Introduction

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

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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 slgA, 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. (1997) 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, 1997; 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...
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. 1998a).

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. 1999a), 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. faecalis 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. infants); 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. salivarius, 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

<table>
<thead>
<tr>
<th>Research group</th>
<th>Feeding mode†</th>
<th>n</th>
<th>Bifidobacteria</th>
<th>Lactobacilli</th>
<th>Bacteroides</th>
<th>Clostridia</th>
<th>Coliforms</th>
<th>Enterobacteria</th>
<th>Streptococci</th>
<th>Staphylococci</th>
<th>Enterococci</th>
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</thead>
<tbody>
<tr>
<td>Bullen et al. (1977)</td>
<td>BF</td>
<td>13</td>
<td>10.3</td>
<td>ND</td>
<td>7.2</td>
<td>3.2</td>
<td>8.8</td>
<td>ND</td>
<td>7.2</td>
<td>ND</td>
<td>ND</td>
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<td>FFa</td>
<td>9</td>
<td>9.5</td>
<td>ND</td>
<td>9.5</td>
<td>7.1</td>
<td>9.5</td>
<td>ND</td>
<td>9.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>FFb</td>
<td>10</td>
<td>7.4</td>
<td>ND</td>
<td>7.6</td>
<td>6.6</td>
<td>9.3</td>
<td>ND</td>
<td>8.6</td>
<td>ND</td>
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<tr>
<td>Stark &amp; Lee (1982)</td>
<td>BF</td>
<td>6</td>
<td>10.6 100</td>
<td>NS</td>
<td>&lt;3.0 100</td>
<td>&lt;3.0 100</td>
<td>ND</td>
<td>6.1 100</td>
<td>ND</td>
<td>ND</td>
<td>6.3 100</td>
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<tr>
<td>FFa</td>
<td>6</td>
<td>10.3 100</td>
<td>NS</td>
<td>9.3 100</td>
<td>6.4 100</td>
<td>ND</td>
<td>9.4 100</td>
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<td>FFb</td>
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<tr>
<td>Yoshioka et al. (1991)</td>
<td>BF</td>
<td>6</td>
<td>10.8</td>
<td>6.6</td>
<td>7.7</td>
<td>ND</td>
<td>ND</td>
<td>8.3</td>
<td>5.7</td>
<td>6.2</td>
<td>ND</td>
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<tr>
<td>FFa</td>
<td>6</td>
<td>10.3</td>
<td>7.0</td>
<td>9.8</td>
<td>ND</td>
<td>ND</td>
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<td>9.0</td>
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<td>FFb</td>
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<tr>
<td>Balmer et al. (1994)*</td>
<td>BF</td>
<td>12</td>
<td>9.0 67</td>
<td>&lt;4.0 50</td>
<td>&lt;4.0 33</td>
<td>6.0 67</td>
<td>8.2 75</td>
<td>ND</td>
<td>6.6 75</td>
<td>&lt;4.0 42</td>
<td></td>
</tr>
<tr>
<td>FFa</td>
<td>26</td>
<td>7.0 65</td>
<td>&lt;4.0 42</td>
<td>9.3 65</td>
<td>&lt;4.0 46</td>
<td>8.5 85</td>
<td>ND</td>
<td>&lt;4.0 15</td>
<td>9.0 77</td>
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<tr>
<td>FFb</td>
<td>25</td>
<td>&lt;4.0 44</td>
<td>&lt;4.0 42</td>
<td>9.0 60</td>
<td>4.0 52</td>
<td>8.0 72</td>
<td>ND</td>
<td>&lt;4.0 16</td>
<td>9.0 96</td>
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<td>Langhendries et al. (1995)</td>
<td>BF</td>
<td>14</td>
<td>8.6 67</td>
<td>ND</td>
<td>10.1 7</td>
<td>NS</td>
<td>7</td>
<td>7.6 79</td>
<td>7.0 29</td>
<td>7.2 21</td>
<td>6.6‡ 36</td>
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<tr>
<td>FFa</td>
<td>20</td>
<td>8.1 60</td>
<td>ND</td>
<td>9.4 15</td>
<td>NS</td>
<td>30</td>
<td>7.4 85</td>
<td>7.4 30</td>
<td>7.7 85</td>
<td>7.3‡ 55</td>
<td>ND</td>
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<tr>
<td>FFb</td>
<td>20</td>
<td>9.8 20</td>
<td>ND</td>
<td>10.2 35</td>
<td>10.7 5</td>
<td>7.8 65</td>
<td>7.5 20</td>
<td>7.8 75</td>
<td>6.4‡ 50</td>
<td>ND</td>
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<tr>
<td>Kleeessen et al. (1995)</td>
<td>BF</td>
<td>20</td>
<td>10.2 95</td>
<td>8.4 85</td>
<td>6.6 80</td>
<td>4.6 45</td>
<td>7.8 95</td>
<td>ND</td>
<td>6.3 95</td>
<td>6.4 100</td>
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<tr>
<td>FFa</td>
<td>10</td>
<td>9.8 90</td>
<td>9.0 80</td>
<td>7.4 50</td>
<td>6.5 60</td>
<td>7.4 100</td>
<td>ND</td>
<td>5.6 90</td>
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<tr>
<td>FFb</td>
<td>10</td>
<td>10.2 89</td>
<td>8.4 56</td>
<td>7.0 89</td>
<td>6.4 89</td>
<td>ND</td>
<td>8.5 100</td>
<td>ND</td>
<td>5.6 78</td>
<td>8.6 89</td>
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<tr>
<td>Gronlund et al. (1999a)*</td>
<td>BF</td>
<td>34</td>
<td>10.8 88</td>
<td>8.3 29</td>
<td>9.4 97</td>
<td>7.4 15</td>
<td>9.6 97</td>
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<td>FFa</td>
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</table>

cfu, Colony forming units; ND, not determined; –, not supplied.

† 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.

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 44 % 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: 10⁷–10⁸ (SD 0·81) log cfu/g faeces and 10⁶–10⁷ (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 10⁷ (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 1·76) 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. 1983; 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. 1999a). 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 by Caesarean section had higher colonisation rates of Clostridium perfringens than the vaginally delivered (57 and 17 % respectively) at 1 month of age (Gronlund et al. 1999a). 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. 1990a,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 (Angerer 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 denaturing 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 unreocgnised 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.
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 H2, CO2 and CH4 (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. 1998a). 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. 1998a). 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. 1998a); thus any ingredient recommendations for use in infant formulas should be treated with great caution.
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. 1998a).

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. 1998a; 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 Midveldt & Midveldt (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. 1998a; 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 trypsinic 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. 1999b). 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. 1999b).

### 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)

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>BF (n 14) FF (n 9)</td>
<td>58·8§ 132·1§</td>
<td>58·1 72·4</td>
<td>30·3 123·1</td>
<td>123·1</td>
</tr>
<tr>
<td>Total SCFA</td>
<td>58·8§ 132·1§</td>
<td>53·8 52·6</td>
<td>25·9 91·9</td>
<td>73·8</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>44·7 98·9</td>
<td>74·9 52·6</td>
<td>85·5 74·6</td>
<td>59·9</td>
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<tr>
<td>Molar ratio</td>
<td>14·1 33·2</td>
<td>2·9 16·2</td>
<td>2·5 15·6</td>
<td>22·8</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>24·0 25·1</td>
<td>5·0 16·6</td>
<td>8·3 12·7</td>
<td>18·5</td>
</tr>
<tr>
<td>Molar ratio</td>
<td>2·9 3·0</td>
<td>0·4 2·2</td>
<td>0·0 4·4</td>
<td>17·9</td>
</tr>
<tr>
<td>n-Butyric acid</td>
<td>– –</td>
<td>0·7</td>
<td>0·0 3·6</td>
<td>14·6</td>
</tr>
<tr>
<td>Molar ratio</td>
<td>– –</td>
<td>1·0 1·4</td>
<td>2·8 11·2</td>
<td>8·61</td>
</tr>
<tr>
<td>Others ‖</td>
<td>1·7 1·9</td>
<td>9·2 9·1</td>
<td>7·0</td>
<td></td>
</tr>
<tr>
<td>Molar ratio</td>
<td>22·4 18·5</td>
<td>– –</td>
<td>0·0 2·8</td>
<td>–</td>
</tr>
</tbody>
</table>

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†, ‡ Values given are means.
§ Values reported are the average of three treatments.
‡ Values given are medians.
§ 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.

Lactose is the main carbohydrate source in human milk and...
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 lactate 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
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. 1998a). 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, glycose 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

### Table 3. List of mammalian digestive enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastro-intestinal lumen</td>
<td></td>
</tr>
<tr>
<td>Salivary α-amylase</td>
<td>Lee (1983); Christian et al. (1999)</td>
</tr>
<tr>
<td>Lingual lipase</td>
<td>Hamosh (1983); Hernell &amp; Blackberg (1983)</td>
</tr>
<tr>
<td>Pepsin</td>
<td>Lentze &amp; Sterchi (1983)</td>
</tr>
<tr>
<td>Pancreatic proteases</td>
<td>Lentze &amp; Sterchi (1983); Holtmann et al. (1997)</td>
</tr>
<tr>
<td>Pancreatic lipase</td>
<td>Hernell &amp; Blackberg (1983); Hamosh (1983)</td>
</tr>
<tr>
<td>Pancreatic α-amylase</td>
<td>Lee (1983); Holtmann et al. (1997); Christian et al. (1999)</td>
</tr>
<tr>
<td>Enterocyte brush border</td>
<td></td>
</tr>
<tr>
<td>Lactase-phlorizin</td>
<td>Lifschitz et al. (1983); Levin (1994); Gudmand-Hoyer &amp; Skovbjerg (1996); Kien et al. (1996); Lebenthal &amp; Lebenthal (1999)</td>
</tr>
<tr>
<td>Peptide hydrolases</td>
<td>Lentze &amp; Sterchi (1983)</td>
</tr>
<tr>
<td>Mammary origin</td>
<td></td>
</tr>
<tr>
<td>Bile salt stimulated lipase</td>
<td>Hernell &amp; Blackberg (1983); Hamosh (1983)</td>
</tr>
<tr>
<td>α-Amylase</td>
<td>Lee (1983); Christian et al. (1999)</td>
</tr>
</tbody>
</table>
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 mammmary 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 1994; 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 H₂ 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 symbiotics 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; Nakha 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; Engser 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-acetylgalactosamine 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 follow-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).
**Probiotics**

Probiotics are live microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989). 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. 1998b). 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

### Table 4. Antimicrobial and bifidogenic properties of major milk proteins

<table>
<thead>
<tr>
<th>Component</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>αs1</td>
<td>Fragment known as israacin has <em>in vivo</em> antibacterial activity against <em>Staphylococcus aureus</em> and <em>Candida albicans</em></td>
<td>Lahov &amp; Regelson (1996)</td>
</tr>
<tr>
<td>αs2</td>
<td>Peptide α-casomorphin reduces gut motility</td>
<td>Schanbacher et al. (1998)</td>
</tr>
<tr>
<td>β-Casein</td>
<td>Peptide β-casomorphin reduces gut motility. May be implicated in the release of latent and immunoregulatory activities from lactoferrin</td>
<td>Schanbacher et al. (1998)</td>
</tr>
<tr>
<td>κ-Casein</td>
<td>κ-Casein glycomacropeptide supports growth of bifidobacteria in the gut</td>
<td>Schanbacher et al. (1998)</td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>Activity against Gram-positive bacteria</td>
<td>Pellegrini et al. (1999)</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td>Inhibits adhesion of <em>sfa</em> and sfaI steel expressing <em>E. coli</em> to immobilised human ileostomy glycoproteins <em>in vivo</em></td>
<td>Ouwehand et al. (1997)</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>Proliferation of <em>Bifidobacterium infantis, B. breve</em> and <em>B. bifidum</em> <em>in vitro</em></td>
<td>Petschow et al. (1999); Schanbacher et al. (1998)</td>
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<td></td>
<td>Inhibition of growth of Gram-negative and Gram-positive enteropathogenic bacteria</td>
<td>Pakkanen &amp; Aalto (1997)</td>
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<td></td>
<td>Bacteriostatic and bactericidal activity of intact bovine lactoferrin and its hydrolysates (lactoferricin)</td>
<td>Saito et al. (1991); Schanbacher et al. (1998); van Hoijdonk et al. (2000)</td>
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</table>

*E. coli, Escherichia coli.*
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 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 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 & Flourié, 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 through 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.

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