differences in gut microflora composition and incidence of infection occur between breast- and formula-fed infants. Human milk components that cannot be duplicated in infant formulae could possibly account for these differences. However, various functional food ingredients such as oligosaccharides, prebiotics, proteins and probiotics could effect a beneficial modification in the composition and activities of gut microflora of infants. The aim of the present review is to describe existing knowledge on the composition and metabolic activities of the gastrointestinal microflora of human infants and discuss various possibilities and opportunities for its nutritional modulation.

Human infants: Colonic microflora: Dietary modulation: Functional foods

Human nutrition is currently receiving much attention for its role in health and disease. In particular, there is ever-increasing interest in understanding the effects of diet in infancy and subsequent implications for later life. As a result, the bioactive and immunomodulatory roles of major dietary components, micronutrients, vitamins, hormones and micro-organisms are being investigated and elucidated (Levy, 1998).

Human milk has always been considered a species-specific complete food (Cuthbertson, 1999) with human breast milk being the 'gold' reference standard for infant nutrition. Human milk, apart from being a nutritious complete food for infants, also contains a myriad of components that have significant bioactive and immunomodulatory roles. Immunoglobulin sIgA, peptide and non-peptide hormones, growth factors, proteins and peptides, lipids, and milk membrane fractions are components whose activities have been reviewed by Goldman *et al.* (l997) and Garofalo & Goldman (1999).

Whenever breast-feeding is not possible or available in adequate amounts, infant formulae may provide a safe, nutritious and healthy food for growth and development. However, such formulae cannot replicate the bioactive and immunomodulatory properties of breast milk because of complex quantitative and qualitative component differences (Hamosh, 1997). This may be one reason why long-term epidemiological research has demonstrated that breast-fed infants are better protected against infections of the gut, respiratory and urinary tracts when compared with those who are formula-fed (Lopez-Alarcon *et al.* 1997; Newburg, l997; Levy, 1998).

The role of the human large intestine as an important nutritional organ is now recognised, in addition to its previously accepted functions in water and electrolyte absorption, as well as the storage and excretion of waste material (Macfarlane & McBain, 1999). The nutritional function of the large intestine arises from the metabolic activities of the resident complex microbiota which heavily populates

Abbreviations: cfu, colony forming units; HMO, human milk oligosaccharides; SCFA, short-chain fatty acids.

* **Corresponding author:** Dr G. R. Gibson, fax +44 118 9357222, email g.r.gibson@reading.ac.uk

(10^{10}–10^{11} microbial cells/g of contents) the organ (Berg, 1996).

The composition and activities of the gastrointestinal microflora can be modified by diet in order to contribute towards improved host health (Gibson & Roberfroid, 1995). Such benefits could arise from: (a) energy salvage from the fermentation of dietary carbohydrates and proteins reaching the colon (Cummings & Macfarlane, 1991); (b) the synthesis of vitamins, primarily of the B and K group (Tamura, 1983; Berg, 1996); (c) production of short-chain fatty acids (SCFA) as bacterial metabolic end products. SCFA can exert an antipathogen effect by lowering the pH of the intestinal lumen thereby facilitating water absorption by the colon (Tamura, 1983; Gibson & Roberfroid, 1995); (d) production of antimicrobial compounds (Tagg *et al.* 1976; Kim, 1993; Yildirim & Johnson, 1998); (e) enhancement of the gut barrier function by competing with pathogens for adhesion receptors on the intestinal mucosa, competition for nutrients and stimulation of host immunity (Beritzoglou *et al.* 1989; Cunningham-Rundles & Lin, 1998; Cebra, 1999).

Whilst it is not possible to produce infant formulae having identical composition and properties to breast milk, potential health benefits could arise from the supplementation of these products with one and/or combinations of functional food ingredients. These may include oligosaccharides, proteins, nucleotides, peptides and probiotics. There is accumulating evidence that such dietary modulation could be beneficial for the host by effecting a health-promoting modification in the composition and the activities of the intestinal microflora (Salminen *et al.* 1998*a*).

The first part of the present review gives a report on the composition and metabolic activities of gut microflora of human infants. In the years to come, our knowledge on the microbial ecology of the infant gut is likely to change and expand with the increased use of high fidelity molecular methodologies already used in gut microbiology. Later, the review describes recent knowledge on the effect of various dietary components on the composition and activities of gut microflora. The potential role of dietary components such as human milk oligosaccharides, nucleotides, proteins, prebiotics and probiotics in beneficially modulating the gut microflora is discussed.

Composition of the infant intestinal microbiota

Shortly after birth the previously sterile infant gut begins to be colonised by an array of bacteria that belongs to the classes of facultative anaerobes and strict anaerobes.

The newborn will first come in contact with bacteria from the birth canal and its surroundings. Factors such as microbial flora of the female genital tract (Brook *et al.* 1979; Hammann, 1982; Tannock *et al.* 1990), sanitary conditions (Mata *et al.* 1969; Lundequist *et al.* 1985), obstetric techniques (Simhon *et al.* 1982), vaginal or Caesarean mode of delivery (Beritzoglou *et al.* 1989; Beritzoglou, 1997; Gronlund *et al.* 1999*a*), geographical distribution of bacterial species (Lundequist *et al.* 1985; Mevissen-Verhage *et al.* 1987; Beritzoglou, 1997) and type of feeding (Bullen *et al.* 1977; Stark & Lee, 1982; Lundequist *et al.* 1985; Mevissen-Verhage *et al.* 1987; Yoshioka *et al.* 1991; Harmsen *et al.* 2000) all have an effect on the level and frequency of various species colonising the infant gut. Some of the factors listed above have been covered in more detail in an earlier review by Heavey & Rowland (1999).

A diverse intestinal flora

Genera and species of facultative anaerobes isolated from infant faeces include *Escherichia* (*E. coli*); *Staphylococcus* (*S. aureus* and *S. epidermidis*); *Streptococcus* (*S. fecalis* and *S. faecium*); *Enterobacter* (*E. cloacae*); *Klebsiella* (*K. pneumoniae*); *Proteus* (*P. mirabilis*); *Citrobacter* (*C. freundii*) and *Pseudomonas* (*Ps. aeruginosa*). The main strict anaerobes colonising the infant intestine belong to *Bifidobacterium* (*B. breve, B. longum, B. adolescentis, B. bifidum, B. infantis*); *Bacteroides* (*B. fragilis, B. distasonis, B. vulgatus, B. ovatus, B. thetaiotaomicron, B. uniformis*); *Clostridium* (*C. perfringens, C. difficile, C. butyricum, C. tertium, C. paraputrificum*); *Lactobacillus* (*L. acidophilus, L. fermentum, L. brevis, L. salivarious, L. plantarum*); *Eubacterium* (*E. aerofaciens, E. lentum, E. rectale*); *Veillonellae* (*V. parvula*); *Peptococcus* (*P. saccharolyticus*) and *Peptostreptococcus* (*P. productus, P. anaerobius*) (Benno *et al.* 1984; Beritzoglou, 1997).

Within the first week of life initial colonisers of the infant gut are thought to be enterobacteria (for example, *E. coli*) and streptococci followed by the more strictly anaerobic bifidobacteria and bacteroides (Bullen *et al.* 1977; Stark & Lee, 1982; Yoshioka *et al.* 1991). Initial colonisation of the gut by facultative anaerobes mediates reduction of the redox potential of the intestinal lumen that in turn is thought to be a prerequisite for subsequent colonisation by the anaerobes (Stark & Lee, 1982).

Intestinal microflora of breast-fed v. bottle-fed infants

Infant faecal flora appears to more or less stabilise at 4 weeks of age and until weaning when introduction of solid foods takes place (Stark & Lee, 1982; Yoshioka *et al.* 1991; Kleessen *et al.* 1995). At this time, the microflora of breast-fed infants undergoes a more dramatic change than for formula-fed infants (Stark & Lee, 1982). A comparison of the composition of infant faecal flora hitherto studied from breast-fed and formula-fed infants at the age of approximately 4 weeks is shown in Table 1.

Formula-fed infants appear to develop a complex microflora with facultative anaerobes, bacteroides and clostridia at higher levels and frequency (Table 1) than in breast-fed infants (Stark & Lee, 1982; Lundequist *et al.* 1985; Mevissen-Verhage *et al.* 1987; Harmsen *et al.* 2000). Bifidobacteria are usually thought to be by far the predominant micro-organisms not only in numbers (cfu)/g wet faeces) but also in frequency in breast-fed infants (Table 1). However, some studies (Simhon *et al.* 1982; Lundequist *et al.* 1985) have suggested that this may not be the case and that coliforms and bacteroides were predominant.

A bifidobacterial flora predominance in formula-fed infants (Table 1) is also common, although in lower numbers and frequency compared with breast-fed infants of the

Table 1. Counts of predominant populations of bacterial genera (log_{10} cfu/g wet weight of faeces) determined at 4 weeks of age in exclusively breast-fed (BF) and formula-fed (FF) infants, together with the percentage of babies colonised by the respective bacterial groups

			Bacterial genera																	
			Bifidobacteria		Lactobacilli		Bacteroides		Clostridia		Coliforms		Enterobacteria		Streptococci		Staphylococci		Enterococci	
Research group	Feeding mode†	*n*	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
Bullen *et al.* (1977)	BF	13	10·3		ND		7·2		3·2		8·8		ND		7·2		ND		ND	
	FFa	9	9·5		ND		9·5		7·1		9·5		ND		9·4		ND		ND	
	FFb	10	7·4		ND		7·6		6·6		9·3		ND		8·6		ND		ND	
Stark & Lee (1982)	BF	6	10·6	100	NS		<3·0	100	<3·0	100	ND		6·1	100	ND		ND		6·3	100
	FFa	6	10·3	100	NS		9·3	100	6·4	100	ND		9·4	100	ND		ND		9·6	100
	FFb		–		–		–		–		–		–		–		–		–	
Yoshioka *et al.* (1991)	BF	6	10·8		6·6		7·7		ND		ND		8·3		5·7		6·2		ND	
	FFa	6	10·3		7·0		9·8		ND		ND		9·3		9·0		5·3		ND	
	FFb		–		–		–		–		–		–		–		–		–	
Balmer *et al.* (1994)*	BF	12	9·0	67	<4·0	50	<4·0	33	6·0	67	8·2	75	ND		ND		6·6	75	<4·0	42
	FFa	26	7·0	65	<4·0	42	9·3	65	<4·0	46	8·5	85	ND		ND		<4·0	15	9·0	77
	FFb	25	<4·0	44	<4·0	42	9·0	60	4·0	52	8·0	72	ND		ND		<4·0	16	9·0	96
Langhendries *et al.* (1995)	BF	14	8·6	57	ND		10·1	7	NS	7	7·6	79	7·0	29	7·2	21	6·8‡	36	ND	
	FFa	20	8·1	60	ND		9·4	15	NS	30	7·4	85	7·4	30	7·7	85	7·3‡	55	ND	
	FFb	20	9·8	20	ND		10·2	35	10·7	5	7·8	65	7·5	20	7·8	75	6·4‡	50	ND	
Kleessen *et al.* (1995)	BF	20	10·2	95	8·4	85	6·6	80	4·6	45	ND		7·8	95	ND		6·3	95	6·4	100
	FFa	10	9·8	90	9·0	80	7·4	50	6·5	60	ND		7·4	100	ND		5·6	90	8·2	100
	FFb	9	10·2	89	8·4	56	7·0	89	6·4	89	ND		8·5	100	ND		5·6	78	8·6	89
Gronlund *et al.* (1999*a*)*	BF	34	10·8	88	8·3	29	9·4	97	7·4	15	9·6	97	ND		ND		ND		ND	
	FFa		–		–		–		–		–		–		–		–		–	
	FFb		–		–		–		–		–		–		–		–		–	

cfu, Colony forming units; ND, not determined; –, not supplied.
* Median counts.
† FFa and FFb refer to formula-fed infants; some authors have examined two case-groups of formula-fed infants (for more information refer to the original references).
‡ Average of *Staphylococcus aureus* and *S. epidermis*.

same age group (Stark & Lee, 1982; Yuhara *et al.* 1983; Benno *et al.* 1984; Mevissen-Verhage *et al.* 1987; Yoshioka *et al.* 1991; Langhendries *et al.* 1995; Kleessen *et al.* 1995).

However, depending on the constituents of the experimental infant formula there are cases in bottle-fed babies where bifidobacteria were not the predominant microorganisms colonising the infant gut. Instead, bacteroides (Simhon *et al.* 1982; Lundequist *et al.* 1985; Langhendries *et al.* 1995), coliforms (Bullen *et al.* 1977; Simhon *et al.* 1982, Balmer *et al.* 1994) and enterococci (Balmer *et al.*1994) have all been seen to be prevalent.

Antimicrobial factors present in human milk (for example, lysozyme, lactoferrin) and not in infant formulae have been considered one reason for the observed lower growth of facultative anaerobes in breast-fed infants. Moreover, human milk has a lower buffering capacity which allows the luminal contents of breast-fed infants to be acidified more easily following bacterial fermentation in the proximal colon (Bullen *et al.* 1977). This may also have an inhibitory effect on growth of clostridia, bacteroides and other anaerobes, which as a result appear in lower numbers in the faeces of breast-fed infants. The, usually seen, bifidobacterial predominance observed in the faeces of both breast- and formula-fed (Table 1) infants may also be due to the fact that these bacteria can tolerate a less reduced environment for growth than other anaerobes (Macfarlane & McBain, 1999).

A lower buffering capacity resulting from a reduced protein and P content in the formula may also contribute towards a prevalence of bifidobacteria (Kawase *et al.* 1983). However, bifidobacteria and/or lactobacilli prevalence was not observed in a study conducted by Rose (1984), irrespective of the type of feeding and level of buffering capacity in the diets received by the infants. In this study, full-term normal infants were allocated to three dietary groups receiving either a standard formula (protein concentration of 18 g/l and buffering capacity 1·5 times that of breast milk) or a low-protein formula (protein concentration of 15 g/l and buffering capacity 1·1 times that of breast milk) or were breast-fed. Instead enterobacteria were present in greater numbers in each group at all times (Rose, 1984).

Feeding of an infant formula, which contained lactulose and mucin, and in addition had a low P content and buffering capacity, to low-birth-weight infants resulted in a lowering of faecal pH with increased organic acid levels and lysozyme activity (Kawase *et al.* 1983). A correlation between the ratio of bifidobacteria and pH in faeces has also been observed. The ratio of bifidobacteria count to total anaerobes was 44·8 % in the faeces of low-birth-weight infants at pH 5·0–5·5, but only 4·4 % at pH 7·0–7·5 (Kawase *et al.* 1983).

From the bifidobacterial species isolated from faeces of thirty-five breast-fed and thirty-five bottle-fed infants (mean bifidobacterial count: l0·74 (SD 0·81) log cfu/g faeces and 10·62 (SD 0·49) log cfu/g faeces respectively) aged 28 to 46 d (Benno *et al.* 1984), *B. breve* occurred most frequently in both breast-fed and bottle-fed infants (89 and 83 %) followed by *B. adolescentis* (49 and 37 % respectively), *B. longum* (43 and 43 % respectively) and finally *B. bifidum* (14 and 26 %). There were no statistically significant differences with regard to the bifidobacterial species counts and frequency of occurrence between the two groups of infants. *B. infantis* was not isolated from any of the samples.

In the study of Yuhara *et al.* (1983), bifidobacteria was the predominant genus isolated from the faeces of thirty breast-fed and forty bottle-fed infants (mean count 10·7 (SD 0·9) log cfu/g faeces and 10·0 (SD 2·2) log cfu/g faeces respectively) aged 33–135 d and 3–134 d respectively. The frequency of occurrence for *B. breve*, *B. adolescentis*, *B. longum* and *B. bifidum* was 90, 40, 67 and 70 % for breast-fed infants respectively, while it was 93, 53, 45 and 23 % for bottle-fed infants. Although the frequency of occurrence of *B. bifidum* in bottle-fed infants was much less than for breast-fed infants, overall there was no statistically significant difference in the numbers and frequencies of occurrence of each species between the two groups. *B. infantis* occurred at very low frequencies of 7 and 8 % in breast-fed and bottle-fed infants (Yuhara *et al.* 1983).

Similarly, in the study of Mevissen-Verhage *et al.* (1987) the predominant bifidobacterial species most frequently isolated from breast-fed and formula-fed infants were *B. breve*, *B. adolescentis*, *B. longum* and *B. bifidum*. Again, *B. infantis* was only isolated infrequently. On the contrary, the most predominant bifidobacterial species in the studies of Kleessen *et al.* (1995) was *B. infantis*, followed by *B. bifidum*, *B. breve*, *B. longum* and *B. adolescentis*.

For the lactobacilli, inconsistent appearance and disappearance during the period from birth until weaning (Stark & Lee, 1982; Lundequist *et al.* 1985) suggests that they are unable to form stable populations in the infant gut. None of the lactobacilli present in maternal vaginal flora appeared to colonise the digestive tract of normally delivered full-term infants (Tannock *et al.* 1990).

For *Bacteroides*, the species most frequently isolated belong to the *B. fragilis* group and are mainly *B. fragilis*, *B. distasonis* and *B. vulgatus*. Generally, bottle-fed infants are more likely to have higher bacteroides and colonisation frequency compared with breast-fed infants (Benno *et al.* 1984; Lundequist *et al.* 1985; Mevissen-Verhage *et al.* 1987; Kleessen *et al.* 1995; Harmsen *et al.* 2000). Dietary Fe, which is incorporated into some infant formulae, can cause increased numbers of bacteroides (Mevissen-Verhage *et al.* 1987; Kleessen *et al.* 1995). In a study carried out by Benno *et al.* (1984), bacteroides were significantly lower ($P<0·05$) in the breast-fed (8·91 (SD l·76) log cfu/g faeces) compared with the formula-fed group of infants (9·9 (SD 0·61) log cfu/g faeces). The most prevalent *Bacteroides* species belonged to the *B. fragilis* group.

Breast-fed infants have significantly less clostridia both in terms of counts and colonisation frequency compared with formula-fed babies (Yuhara *et al.* l983; Benno *et al.* 1984; Kleessen *et al.* 1995). The most common *Clostridium* species isolated have been *C. difficile*, *C. perfringens*, *C. paraputrificum* and *C. tetrium* (Benno *et al.* 1984). *Clostridium perfringens* was most frequently isolated (60–80 %) from the faecal specimens of breast-fed and

formula-fed infants 3–6 weeks old (Mevissen-Verhage *et al.* 1987).

The development of anaerobic microflora in infants delivered by Caesarean section appears to be delayed and bifidobacteria did not reach normal levels for 4–8 weeks (Bennet & Nord, 1987). Bifidobacteria and lactobacilli colonisation rates in Caesarean-delivered infants reached the rates of vaginally delivered infants at 1 month and 10 d, respectively (Gronlund *et al.* 1999*a*). In the Caesarean-delivered infants no permanent colonisation with bacteria of the *Bacteroides fragilis* group was seen before the infants reached 2 months of age. Infants born by Caesarean section had higher colonisation rates of *Clostridium perfringens* than the vaginally delivered infants (57 and 17 % respectively) at 1 month of age (Gronlund *et al.* 1999*a*). *Clostridium perfringens* colonised 26 and 90 % of the infants delivered by Caesarean section within 48 h after birth and the first 14 d of life, respectively. Breast-feeding led to the repression of *Clostridium perfringens*, whereas bottle-feeding allowed its maintenance (Beritzoglou *et al.* 1989).

New opportunities for the study of microbial ecology using molecular techniques

The microflora composition (Table 1) residing in the gastrointestinal tract of infants has largely been determined by standard culture techniques and phenotypic characterisations, i.e. based on colony morphology and various biochemical markers such as enzyme activities and metabolic end products. However, these traditional cultural methods can only elucidate part of the overall microbial diversity occurring in the infant colon since they are applicable only to cultivable bacteria and quite often the chosen media are not selective for the required bacterial genera or species (Holdeman *et al.* 1977; Silvi *et al.* 1996; Hartemink & Rombouts, 1999).

A generation of new and more reliable information on the diversity of gut microflora in animals and man is now accumulating with the application of molecular-based techniques. The principle underlying these applications in the study of microbial diversity stems from the fact that a comparison of nucleotide sequences of individual genes would suffice for the elucidation of evolutionary and phylogenetic relationships between micro-organisms. In this sense, the application of nucleic acid probes, which are fragments of single-stranded nucleic acid (mainly DNA) that bind to complementary DNA or RNA (target nucleic acid), has created opportunities for the rapid identification of micro-organisms (Schleifer *et al.* 1993).

In prokaryotes the comparative analysis of ribosomal RNA, in particular the 16S and 23S rRNA genes, has been a breakthrough for the identification of bacteria from genus down to species or strain level (Amann *et al.* 1990*a*,*b*; Langendijk *et al.* 1995; Wang *et al.* 1996; Franks *et al.* 1998). In particular, techniques such as the polymerase chain reaction (Mullis *et al.* 1986), gene sequencing (Suau *et al.* 1999) and *in situ* hybridisation (Anqerer *et al.* 1987; Schleifer *et al.* 1993) are routinely used. A simplified schematic representation of the molecular techniques currently in use for the study of gut microbial ecology is given in Fig. 1. The methods for the analysis of the intestinal microflora have been recently reviewed in detail by O'Sullivan (1999).

Polymerase chain reaction using 16S rRNA targeted primers has been successfully applied for the detection and quantification of predominant anaerobes in human adult and infant faeces (Wang *et al.* 1996), as well as the tracking of a probiotic *Bifidobacterium* in the stools of infants fed an instant milk formula containing the strain (Kok *et al.* 1996). Millar *et al.* (1996) used 16S rRNA gene polymerase chain reaction combined with denaturating gel gradient electrophoresis in their research into potential agents causing the pathogenesis of necrotising enterocolitis in infants. Uncultured bacteria thought to be a causative agent in the pathogenesis of necrotising enterocolitis were also present in samples from healthy infants. However, the possibility of unrecognised bacteria that could be associated with the mucosa of the small intestine of infants with necrotising enterocolitis was not excluded.

In a recent study by Harmsen *et al.* (2000) the intestinal flora development of breast- and formula-fed infants during the first 20 d of life was investigated using oligonucleotide probes and fluorescent *in situ* hybridisation, in addition to a conventional cultural approach. Both groups of infants were initially colonised by a diverse (adult-type) flora during the first 6 d of life, but in the following days a bifidobacterial dominant flora was established in breast-fed infants. In most formula-fed infants similar amounts of *Bacteroides* and bifidobacteria were found. It was noted that in the formula-fed infants, while the bacteroides numbers equalled those of bifidobacteria according to fluorescent *in situ* hybridisation, they were 100–1000-fold lower according to culture-based studies. This suggested that there may be a problem in culturing this group of anaerobic bacteria, which can lead towards a large bias. This, in addition to the general observation of low recovery of anaerobes through conventional cultivation methods compared with total cell counts obtained with the DNA stain 4′,6-diamidino-2-phenylindole, may change the size of the relative contribution that various genera make in the overall gut microbial population. Thus, while bifidobacterial numbers, as determined by fluorescent *in situ* hybridisation and conventional culture methods, did not differ significantly (Langendijk *et al.* 1995) their contribution to the total adult faecal flora was found to be only around 1 % (Langendijk *et al.* 1995) or 3 % (Franks *et al.* 1998).

It is expected that the use of the new more powerful molecular techniques in gut microbiology will rapidly advance our knowledge and understanding of gut microbial ecology and diversity in the near future.

Fermentation capacity of the infant intestinal microbiota

Study of the colonic contents of sudden-death victims has shown that the human faecal microflora could be considered as representative of that found in the large intestine (Moore *et al.* 1978; Macfarlane *et al.* 1998). Colonic bacteria thrive on a number of materials that become available for fermentation as they flow from the ileum

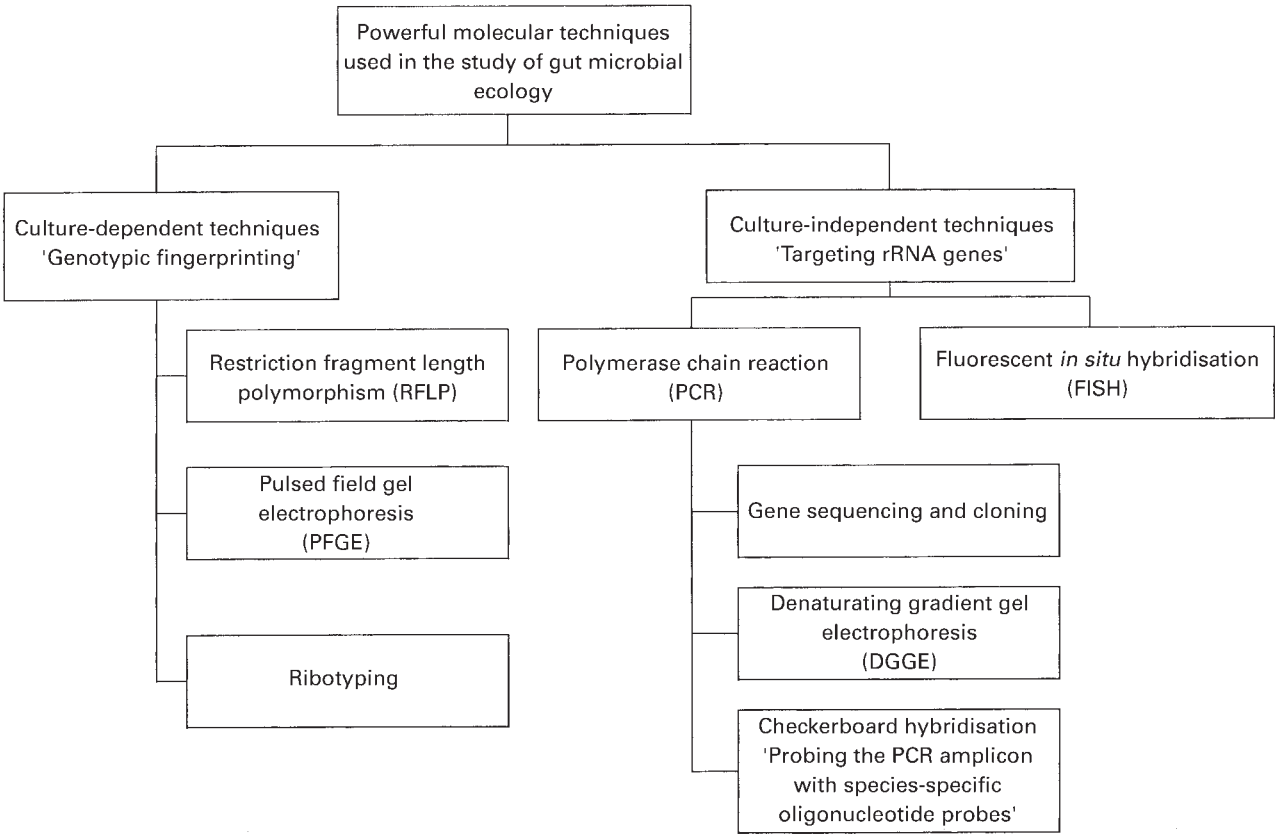

Fig. 1. Simplified schematic representation of the powerful molecular techniques used in the study of gut microbial ecology.

into the large intestine. Undigested and/or unabsorbed foodstuffs (for example, mainly carbohydrates and proteins) from the small intestine and various host secretions (for example, pancreatic juice, bile, mucus and sloughed epithelial cells) firstly become available to bacteria resident in the caecum. As a result, substrates available for fermentation deplete as bowel contents move distally towards the recto-sigmoid region, thereby giving rise to varying fermentation patterns along the length of the colon.

Microflora fermentation metabolites and associated characteristics

Fermentation by the colonic microflora results in the production of SCFA as major fermentation end products, and gases including H_2, CO_2 and CH_4 (Cummings & Macfarlane, 1991; Gibson & Roberfroid, 1995). SCFA are rapidly absorbed by the colonic mucosa facilitating water absorption from the colonic lumen and thus may confer some protection against diarrhoea (Cummings & Macfarlane, 1991).

During saccharolytic metabolism in the colon acetate, propionate and butyrate are the main SCFA produced, while lactate, ethanol, succinate, formate, valerate and caproate also constitute significant products. The molar ratios of SCFA formed from carbohydrate fermentation depend on the type of substrate fermented. For example, the metabolism of pectin and starch by human intestinal bacteria is known to generate high amounts of acetate and butyrate respectively (Cummings & Macfarlane, 1991; Wang & Gibson, 1993; Bourquin *et al.* 1996; Salminen *et al.* 1998*a*). More recently, it has been shown (Olano-Martin *et al.* 2000) that the *in vitro* fermentation of dextran and oligodextran (i.e. dextran hydrolysate (Mountzouris *et al.* 1999)) by human intestinal microflora yielded almost double the amount of butyrate compared with maltodextrin (i.e. starch hydrolysate). Butyrate attracts attention for its possible biological properties against colon cancer (Salminen *et al.* 1998*a*). *In vitro*, butyrate was shown not only to induce apoptosis in colonic tumour cell lines but also to be the most effective inducer of apoptosis compared with propionate and acetate (Hague *et al.* 1995). However the exact mechanisms underpinning the role of butyrate on cellular proliferation and differentiation in the normal colon still remain to be elucidated (Wachtershauser & Stein, 2000). The role of butyrate as growth-stimulatory or growth-inhibitory for colonic epithelial cells may depend on the availability of other energy sources (Singh *et al.* 1997).

However, the fact that the amount of butyrate found in infant faeces (Table 2) and their *in vitro* incubations with carbohydrates is low may indicate that this metabolite might not be as important for the colonic enterocytes in the developing intestine of pre-weaned human neonates as is suggested for those of the adult (Parrett & Edwards, 1997; Salminen *et al.* 1998*a*); thus any ingredient recommendations for use in infant formulas should be treated with great caution.

Table 2. Faecal short-chain fatty acid (SCFA) concentrations (mmol/kg wet weight faeces) of breast-fed (BF), formula-fed (FF) infants and adults and their respective molar ratios

(Mean or median values)

	Lifschitz *et al.* (1990)*		Siigur *et al.* (1993)†		Parrett & Edwards (1997)†		Gibson *et al.* (1995)*‡
Research group ...	BF (*n* 14)	FF (*n* 9)	BF (*n* 13)	FF (*n* 21)	BF (*n* 9)	FF (*n* 8)	Adults (*n* 8)
Total SCFA	58·8§	132·1§	58·1	72·4	30·3	123·1	123·1
Acetic acid	44·7	98·9	53·8	52·6	25·9	91·9	73·8
Molar ratio	76·0	74·9	92·6	72·6	85·5	74·6	59·9
Propionic	14·1	33·2	2·9	16·2	2·5	15·6	22·8
Molar ratio	24·0	25·1	5·0	16·6	8·3	12·7	18·5
n-Butyric acid	–	–	0·4	2·2	0·0	4·4	17·9
Molar ratio			0·7	3·0	0·0	3·6	14·6
Others ‖	–	–	1·0	1·4	2·8	11·2	8·61
Molar ratio			1·7	1·9	9·2	9·1	7·0
Lactic acid	22·4	18·5	–	–	0·0	2·8	–

–, Not supplied or determined.
* Values given are means.
† Values given are medians.
‡ Values reported are the average of three treatments.
§ Total SCFA have been calculated as the sum of acetic and propionic acid concentrations given.
‖ Calculated by subtracting the sum of acetic, propionic and n-butyric acid from the total SCFA.

The human gut microflora has also a high proteolytic activity which mainly results in the production of branched SCFA such as isobutyrate and isovalerate but also other metabolites such as ammonia, phenols, indoles and amines that can be potentially toxic for the host (Macfarlane *et al.* 1988; Salminen *et al.* 1998*a*).

Faecal SCFA are the net outcome of the overall fermentation and absorption taking place in the colon but give limited information on the events occurring along the length of the large bowel. It has been estimated that around 95 % of the SCFA generated in the colon is absorbed (Cummings & Macfarlane, 1991). However due to the inaccessibility of intestinal contents, faecal SCFA have been extensively used in the study of gut microbial ecology and function in the same manner that faecal inocula have been used in numerous *in vitro* models for investigating the fermentability of various substrates by human adult flora (Gibson *et al.* 1995; Salminen *et al.* 1998*a*; Olano-Martin *et al.* 2000).

Faecal SCFA profiles in infants (Table 2) differ mainly according to the type of feeding. In breast-fed infants, acetic acid accounts for most of the total SCFA. Formula-fed infants also have acetate as the predominant SCFA in faeces but propionate and, to a lesser extent, butyrate have higher molar ratios compared with breast-fed infants (Table 2). Generally, higher amounts of faecal SCFA have been determined in formula-fed compared with breast-fed infants. In the study of Midtvedt & Midtvedt (1992), children who received both breast milk and formula supplement had values of SCFA between those in the groups that received either breast milk or formula. It was hypothesised that these differences occur because human milk is better utilised by the infant, thus less of the unabsorbed components reach the colon and subsequently less SCFA can be produced. Generally, faecal SCFA concentration in infants was generally lower than that in adults (Table 2).

Breast-fed infants tend to have a more acidic stool pH ranging from pH 5 to 6 compared with a neutral pH found in the faeces of formula-fed infants (Fig. 2) despite the fact that breast-fed infants have lower amounts of faecal SCFA compared with formula-fed ones (Table 2). This could possibly be explained considering the lower buffering capacity of human milk compared with infant formulae that allows the intestinal contents of breast-fed infants to be acidified easier (Bullen *et al.* 1977; Rose, 1984).

Microbial enzyme activities or metabolic endpoints resulting in compounds with potentially toxic or beneficial effects belong to the microflora-associated characteristics that are also of relevance to gut physiology and pathophysiology (Salminen *et al.* 1998*a*; Mackie *et al.* 1999). Norin *et al.* (1985) studied the following biochemical characteristics in faeces from children of 0–61 months of age: conversion of cholesterol to coprostanol and bilirubin to urobilins; inactivation of trypsin; degradation of mucin. Their results indicated that the establishment of a microflora capable of performing the examined biochemical functions is a long-drawn-out process and was established within the second year of life. Only tryptic activity was present in faeces from all children up to 21 months of age (Norin *et al.* 1985). The faecal bacterial enzyme activities β-glucosidase, β-glucuronidase and urease were studied in twenty-nine full-term healthy infants during the first 6 months of life (Gronlund *et al.* 1999*b*). It was shown that mode of delivery had no influence on the faecal enzyme activities. The type of milk (breast-fed *v.* formula-fed) that infants receive during the first months of life was found to affect the faecal enzyme activities. Formula-fed infants had significantly higher urease activity at 1–2 months of age and higher median activity of β-glucuronidase at 6 months of age (Gronlund *et al.* 1999*b*).

Carbohydrate fermentation capacity

Lactose is the main carbohydrate source in human milk and

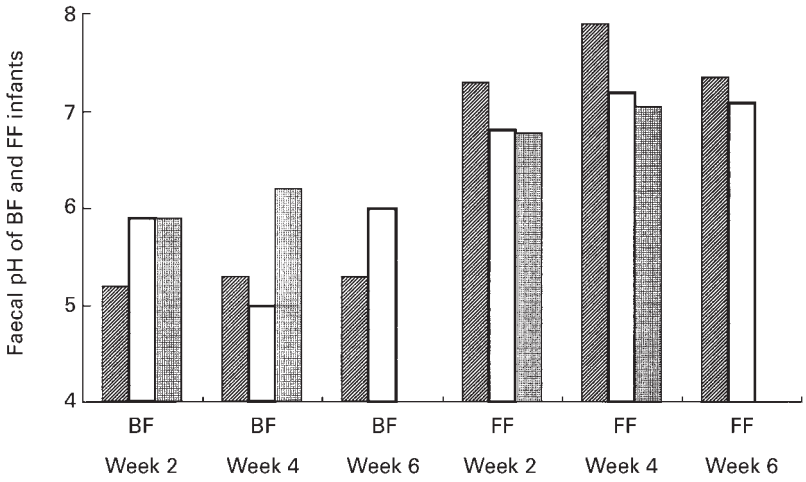

Fig. 2. Faecal pH values of breast-fed (BF) and formula-fed (FF) human infants during the first 2, 4 and 6 weeks of age. Data were taken from the studies of Bullen *et al.* (1977) (▨), Simhon *et al.* (1982) (□) and Kleessen *et al.* (1995) (▦).

infant formulae. Irrespective of the type of feeding, a proportion of lactose that escapes digestion and absorption in the small intestine becomes available for fermentation by the colonic microflora in both breast-fed and formula-fed infants. Since lactose would be the main carbohydrate fermented in the colon, differences in faecal SCFA profiles could be mainly due to variability in the intestinal microflora between the two feeding groups (Table 1), and possible differences in the ability of the microflora to ferment carbohydrates (Siigur *et al.* 1993; Parrett & Edwards, 1997). Another reason for these differences could also be the fact that human milk contains a significant amount of complex oligosaccharides not found in bovine milk or infant formulae (Kunz & Rudloff, 1993). Human milk oligosaccharides (HMO) have potential anti-infective function in human newborns (Coppa *et al.* 1993; Kunz & Rudloff, 1993; Peterson *et al.* 1998) while they undergo fermentation in the large intestine, since they mainly escape digestion and absorption in the small intestine (Brand-Miller *et al.* 1998; Engfer *et al.* 2000; Gnoth *et al.* 2000). In particular, N-acetylglucosamine oligosaccharides have been shown to favour the growth of *Bifidobacterium* species (Kunz & Rudloff, 1993). Bifidobacteria are known producers of lactate and acetate and their predominance in the intestinal microflora of breast-fed infants (Table 1) possibly explains the high proportion of acetate in the faeces of this group of infants (Table 2). A low amount of acetate has been shown to be associated with infantile diarrhoea and upper respiratory tract infections irrespective of the type of feeding (Siigur *et al.* 1993).

Breast-fed infants have been reported to have detectable amounts of reducing sugars excreted in their faeces (Wharton *et al.* 1994) and HMO excreted in their urine and faeces (Kunz & Rudloff, 1993; Brand-Miller *et al.* 1998). Faeces from breast-fed infants contained significantly more lactose than stools from formula-fed infants (2·05 *v.* 0·57 μmol/g wet weight faeces respectively), while there were no significant differences in the amounts of faecal hexose (8·05 *v.* 6·31 μmol/g wet weight faeces respectively) and lactate (Table 3) between the two groups (Lifschitz *et al.* 1990). However, a brief estimation based on data relating to energy and nutrient intake (Lambert & Hall, 1995; De Bruin *et al.* 1998) and faecal excretion (Sievers *et al.* 1993) in infants would indicate that the overall amount of dietary carbohydrate recovered in faeces from healthy infants (Lifschitz *et al.* 1990) was very low and could not be more than 0·1 % of the amount of lactose ingested.

In vitro studies using faecal microflora from breast-fed and formula-fed infants as the inocula have shown that an acidic pH of 5·5 would result in relatively less lactose being hydrolysed from the microflora originating from breast-fed infants, while it would result in a lower fermentation of lactose breakdown products (i.e. hexose) from the gut microflora of formula-fed infants (Lifschitz *et al.* 1990). The possible *in vivo* consequence of this would be that breast-fed infants would be less likely to suffer osmotic diarrhoea as compared with formula-fed infants who would have a higher osmotic load in their large bowel due to the presence of unfermented hexose. However, the fact that bottle-fed infants usually have higher concentrations of total faecal SCFA (Table 2), and lower concentration of lactose and hexose in their stools (Lifschitz *et al.* 1990) did not support this hypothesis.

The intestinal microflora from formula-fed infants (median age 6 weeks) had a comparable fermentation capacity to that from breast-fed infants (median age 5 weeks). This was determined by *in vitro* incubations of faecal cultures from formula- and breast-fed infants with a variety of dietary carbohydrates (i.e. glucose, lactose, fructo-oligosaccharides and soyabean polysaccharide) for 24 h (Parrett & Edwards, 1997). In all cases, the fermentation capacity in both infant groups was lower than that of adults. The microflora from formula- and breast-fed infants was shown to have a similar fermentation capacity for simple sugars and oligosaccharides but was equally poor at fermenting soyabean polysaccharide (Parrett & Edwards, 1997).

In vitro fermentation capacities of breast-fed infants for complex carbohydrates (i.e. soyabean polysaccharide and

Table 3. List of mammalian digestive enzymes

Enzyme	References
Gastro-intestinal lumen	
Salivary α-amylase	Lee (1983); Christian *et al.* (1999)
Lingual lipase	Hamosh (1983); Hernell & Blackberg (1983)
Pepsin	Lentze & Sterchi (1983)
Pancreatic proteases	Lentze & Sterchi (1983); Holtmann *et al.* (1997)
Pancreatic lipase	Hernell & Blackberg (1983); Hamosh (1983)
Pancreatic α-amylase	Lee (1983); Holtmann *et al.* (1997); Christian *et al.* (1999)
Enterocyte brush border	
Lactase-phlorizin	Lifschitz *et al.* (1983); Rings *et al.* (1994); Levin (1994); Gudmand-Hoyer & Skovbjerg (1996); Kien *et al.* (1996); Lebenthal & Lebenthal (1999)
Maltase-glucoamylase	Lee (1983); Levin (1994); Gudmand-Hoyer & Skovbjerg (1996); Lebenthal & Lebenthal (1999)
Sucrase-isomaltase	Levin (1994); Treem (1995); Gudmand-Hoyer & Skovbjerg (1996); Lebenthal & Lebenthal (1999)
Trehalase	Levin (1994); Gudmand-Hoyer & Skovbjerg (1996); Lebenthal & Lebenthal (1999)
Peptide hydrolases	Lentze & Sterchi (1983)
Mammary origin	
Bile salt stimulated lipase	Hernell & Blackberg (1983); Hamosh (1983)
α-Amylase	Lee (1983); Christian *et al.* (1999)

guar gum) was shown to increase progressively and not be significantly developed until late weaning (Parrett *et al.* 1997). One reason for this may be that before weaning, the intestinal microflora of infants is primarily adapted to lactose, hexoses and oligosaccharides from milk and therefore the enzymes needed to ferment complex carbohydrates may not be present or sufficiently active (Parrett & Edwards, 1997). It has been suggested that continued ingestion of complex carbohydrates may effect a change in the colonic microflora of infants, by inducing enzymes or altering bacterial populations such that their ability to ferment these substrates was increased (Tamura, 1983; Parrett *et al.* 1997). This change was also evidenced by increased levels of propionate and butyrate observed in cultures of faeces from breast-fed infants at early and late weaning compared with pre-weaning (Parrett *et al.* 1997). The presence of propionate and butyrate gives evidence for development of a more complex flora since these SCFA are produced by bacteria belonging mainly to the bacteroides and clostridia genera (Cummings & Macfarlane, 1991).

Stark & Lee (1982) have shown that the introduction of solids in the diet of formula-fed infants did not result in a major disturbance in the microbial ecology of the large bowel as was the case for breast-fed infants. In this sense, the more complex microflora of formula-fed infants (Table 1) could confer a significant adaptation advantage to dietary complex carbohydrates when weaning occurs but this possible adaptation will have to be determined in future studies.

Nutritional modulation of the infant intestinal microflora

The role of intestinal microflora in health and disease is becoming increasingly recognised (Macfarlane & McBain, 1999). It is now evident that the composition and activities of the intestinal microflora can be modulated through diet (Gibson & Roberfroid, 1995; Salminen *et al.* 1998*a*). In particular, carbohydrates are the principal nutritional components in the diet that are used metabolically by the host for the generation of maintenance energy, growth and development. Human and most mammalian milks have lactose as the main carbohydrate source. Infant formulae contain the following carbohydrates: lactose, maltose, sucrose, maltodextrins, glucose syrup or dried glucose syrup, gluten-free pre-cooked starch; gelatinised starch (Jukes, 1997). Most formulae for term infants follow the human milk model and have lactose as the main carbohydrate.

Humans are well-equipped with an enzymic system (Table 3) for the digestion of dietary components. The resulting breakdown products such as simple sugars, peptides and fatty acids can be metabolised by the host following absorption from intestinal enterocytes. Dietary components that totally, or even partially, escape digestion in the above enzymic system (Table 3) will arrive in the hindgut where they are then subject to metabolic activities of the colonic microflora.

Digestive enzymes

The digestive system of mammals comprises enzymes that are secreted in the gastrointestinal lumen and located in the brush border membrane of enterocytes performing epithelial digestion of dietary components (Table 3).

Healthy infants are enzymically well adapted for the digestion of various dietary components such as proteins, fats and carbohydrates. Brush border peptide hydrolases are functional and appear very early in gestation with activities similar to those found in the intestinal tract of children and adults (Lentze & Sterchi, 1983). Pancreatic lipase and bile salt concentrations in newborn infants are low but a reasonably good absorption of fat seen in infants is due to the presence of lingual lipase, which significantly increases the lipolytic activity (Hamosh, 1983). Breast-fed

infants benefit additionally from the presence of the bile salt-stimulated lipase of human milk (Hernell & Blackberg, 1983). Disaccharidases located in the brush border membranes of small intestinal enterocytes are active by the 10th week of gestation and increase up to 40 weeks (Lebenthal & Lebenthal, 1999). Pancreatic α-amylase in the neonatal duodenum and infants under 3 months of age is absent, or very low, compared with concentrations found in adults (Lee, 1983; Christian *et al.* 1999). Despite this deficiency, young infants (i.e. less than 6 months) seem to be able to tolerate a moderate amount of starch. This is because two other enzymes, namely the brush border maltase–glucoamylase and mammary amylase, are also involved in starch hydrolysis (Lee, 1983). Lactose is hydrolysed in the small intestine by epithelial lactase (Rings *et al.* 1994) and the generated glucose and galactose are subsequently absorbed by active transport into enterocytes (Levin, 1994). Lactose is a slowly absorbed carbohydrate whose predominant presence in milk also influences bacterial metabolism (Kien *et al.* 1996; Vanderhoof, 1998). Lifschitz *et al.* (1983) showed that in breast-fed infants lactose that escaped absorption in the upper gut was fully utilised in the colon, as evidenced by breath H_2 measurements, stool pH over 5·5 and the absence of reducing sugars (for example, glucose) in the stools.

Currently, there is a great deal of scientific and commercial interest directed towards an elucidation of the role and effects of a range of non-immunological nutritional components such as human milk oligosaccharides, proteins and nucleotides on the gastrointestinal flora. In addition probiotics, prebiotics and synbiotics also represent a promising approach for rational dietary modulation of the gut microflora.

Human milk oligosaccharides

Human milk is known to contain significant amounts of over 130 lactose-derived oligosaccharides, whilst cows' milk contains only trace amounts (Kunz, 1998). HMO can range from 0·7 up to 8 g/l (Kunz & Rudloff, 1993; Kunz, 1998; Nakhla *et al.* 1999) and are therefore one of the four main components of human breast milk in addition to lactose, fat and protein. From HMO, lacto-N-tetraose and their monofucosylated derivatives account for up to 50–70 % of the total HMO (Kunz & Rudloff, 1993). Some HMO are known to be potent inhibitors of bacterial adhesion to epithelial cells by acting as receptor analogues to mucosal adhesion molecules (Kunz & Rudloff, 1993; Kunz, 1998; Peterson *et al.* 1998). Among the HMO, lacto-N-tetraose and lacto-N-neotetraose act as cell surface receptors for *Streptococcus pneumoniae*, fucosylated oligosaccharides are receptors for *E. coli* and sialated oligosaccharides are recognised receptor sites for influenza viruses A, B and C, *Campylobacter pylori* and *Mycoplasma pneumoniae* (Kunz & Rudloff, 1993). HMO, in a free or protein-bound form, are mainly located in the soluble (whey) fraction of milk and have been identified as potential ligands for selectins (Schwertmann *et al.* 1996). From this, it can be postulated that HMO may contribute towards the lower incidence of gastrointestinal, respiratory and urinary infections seen in breast-fed infants compared with those who are formula-fed.

HMO are resistant to enzymic hydrolysis in the upper gastrointestinal tract (Brand-Miller, 1998; Engfer *et al.* 2000; Gnoth *et al.* 2000) and have also been shown to favour *Bifidobacterium* proliferation *in vitro* (Gyorgy *et al.* 1954). In particular, N-acetylglucosamine containing oligosaccharides, together with lactose, were shown to stimulate the growth of *Bifidobacterium bifidum* (Gyorgy *et al.* 1974).

Proteins and peptides

The whey fraction of human and bovine milk contains proteins such as α-lactalbumin, β-lactoglobulin (bovine milk) and lactoferrin that have been shown to exert antimicrobial function and bifidogenic properties (Saito *et al.* 1991; Ouwehand *et al.* 1997; Pakkanen & Aalto, 1997; Schanbacher *et al.* 1998; Pelligrini *et al.* 1999; Petschow *et al.* 1999; Pihlanto-Leppala *et al.* 1999; van Hoijdonk *et al.* 2000). Similarly, some proteins of the casein fraction can have an effect on the intestinal microflora through regulation of gut motility, antibacterial action and bifidogenic properties (Zucht *et al.* 1995; Lahov & Regelson, 1996; Schanbacher *et al.* 1998). Some of these antimicrobial and bifidogenic properties are summarised in Table 4.

The protein composition of human and bovine milk including amino acids has been listed by Heine *et al.* (1991). Addition of any of these proteins, or their peptides, to infant formulae should consider possible changes in the amino acid pattern of the new product, as well as the required safety evaluation tests that need to be made before feeding.

Nucleotides

Nucleotides are low-molecular-weight biological components that form the building blocks of the nucleic acids and play major roles in multiple biochemical processes fundamental to cellular metabolism and function. The biological effects of dietary nucleotides refer to immune function, Fe absorption, lipid metabolism, gastrointestinal growth and development, hepatic morphology and function and have been extensively reviewed by Boza (1998), Cosgrove (1998) and Schlimme *et al.* (2000). Supplementation of infant formulae and fol1ow-on formulae with nucleotides is allowed in the European Union (Schlimme *et al.* 2000).

Their effects on the gut microflora have not hitherto been thoroughly investigated. However, one study did not support their use in infant formulae for improving the gut microflora composition (Balmer *et al.* 1994). In another study, positive changes in the gut microflora of infants given the nucleotide-supplemented formula were seen as denoted by a higher percentage of bifidobacteria and a lower percentage of enterobacteria in faeces compared with the unsupplemented control formula. The numbers remained different from the respective percentages seen in breast-fed infants (Gil *et al.* 1986).

Table 4. Antimicrobial and bifidogenic properties of major milk proteins

Component	Function	References
α_{s1}	Fragment known as isracidin has *in vivo* antibacterial activity against *Staphylococcus aureus* and *Candida albicans*	Lahov & Regelson (1996)
	Peptide α-casomorphin reduces gut motility	Schanbacher *et al.* (1998)
α_{s2}	Fragment named casocidin-I acts as antibacterial agent that can inhibit the growth of *E. coli* and *Staphylococcus carnosus in vitro*	Zucht *et al.* (1995)
β-Casein	Peptide β-casomorphin reduces gut motility. May be implicated in the release of latent and immunoregulatory activities from lactoferrin	Schanbacher *et al.* (1998)
κ-Casein	κ-Casein glycomacropeptide supports growth of bifidobacteria in the gut	Schanbacher *et al.* (1998)
	Bifidobacterial-rich microflora in the gut after enteropathogenic *E. coli* infection in rhesus monkeys	WM Bruck, SL Kelleher, GR Gibson, KE Nielsen, DEW Chatterton and B Lonnerdal (unpublished results)
α-Lactalbumin	Activity against Gram-positive bacteria	Pellegrini *et al.* (1999)
	Promotion of bifidobacterial-rich microflora in the gut after enteropathogenic *E. coli* infection in rhesus monkeys	WM Bruck, SL Kelleher, GR Gibson, KE Nielsen, DEW Chatterton and B Lonnerdal (unpublished results)
	α-Lactalbumin hydrolysates suppress growth of *E. coli* JM 103 *in vitro*	Pihlanto-Leppala *et al.* (1999)
β-Lactoglobulin	Inhibits adhesion of sfaI and mainly sfaII expressing *E. coli* to immobilised human ileostomy glycoproteins *in vitro*	Ouwehand *et al.* (1997)
	β-Lactoglobulin hydrolysates suppress growth of *E. coli* JM 103 *in vitro*	Pihlanto-Leppala *et al.* (1999)
Lactoferrin	Proliferation of *Bifidobacterium infantis, B. breve* and *B. bifidum in vitro*	Petschow *et al.* (1999); Schanbacher *et al.* (1998)
	Inhibition of growth of Gram-negative and Gram-positive enteropathogenic bacteria	Pakkanen & Aalto (1997)
	Bacteriostatic and bactericidal activity of intact bovine lactoferrin and its hydrolysates (lactoferricin)	Saito *et al.* (1991); Schanbacher *et al.* (1998); van Hoijdonk *et al.* (2000)

E. coli, *Escherichia coli.*

Probiotics

Probiotics are live microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, l989). The most common probiotic micro-organisms are: a) Lactobacilli, i.e. *L. acidophilus*, *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, *L. reuteri*, *L. brevis*, *L. cellobiosus*, *L. curvatus*, *L. fermentum*, *L. plantarum;* b) Gram-positive cocci, i.e. *Lactococcus lactis* subsp. *cremonis*, *Streptococcus salivarius* subsp. *thermophilus*, *Enterococcus faecium*, *Staphylococcus diaacecetylactis*, *S. intermedius*; c) Bifidobacteria, i.e. *B. bifidum*, *B. adolescentis*, *B. animalis*, *B. infantis*, *B. longum*, *B. thermophilum* (Collins & Gibson, 1999).

Probiotic supplementation in infant formulae has shown that some strains may persist in the infant gut (Bennet *et al.* 1992; Millar *et al.* 1993; Langhendries *et al.* 1995) and lower stool pH (Langhendries *et al.* 1995). For *Lactobacillus*, there was no evidence that administration had any positive clinical benefit on a group of premature infants since the faecal reservoir of potential nosocomial pathogens was not reduced (Millar *et al.* 1993). The application of two non-pathogenic and antibiotic-susceptible *E. coli* strains to premature infants was successful in significantly reducing their colonisation by antibiotic-resistant enteropathogens through colonisation antagonistic abilities (Lari *et al.* 1990). Supplementation of *Lactobacillus GG* (Isolauri *et al.* 1991) and *Bifidobacterium bifidum* with *Streptococcus thermophilus* (Saavedra *et al.* 1994) was successful in treatment and prevention of rotavirus diarrhoea in children and infants respectively. Treatment and prevention of rotavirus-induced diarrhoea is possibly one of the best-documented health effects of probiotics (Salminen *et al.* 1998*b*). In a recent study with rats it was shown that *Bifidobacterium infantis* supplementation resulted in intestinal colonisation and a significant reduction in the incidence of necrotising enterocolitis comparing controls with *E. coli*-treated animals (Caplan *et al.* 1999).

Inhibition of the *in vitro* adhesion of enteropathogenic *E. coli* to HT-29 epithelial cells by *Lactobacillus plantarum* 299v and *Lactobacillus* GG is thought to be mediated through the ability of the above probiotics to increase expression of MuC2 and MuC3 intestinal mucins (Mack *et al.* 1999). It has been suggested that the increased intestinal mucin production could prevent the attachment of enteropathogens through steric hindrance or greater competitive inhibition for attachment sites on mucins (Mack *et al.* 1999).

Careful assessment is also needed in the case of

immunocompromised individuals or those under antibiotic treatment as probiotic supplementation could possibly create complications (Pletincx *et al.* 1995).

Probiotics need to endure a range of physicochemical factors during transit through the stomach (for example, acid, pepsin) and small intestine (for example, proteolytic enzymes, lysozyme bile salts) in order to survive. Less than 10 % of the administered probiotic *Lactobacillus* and *Bifidobacterium* species survived during a study simulating *in vitro* upper gastrointestinal tract transit (Charteris *et al.* 1998). However, it was suggested that the presence of milk proteins markedly improved gastric transit tolerance up to 100 % and that the presence of mucin and milk proteins exerted a protective effect during upper gastrointestinal transit (Charteris *et al.* 1998).

The ability of probiotics to persist in the gut will partly depend on their binding to enterocytes and intestinal mucus. The *in vitro* ability to adhere to intestinal mucus isolated from human faeces depended mainly on the probiotic strain used (Ouwehand *et al.* 1999) and also the donor age, with infant mucus supporting lower attachment (Kirjavainen *et al.* 1998).

Prebiotics

Prebiotics have been defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health (Gibson & Roberfroid, 1995). Prebiotics should reduce harmful putative bacteria such as coliforms and clostridia and increase lactic acid-producing bacteria such as bifidobacteria and lactobacilli. Among the most common prebiotics are fructo-oligosaccharides, galacto-oligosaccharides and lactulose (Collins & Gibson, 1999). There are a number of studies supporting beneficial effects on the adult human intestinal microflora of fructo-oligosaccharides (Gibson *et al.* 1995; Bouhnik *et al.* 1999), galacto-oligosaccharides (Tanaka *et al.* 1983; Bouhnik *et al.* 1997; Sako *et al.* 1999) lactulose (Kawase *et al.* 1983; Ballongue *et al.* 1997; Salminen & Salminen, 1997) and isomalto-oligosaccharides (Kohmoto *et al.* 1991; Kaneko *et al.* 1994).

It is likely that inclusion of such dietary prebiotic components in moderate amounts may benefit formula-fed infants by establishing an intestinal flora with more bifidobacteria and fewer coliforms, clostridia and bacteroides. The carbohydrate could be added in addition to the existing lactose concentrations since formulae do not contain HMO. In this way, the prebiotic could also contribute positively towards host energy as a result of its metabolism by the intestinal microflora. However, it needs to be considered that the infant faecal flora appears not to have a similar fermentation capacity for oligosaccharides and complex carbohydrates compared with the adult (Parrett & Edwards, 1997; Parrett *et al.* 1997), implying that very careful assessment is needed to prevent carbohydrate overload of the intestine. Intestinal overload could result in undesirable gastrointestinal symptoms such as diarrhoea (Cummings *et al.* 2001; Livesey, 2001; Marteau & Flourie, 2001).

Synbiotics

A combined approach would be that of a synbiotic ((i.e. probiotic(s) mixed with prebiotic(s)). The combination could enhance the survival of the probiotic micro-organism as its specific substrate is readily available for fermentation. It could be expected that the prebiotic substrate could confer protection to the probiotic organism during transit though the upper gastrointestinal tract, by protecting it against gastric acidity (protection effectiveness dependent on the prebiotic's sugar constituents and type of moieties linkage) and proteolytic attacks from gastric and pancreatic proteases most likely through mechanisms of coating the surface of probiotic micro-organism and steric hindrance, but this remains to be investigated. Similar protective effect of milk proteins and mucin on probiotics has been reported by Charteris *et al.* (1998). Examples of synbiotics include bifidobacteria combined with fructo-oligosaccharides, lactobacilli combined with lactitol and bifidobacteria combined with galacto-oligosaccharides. An overview of the concept has been given by Gibson & Roberfroid (1995) and Collins & Gibson (1999).

Conclusions

The gut microflora of breast-fed infants and formula-fed infants differs, with formula-fed infants having a complex microflora with facultative anaerobes, bacteroides and clostridia at higher levels and frequency than in breast-fed infants. It might be that the gut microflora confers protection to infections and disease since there is now evidence that nutrition and the indigenous microbiota may influence immune response of the gastrointestinal tract and therefore host defence (Cunningham-Rundles & Lin, 1998). There exists a great deal of potential for modulating the gastrointestinal microflora of infants using dietary components and micro-organisms. Supplementation of infant formulae with one and/or combinations of functional food ingredients may promote improved long-term health and development of the neonatal gut. It could also contribute towards disease-preventative and therapeutic characteristics of commercial products. Carefully balanced experiments both *in vitro* and *in vivo* are needed to critically examine the potential of these components in infant nutrition. These should be consolidated through the use of up-to-date methodologies such as culture-independent molecular analyses that can provide more specific and sensitive means of identifying, quantifying and understanding gut microbial ecology. The field is still in its infancy and it has to grow with care.

References

Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R & Stahl DA (1990*a*) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Applied and Environmental Microbiology* **56**, 1919–1925.

Amann RI, Krumholz L & Stahl DA (1990*b*) Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic and environmental studies in microbiology. *Journal of Bacteriology* **172**, 762–770.

Anqerer LM, Stoler MH & Anqerer RC (1987) In situ hybridisation. In *Applications to Nemobiology*, pp. 42–70 [K Valentino, JH Eberwine and JD Barchas, editors]. Oxford: Oxford University Press.

Ballongue J, Schumann C & Ouignon P (1997) Effects of lactulose and lactitol on colonic microflora and enzymatic activity. *Scandinavian Journal of Gastroenterology* **32**, 41–44.

Balmer SE, Hanvey LS & Wharton BA (1994) Diet and faecal flora in the newborn: nucleotides. *Archives of Disease in Childhood* **70**, Fl37–Fl40.

Bennet R & Nord CE (1987) Development of the fecal anaerobic microflora after Caesarean section and treatment with antibiotics in newborn infants. *Infection* **15**, 332–336.

Bennet R, Nord CE & Zetterstrom R (1992) Transient colonisation of the gut of newborn infants by orally administered bifidobacteria and lactobacilli. *Acta Paediatrica* **81**, 784–787.

Benno Y, Sawada K & Mitsuoka T (1984) The intestinal microflora of infants: composition of faecal flora in breast-fed and bottle-fed infants. *Microbiology and Immunology* **28**, 975–986.

Berg RD (1996) The indigenous gastrointestinal microflora. *Trends in Microbiology* **4**, 430–435.

Beritzoglou E (1997) The intestinal microflora during the first weeks of life. *Anaerobe* **3**, 173–177.

Beritzoglou E, Romond MB & Romond C (1989) Modulation of *Clostridium perfringens* intestinal colonisation in infants delivered by Caesarean section. *Infection* **17**, 232–236.

Bouhnik Y, Flourie B, D'Agay-Abensour L, Pochart P, Gramet G, Durand M & Rambaud JC (1997) Administration of transgalacto-oligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. *Journal of Nutrition* **127**, 444–448.

Bouhnik Y, Vahedi K, Achour L, Attar A, Salfati J, Pochart P, Marteau P, Flourie B, Bornet F & Rambaud JC (1999) Short-chain fructo-oligosaccharide administration dose-dependently increases fecal bifidobacteria in healthy humans. *Journal of Nutrition* **129**, 113–116.

Bourquin LD, Titgemeyer EC & Fahey GC (1996) Fermentation of various dietary fiber sources by human fecal bacteria. *Nutrition Research* **16**, 1119–1131.

Boza J (1998) Nucleotides in infant nutrition. *Monatsschrift Inderheilkunde* **146**, S39–S48.

Brand-Miller J, McVeagh P, McNeil Y & Messer M (1998) Digestion of human milk oligosaccharides by healthy infants evaluated by the lactulose hydrogen breath test. *Journal of Pediatrics* **133**, 95–98.

Brook I, Barrett C, Brinkman CR, Martin WJ & Finegold SM (1979) Aerobic and anaerobic bacterial flora of the maternal cervix and newborn gastric fluid and conjunctiva: a prospective study. *Pediatrics* **63**, 451–455.

Bullen CL, Tearle PV & Stewart MG (1977) The effect of "humanized" milks and supplemented breast feeding on the faecal flora of infants. *Journal of Medical Microbiology* **10**, 403–413.

Caplan MS, Miller-Catchpole R, Kaup S, Russell T, Lickerman M, Amer M, Xiao Y & Thomson R (1999) Bifidobacterial supplementation reduces the incidence of necrotising enterocolitis in a neonatal rat model. *Gastroenterology* **117**, 577–583.

Cebra JJ (1999) Influences of microbiota on intestinal immune system development. *American Journal of Clinical Nutrition* **69**, 1046S–1051S.

Charteris WP, Kelly PM, Morelli L & Collins JK (1998) Development and application of an *in vitro* methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in the upper human gastrointestinal tract. *Journal of Applied Microbiology* **84**, 759–768.

Christian M, Edwards C & Weaver L (1999) Starch digestion in infancy. *Journal of Pediatic Gastroenterology and Nutrition* **29**, 116–124.

Collins MD & Gibson GR (1999) Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *American Journal of Clinical Nutrition* **69**, 1052S–1057S.

Coppa GV, Gabrielli O, Pierani P, Catassi C, Carlucci A & Giorgi PL (1993) Changes in carbohydrate composition in human milk over 4 months of lactation. *Pediatrics* **91**, 637–641.

Cosgrove M (1998) Nucleotides. *Nutrition* **14**, 748–751.

Cummings J, Lee A & Storey D (2001) Workshop: physiology and tolerance of LDCs. *British Journal of Nutrition* **85**, S59–S60.

Cummings JH & Macfarlane GT (1991) The control and consequences of bacterial fermentation in the human colon. *Journal of Applied Bacteriology* **70**, 443–459.

Cunningham-Rundles S & Lin DHL (1998) Nutrition and the immune system of the gut. *Nutrition* **14**, 573–579.

Cuthbertson WJF (1999) Evolution of infant nutrition. *British Journal of Nutrition* **81**, 359–371.

De Bruin NC, Degenhart HJ, Gal S, Westerterp KR, Stijnen T & Visser HKA (1998) Energy utilisation and growth in breast-fed and formula-fed infants measured prospectively during the first year of life. *American Journal of Clinical Nutrition* **67**, 885–896.

Engfer MB, Stahl B, Finke B, Sawatzki G & Daniel H (2000) Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract. *American Journal of Clinical Nutrition* **71**, 1589–1596.

Franks AH, Harmsen HJM, Raangs GC, Jansen GJ, Schut F & Welling GW (1998) Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Applied and Environmental Microbiology* **64**, 3336–3345.

Fuller R (1989) Probiotics in man and animals. *Journal of Applied Bacteriology* **66**, 365–378.

Garofalo RP & Goldman AS (1999) Expression of functional immunomodulatory and anti-inflammatory factors in human milk. *Clinics in Perinatology* **26**, 361–377.

Gibson GR, Beatty ER, Wang X & Cummings JH (1995) Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* **108**, 975–982.

Gibson GR & Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition* **125**, 1401–1412.

Gil A, Corral E, Martinez-Valverde A & Molina JA (1986) Effects of the addition of nucleotides to an adapted milk formula on the microbial pattern of feces in at term newborn infants. *Journal of Clinical Nutrition and Gastroenterology* **1**, 127–132.

Gnoth MJ, Kunz C, Kinne-Saffran E & Rudloff S (2000) Human milk oligosaccharides are minimally digested in vitro. *Journal of Nutrition* **130**, 3014–3020.

Goldman AS, Chheda S & Garofalo R (1997) Spectrum of immunomodulating agents in human milk. *International Journal of Pediatric Hematology/Oncology* **4**, 491–497.

Gronlund MM, Lehtonen OP, Eerola E & Kero P (1999*a*) Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. *Journal of Pediatic Gastroenterology and Nutrition* **28**, 19–25.

Gronlund MM, Salminen S, Mykkanen H, Kero P & Lehtonen OP (1999*b*) Development of intestinal bacterial enzymes in infants – relationship to mode of delivery and type of feeding. *Acta Pathologica, Microbiologica et Immunologica Scandinavica* **107**, 655–660.

Gudmand-Hoyer E & Skovbjerg H (1996) Disaccharide digestion and maldigestion. *Scandinavian Journal of Gastroenterology* **31**, 111–121.

Gyorgy P, Jeanloz RW, von Nicolai H & Zilliken F (1974) Undialyzable factors for *Lactobacillus bifidus* var *pennsylvanicus*: protective effects of sialic acid bound to glycoproteins and oligosaccharides against bacterial degradation. *European Journal of Biochemistry* **43**, 29–33.

Gyorgy P, Norris RF & Rose CS (1954) Bifidus factor I. A variant of *Lactobacillus bifidus* requiring a special growth factor. *Archives of Biochemistry and Biophysics* **48**, 193–201.

Hague A, Elder DJE, Hicks DJ & Paraskeva C (1995) Apoptosis in colorectal tumour cells: induction by the short chain fatty acids butyrate, propionate and acetate and by the bile salt deoxycholate. *International Journal of Cancer* **60**, 400–406.

Hammann R (1982) A reassessment of the microbial flora of the female genital tract, with special reference to the occurrence of *Bacteroides* species. *Journal of Medical Microbiology* **15**, 293–302.

Hamosh M (1983) Lingual lipase and fat digestion in the neonatal period. *Journal of Pediatic Gastroenterology and Nutrition* **2**, S236–S241.

Hamosh M (1997) Introduction: Should infant formulas be supplemented with bioactive components and conditionally essential nutrients present in human milk. *Journal of Nutrition* **127**, 971S–974S.

Harmsen HJM, Wildeboer ACM, Raangs GC, Wagendorp AA, Klijn N, Bindels JC & Welling GW (2000) Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *Journal of Pediatic Gastroenterology and Nutrition* **30**, 61–67.

Hartemink R & Rombouts FM (1999) Comparison of media for the detection of bifidobacteria, lactobacilli and total anaerobes from faecal samples. *Journal of Microbiological Methods* **36**, 181–192.

Heavey PM & Rowland IR (1999) The gut microflora of the developing infant: microbiology and metabolism. *Microbial Ecology in Health and Disease* **11**, 75–83.

Heine WE, Klein PD & Reeds PJ (1991) The importance of alpha-lactalbumin in infant nutrition. *Journal of Nutrition* **131**, 277–283.

Hernell O & Blackberg L (1983) Bile-salt-stimulated lipase of human milk and lipid digestion in the neonatal period. *Journal of Pediatic Gastroenterology and Nutrition* **2**, S242–S247.

Holdeman LV, Cato EP & Moore WEC (1977) *Anaerobic Laboratory Manual*, 4th ed. Blacksburg: Virginia Polytechnic and State University.

Holtmann G, Kelly DG, Sterbby B & DiMagno EP (1997) Survival of human pancreatic enzymes during small bowel transit: effect of nutrients, bile acids, and enzymes. *American Journal of Physiology* **273**, G553–G558.

Isolauri E, Juntunen M, Rautanen T, Sillanaukee P & Koivula T (1991) A human *Lactobacillus* strain (*Lactobacillus casei* sp strain *GG*) promotes recovery from acute diarrhea in children. *Pediatrics* **88**, 90–97.

Jukes DJ (1997) Infant formula and follow-on formula. In *Food Legislation of the UK. A Concise Guide*, pp. 42–47. Oxford: Butterworth Heinemann.

Kaneko T, Kohmoto T, Kikuchi H, Shiota M, Iino H & Mitsuoka T (1994) Effects of isomaltooligosaccharides with different degrees of polymerisation on human fecal bifidobacteria. *Bioscience Biotechnology and Biochemistry* **58**, 2288–2290.

Kawase K, Suzuki T, Kiyosawa I, Okonogi S, Kawashima T & Kuboyama M (1983) Effects of composition of infant formulas on the intestinal microflora of infants. *Bifidobacteria Microflora* **2**, 25–31.

Kien CL, McClead RE & Cordero L Jr (1996) In vivo lactose digestion in preterm infants. *American Journal of Clinical Nutrition* **64**, 700–705.

Kim WJ (1993) Bacteriocins of lactic acid bacteria: their potential as food biopreservative. *Food Reviews International* **9**, 299–313.

Kirjavainen PV, Ouwehand AC, Isolauri E & Salminen SJ (1998) The ability of probiotic bacteria to bind to human intestinal mucus. *FEMS Microbiology Letters* **167**, 185–189.

Kleessen B, Bunke H, Tovar K, Noack J & Sawatzki G (1995) Influence of two infant formulas and human milk on the development of the faecal flora in newborn infants. *Acta Paediatrica* **84**, 1347–1356.

Kohmoto T, Fukui F, Takaku H & Mitsuoka T (1991) Dose-response test of isomaltooligosaccharides for increasing fecal bifidobacteria. *Agricultural and Biological Chemistry* **55**, 2157–2159.

Kok RG, De Waal A, Schut F, Welling GW, Weenk G & Hellingwerf KJ (1996) Specific detection and analysis of a probiotic *Bifidobacterium* strain in infant feces. *Applied and Environmental Microbiology* **62**, 3668–3672.

Kunz C (1998) Complex oligosaccharides in infant nutrition. *Monatsschrift Inderheilkunde* **146**, S49–S56.

Kunz C & Rudloff S (1993) Biological functions of oligosaccharides in human milk. *Acta Paediatrica* **82**, 903–912.

Lahov E & Regelson W (1996) Antibacterial and immunostimulating casein-derived substances from milk: casecidin, isracidin peptides. *Food and Chemical Toxicology* **34**, 131–145.

Lambert EJ & Hall MA (1995) Infant nutrition part 1: preweaning (0–4 months). *British Journal of Hospital Medicine* **53**, 567–569.

Langendijk PS, Schut F, Jansen GJ, Raangs GC, Kamphuis GR, Wilkinson MHF & Welling GW (1995) Quantitative in situ hybridisation of *Bifidobacterium* spp. with genus-specific 16S-rRNA-targeted probes and its application in fecal samples. *Applied and Environmental Microbiology* **61**, 3069–3075.

Langhendries JP, Detry J, Van Hees J, Lamboray JM, Darimont J, Mozin MJ, Secretin MC & Senterre J (1995) Effect of a fermented infant formula containing viable bifidobacteria on the fecal flora composition and pH of healthy full-term infants. *Journal of Pediatric Gastroenterology and Nutrition* **21**, 177–181.

Lari AR, Gold F, Borderon JC, Laugier J & Lafont JP (1990) Implantation and in vivo antagonistic effects of antibiotic-susceptible *Escherichia coli* strains administered to premature newborns. *Biology of the Neonate* **58**, 73–78.

Lebenthal A & Lebenthal E (1999) The ontogeny of the small intestinal epithelium. *Journal of Parenteral and Enteral Nutrition* **23**, S3–S6.

Lee PC (1983) Digestibility of starches and modified food starches. *Journal of Pediatric Gastroenterology and Nutrition* **2**, S227–S232.

Lentze MJ & Sterchi EE (1983) Hydrolysis of proteins and proteolytic activity. *Journal of Pediatic Gastroenterology and Nutrition* **2**, S252–S259.

Levin RJ (1994) Digestion and absorption of carbohydrates – from molecules and membranes to humans. *American Journal of Clinical Nutrition* **59**, 690S–698S.

Levy J (1998) Immunonutrition: the pediatric experience. *Nutrition* **14**, 641–647.

Lifschitz CH, Smith EO & Garza C (1983) Delayed complete functional lactase sufficiency in breast-fed infants. *Journal of Pediatic Gastroenterology and Nutrition* **2**, 478–482.

Lifschitz CH, Wolin MJ & Reeds PJ (1990) Characterisation of carbohydrate fermentation in feces of formula-fed and breast-fed infants. *Pediatric Research* **27**, 165–169.

Livesey G (2001) Tolerance of low-digestible carbohydrates: a general view. *British Journal of Nutrition* **85**, S7–S16.

Lopez-Alarcon M, Villalpando S & Fajardo A (1997) Breast-feeding lowers the frequency and duration of acute respiratory infection and diarrhea in infants under six months of age. *Journal of Nutrition* **127**, 436–443.

Lundequist B, Nord CE & Winberg J (1985) The composition of the faecal microflora of breastfed and bottle fed infants from birth to eight weeks. *Acta Paediatrica Scandinavica* **74**, 45–51.

Macfarlane GT, Allison C, Gibson SAW & Cummings JH (1988) Contribution of the microflora on the proteolyis in the human large intestine. *Journal of Applied Bacteriology* **64**, 37–46.

Macfarlane GT & McBain AJ (1999) The human colonic microbiota. In *Colonic Microbiota, Nutrition and Health*, pp. 3–28 [GR Gibson and MB Roberfroid, editors]. Holland: Kluwer Scientific Publishers.

Macfarlane GT, Macfarlane S & Gibson GR (1998) Validation of a three-stage continuous culture system for investigating the effect of retention time on the ecology and metabolism of bacteria in the human colon. *Microbial Ecology* **35**, 180–187.

Mack DR, Michail S, Wei S, McDougall L & Hollingsworth MA (1999) Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. *American Journal of Physiology* **276**, G941–G950.

Mackie RI, Sghir A & Gaskins HR (1999) Developmental microbial ecology on the neonatal gastrointestinal tract. *American Journal of Clinical Nutrition* **69**, 1035S–1045S.

Marteau P & Flourie B (2001) Tolerance to low-digestible carbohydrates: symptomatology and methods. *British Journal of Nutrition* **85**, S17–S21.

Mata LJ, Carrillo C & Villatoro E (1969) Fecal microflora in healthy persons in a preindustrial region. *Applied Microbiology* **17**, 596–602.

Mevissen-Verhage EAE, Marcelis JH, De Vos MN, Harmsen-Van Amerongen WCM & Verhoef J (1987) *Bifidobacterium*, *Bacteroides*, and *Clostridium* spp. in fecal samples from breast-fed and bottle-fed infants with and without iron supplement. *Journal of Clinical Microbiology* **25**, 285–289.

Midtvedt AC & Midtvedt T (1992) Production of short chain fatty acids by the intestinal microflora during the first 2 years of human life. *Journal of Pediatric Gastroenterology and Nutrition* **15**, 395–403.

Millar MR, Bacon C, Smith SL, Walker V & Hall MA (1993) Enteral feeding of premature infants with *Lactobacillus* GG. *Archives of Disease in Childhood* **69**, 483–487.

Millar MR, Linton CJ, Cade A, Glancy D, Hall M & Jalal H (1996) Application of 16S rRNA gene PCR to study bowel flora of preterm infants with and without necrotising enterocolitis. *Journal of Clinical Microbiology* **34**, 2506–2510.

Moore WEC, Cato EP & Holdeman LV (1978) Some current concepts in intestinal bacteriology. *American Journal of Clinical Nutrition* **31**, S33–S42.

Mountzouris KC, Gilmour SG, Grandison AS & Rastall RA (1999) Modelling of oligodextran production in an ultra-filtration stirred-cell membrane reactor. *Enzyme and Microbial Technology* **24**, 75–85.

Mullis K, Faloona F, Scharf S, Saiki R, Horn G & Erlich H (1986) Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. *Cold Harbor Symposia on Quantitative Biology* LI, **263–273.**

Nakhla T, Fu D, Zopf D, Brodsky NL & Hurt H (1999) Neutral oligosaccharide content of preterm human milk. *British Journal of Nutrition* **82**, 361–367.

Newburg DS (1997) Do the binding properties of oligosaccharides in milk protect human infants from gastrointestinal bacteria? *Journal of Nutrition* **127**, 980S–984S.

Norin KE, Gustafsson BE, Lindblad BS & Midtvedt T (1985) The establishment of some microflora associated biochemical characteristics in feces from children during the first years of life. *Acta Paediatrica Scandinavica* **74**, 207–212.

Olano-Martin E, Mountzouris KC, Gibson GR & Rastall RA (2000) *In vitro* fermentability of dextran oligodextran and maltodextrin by human gut bacteria. *British Journal of Nutrition* **83**, 247–255.

O'Sullivan DJ (1999) Methods of analysis of the intestinal microflora. In *Probiotics a Critical Review*, pp. 23–44 [GW Tannock, editor]. Norfolk, England: Horizon Scientific Press.

Ouwehand AC, Kirjavainen PV, Gronlund MM, Isolauri E & Salminen SJ (1999) Adhesion of probiotic micro-organisms to intestinal mucus. *International Dairy Journal* **9**, 623–630.

Ouwehand AC, Salminen SJ, Skurnik M & Conway PL (1997) Inhibition of pathogen adhesion by β-lactoglobulin. *International Dairy Journal* **7**, 685–692.

Pakkanen R & Aalto J (1997) Growth factors and antimicrobial factors of bovine colostrum. *International Dairy Journal* **7**, 285–297.

Parrett AM & Edwards CA (1997) In vitro fermentation of carbohydrate by breast and formula fed infants. *Archives of Disease in Childhood* **76**, 249–253.

Parrett AM, Edwards CA & Lokerse E (1997) Colonic fermentation capacity in vitro: development during weaning in breast-fed infants is slower for complex carbohydrates than for sugars. *American Journal of Clinical Nutrition* **65**, 927–933.

Pellegrini A, Thomas U, Bramaz N, Hunziker P & Von Fellenberg R (1999) Isolation and identification of three bactericidal domains in the bovine alpha-lactalbumin molecule. *Biochimica et Biophysica Acta* **1426**, 439–448.

Peterson JA, Patton S & Hamosh M (1998) Glycoproteins of the human milk fat globule in the protection of the breast fed infant against infections. *Biology of the Neonate* **74**, 143–162.

Petschow BW, Talbott RD & Batema RP (1999) Ability of lactoferrin to promote the growth of *Bifidobacterium* spp. in vitro is independent of receptor binding capacity iron saturation level. *Journal of Medical Microbiology* **48**, 541–549.

Pihlanto-Leppala A, Marnila P, Hubert L, Rokka T, Korhonen HJT & Karp M (1999) The effect of α-lactalbumin and β-lactoglobulin hydrolysates on the metabolic activity of *Escherichia coli* JM 103. *Journal of Applied Microbiology* **87**, 540–545.

Pletincx M, Legein J & Vandenplas Y (1995) Fungemia with *Saccharomyces boulardii* in a 1-year-old girl with protracted diarrhea. *Journal of Pediatic Gastroenterology and Nutrition* **21**, 113–115.

Rings EHHM, Van Beers EH, Krasinski SD, Verhave M, Montgomery RK, Grand RJ, Dekker J & Buller HA (1994) Lactase; origin, gene expression, localisation, and function. *Nutrition Research* **14**, 775–797.

Rose SJ (1984) Bacterial flora of breast-fed infants. *Pediatrics* **74**, 563.

Saavedra JM, Bauman NA, Oung I, Perman JA & Yolken RH (1994) Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *Lancet* **344**, 1046–1049.

Saito H, Miyakawa H, Tamura Y, Shimamura S & Tomita M (1991) Potent bactericidal activity of bovine lactoferrin hydrolysate produced by heat treatment at acidic pH. *Journal of Dairy Science* **74**, 3724–3730.

Sako T, Matsumoto K & Tanaka R (1999) Recent progress on research and applications of non-digestible galacto-oligosaccharides. *International Dairy Journal* **9**, 69–80.

Salminen S, Bouley C, Boutron-Ruault MC, Cummings JH, Franck A, Gibson GR, Isolauri E, Moreau MC, Roberfroid M & Rowland IR (1998*a*) Functional food science and gastrointestinal physiology and function. *British Journal of Nutrition* **80**, S147–S171.

Salminen S, Ouwehand AC & Isolauri E (1998*b*) Clinical applications of probiotic bacteria. *International Dairy Journal* **8**, 563–572.
Salminen S & Salminen E (1997) Lactulose, lactic acid bacteria, intestinal microbiology and mucosal protection. *Scandinavian Journal of Gastroenterology* **32**, 45–48.
Schanbacher FL, Talhouk RS, Murray FA, Gherman LI & Willett LB (1998) Milk-borne bioactive peptides. *International Dairy Journal* **8**, 393–403.
Schleifer KH, Ludwig W & Amann R (1993) Nucleic acid probes. In *Handbook of New Bacterial Systematics*, pp. 463–510 [M Goodfellow and AG O'Donnell, editors]. London: Academic Press.
Schlimme E, Martin D & Meisel H (2000) Nucleosides and nucleotides: natural bioactive substances in milk and colostrum. *British Journal of Nutrition* **84**, S59–S68.
Schwertmann A, Rudloff S & Kunz C (1996) Potential ligands for cell adhesion molecules in human milk. *Annals of Nutrition & Metabolism* **40**, 252–262.
Sievers E, Oldigs HD, Schulz-Lell G & Schaub J (1993) Fecal excretion in infants. *European Journal of Pediatrics* **152**, 452–454.
Siigur U, Ormisson A & Tamm A (1993) Faecal short-chain fatty acids in breast fed and bottle-fed infants. *Acta Paediatrica* **82**, 536–538.
Silvi S, Rumney CJ & Rowland IR (1996) An assessment of three selective media for bifidobacteria in faeces. *Journal of Applied Bacteriology* **81**, 561–564.
Simhon A, Douglas JR, Drasar BS & Soothill JF (1982) Effect of feeding on infants' faecal flora. *Archives of Disease in Childhood* **57**, 54–58.
Singh B, Halestrap AP & Paraskeva C (1997) Butyrate can act as a stimulator of growth or inducer of apoptosis in human colonic epithelial cell lines depending on the presence of alternative energy sources. *Carcinogenesis* **18**, 1265–1270.
Stark PL & Lee A (1982) The microbial ecology of the large bowel of breast-fed and formula-fed infants during the first year of life. *Journal of Medical Microbiology* **15**, 189–203.
Suau A, Bonnet R, Sutren M, Godon JJ, Gibson GR, Collins MD & Dore J (1999) Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Applied and Environmental Microbiology* **65**, 4799–4807.
Tagg JR, Dajani AS & Wannamaker LW (1976) Bacteriocins of Gram-positive bacteria. *Bacteriological Reviews* **40**, 722–756.
Tamura Z (1983) Nutriology of Bifidobacteria. *Bifidobacteria Microflora* **2**, 3–16.
Tanaka R, Takayama H, Morotomi M, Kuroshima T, Ueyama S, Matsumoto K, Kuroda A & Mutai M (1983) Effects of administration of TOS and *Bifidobacterium breve* 4006 on the human fecal flora. *Bifidobacteria Microflora* **2**, 17–24.
Tannock GW, Fuller R, Smith SL & Hall MA (1990) Plasmid profiling of members of the family *Enterobacteriaceae*, lactobacilli and bifidobacteria to study the transmission of bacteria from mother to infant. *Journal of Clinical Microbiology* **28**, 1225–1228.
Treem WR (1995) Congenital sucrase-isomaltase deficiency. *Journal of Pediatric Gastroenterology and Nutrition* **21**, 1–14.
Vanderhoof JA (1998) Immunonutrition: the role of carbohydrates. *Nutrition* **14**, 595–598.
Van Hoijdonk ACM, Kussendrager KD & Steijns JM (2000) *In vivo* antimicrobial and antiviral activity of components in bovine milk and colostrum involved in non-specific defence. *British Journal of Nutrition* **84**, S127–S134.
Wachtershauser A & Stein J (2000) Rationale for the luminal provision of butyrate in intestinal diseases. *European Journal of Nutrition* **39**, 164–171.
Wang RF, Cao WW & Cerniglia CE (1996) PCR detection and quantification of predominant anaerobic bacteria in human and animal fecal samples. *Applied and Environmental Microbiology* **62**, 1242–1247.
Wang X & Gibson GR (1993) Effects of the *in vitro* fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *Journal of Applied Bacteriology* **75**, 373–380.
Wharton BA, Balmer SE, Berger HM & Scott PH (1994) Protein nutrition, fecal flora and iron-metabolism – the role of milk-based formulae. *Acta Paediatrica* **83**, 24–30.
Yildirim Z & Johnson MG (1998) Characterisation and antimicrobial spectrum of bifidocin B, a bacteriocin produced by *Bifidobacterium bifidum* NCFB 1454. *Journal of Food Protection* **61**, 47–51.
Yoshioka H, Fujita K, Sakata H, Murono K & Iseki K (1991) Development of the normal intestinal flora and its clinical significance in infants and children. *Bifidobacteria Microflora* **10**, 11–17.
Yuhara T, Isojima S, Tsuchiya F & Mitsuoka T (1983) On the intestinal flora of bottle-fed infants. *Bifidobacteria Microflora* **2**, 33–39.
Zucht HD, Raida M, Aderman K, Magert HJ & Forssmann WG (1995) Casocidin-I: a casein-α_{s2} derived peptide exhibits antibacterial activity. *FEBS Letters* **372**, 185–188.