Abstract:

The invention relates to compositions comprising bifidobacteria and galactooligosaccharides and fructooligosaccharides that support the introduction of solid weaning foods in the diet of infants that receive infant milk formula or breast milk.

Title:

A COMPOSITION COMPRISING BIFIDOBACTERIUM INFANTIS AND FRUCTO- AND GALACTO-OLIGOSACCHARIDES FOR THE PREVENTION OF INTESTINAL DISCOMFORT IN INFANTS

Compositions comprising bifidobacteria and galactooligosaccharides and fructooligosaccharides are provided that support the introduction of solid weaning foods in the diet of infants that receive infant milk formula or breast milk.
A COMPOSITION COMPRISING BIFIDOBACTERIUM INFANTIS AND FRUCTO AND GALACTO-OLIGOSACCHARIDES FOR THE PREVENTION OF INTESTINAL DISCOMFORT IN INFANTS

FIELD OF THE INVENTION
The present invention relates to compositions that support the introduction of solid weaning foods in the diet of infants that receive infant milk formula or breast milk.

BACKGROUND OF THE INVENTION
Probiostart™ is formulated for infants and children to support a healthy digestion and immunity. One pack of probiostart contains 10 sachets, a net weight of 1.5 g/sachet and each sachet includes fructooligosaccharides and probiotic culture /Lactobacillus acidophilus/ Rossell-52, /Bifidobacterium infantis/ Rosell-33 and /Bifidobacterium bifidum/ Rosell-71).

Scholtens et al., J Pediatr Gastroenterol Nutr, 2006, 42:553-559 describe that addition of prebiotic oligosaccharides to solid foods induces an increase in the faecal proportion of bifidobacteria in the intestinal microbiota in the weaning period.

WO 02/060276 relates to a probiotic combination comprising different combinations of lactobacilli, propionic acid bacteria and/or bifidobacteria. The probiotics are preferably combined with a suitable prebiotic to form a synbiotic. The combination of the invention can be consumed as such or combined with a suitable foodstuff or pharmaceutical product, and it is therapeutically useful for example for stimulating the immune system and for general health improvement.

SUMMARY OF THE INVENTION
The present inventors found that the intestinal flora of infants changes significantly when introducing solid and spoonable food besides the infant milk formula or breast feeding. It was found that the percentage of /Bifidobacterium infantis/ (B.infantis) decreased significantly while other bacteria remained stable. Additionally, the distribution of the various short chain fatty acids (SCFA's) changed after the introduction of solid foods.
Particularly, the percentage of fecal acetate decreased significantly while the percentage of propionate significantly increased, see example 1.

Introducing solid foods into the infant's diet often gives rise to intestinal problems. These problems are particularly caused by changes in the intestinal environment, particularly if changes in the diet occur suddenly. The present inventors found that important characteristics are changed when solid foods are introduced. It is preferred that when solid foods are introduced in the infant's diet, e.g. besides human milk or infant milk formula, changes in the flora and SCFA patterns are reduced and smoothened so the changes occur more gradually, and that the infant does not suffer from intestinal discomfort. The present invention provides a method and a composition that reduces the changes in bifidobacteria population and SCFA levels, thereby preventing and/or reducing intestinal discomfort.

The present invention relates to the administration of *B. infantis* in combination with galactooligosaccharides and fructooligosaccharides in order to blunt the changes caused by the ingestion of solid weaning foods. Supplementation of *B. infantis* limits the reduction in the intestinal *B. infantis* observed when solid foods are introduced in the diet. The mixture of galactooligosaccharides and fructooligosaccharides optimally increases the acetate production, while resulting in a limited propionate production.

A main metabolic product of *B. infantis* is acetate. The reduction in acetate production is believed to be, at least partly, a result of the reduced occurrence of *B. infantis*. Hence, the co-administration *B. infantis* with galactooligosaccharides and fructooligosaccharides synergistically increases the acetate production, while keeping the extra propionate production to a minimum.

The present inventors found that the mixture of galactooligosaccharides and fructooligosaccharides is more effective in stimulating acetate production, while resulting in a limited propionate production than other prebiotics, see example 2. A limited
propionate production is desired as in infants, upon introduction of solid foods in the diet, the propionate production is already increased.

Advantageously, the combination of *B. infantis*, galactooligosaccharides and fructooligosaccharides is added to the solid weaning foods such as fruit purees or other foods for infants.

Preferably the present symbiotic combination is administered over a prolonged period of time, e.g. 2 or 3 months, i.e. the transitional period from infant milk formula to more solid foods. Hence, in a further aspect the present invention provides a container (e.g. a sachet) with a specific dosage of *B. infantis*, galactooligosaccharides and fructooligosaccharides. The present invention also provides a pack containing multiple containers with a specific dosage of *B. infantis*, galactooligosaccharides and fructooligosaccharides.

**DETAILED DESCRIPTION OF THE INVENTION**

The weaning period is the period wherein infants gradually get accustomed to the adult solid foods. Usually infants are introduced to solid foods at the age of 4 to 6 months. Herein weaning is defined as the transition from milk feeding to solid foods. Weaning foods as used herein refers to the food composition(s) that are used in infants in the weaning period.

The present invention concerns the use of a composition comprising *Bifidobacterium infantis* and galactooligosaccharides and fructooligosaccharides for the preparation of a food product for treatment and/or prevention of intestinal discomfort in an infant due to introduction of weaning foods in the diet of said infant.

In one embodiment of the invention, the food product is prepared by combining a composition a) comprising *Bifidobacterium infantis* and galactooligosaccharides and fructooligosaccharides and a composition b) which is a ready-to-use food product, wherein the food product is preferably a weaning food. Preferably composition b) has a
viscosity of at least 100 mPas at a shear rate of 100 s\(^{-1}\) at 20°C. Preferably the weight ratio of composition a) to composition b) is between 0.005 and 0.1. Preferably the amount of composition a) is between 0.5 g to 10 g.

Thus the present invention also relates to a composition c) for treatment and/or prevention of intestinal discomfort in an infant due to introduction of food products in the infant's diet, wherein composition c) is prepared by combining 0.5 g to 10 g of a composition a) with a composition b) wherein:

composition a) comprises

i. *Bifidobacterium infantis*; and

ii. galactooligosaccharides and fructooligosaccharides;

composition b) is a food product having a viscosity of at least 100 mPas at a shear rate of 100s\(^{-1}\) at 20°C; preferably the food product is a weaning food; and

the weight ratio of composition a) to composition b) is between 0.005 and 0.1.

In a further aspect the present invention relates to a method for making a food product comprising adding a composition a) to a composition b), to obtain a composition c) wherein:

composition a) comprises

i. *Bifidobacterium infantis*; and

ii. galactooligosaccharides and fructooligosaccharides:

composition b) is a food product having a viscosity of at least 100 mPas at a shear rate of 100s\(^{-1}\) at 20°C, preferably the food product is a weaning food having a viscosity of at least 100 mPas at a shear rate of 100s\(^{-1}\) at 20°C; and

the weight ratio of composition a) to composition b) is between 0.005 and 0.1.

In a further aspect the present invention relates to a packaged powder composition comprising between 0.5 and 10 g powder, including galactooligosaccharides, fructooligosaccharides and *Bifidobacterium infantis*. The present invention also provides a container comprising at least 5 of such packaged compositions.
**Bifidobacterium**

The present composition preferably comprises *Bifidobacterium infantis* (*B.infantis*), more preferably *Bifidobacterium infantis*-*longum*. Preferably the present composition contains a bacterium which has at least 95 % identity with the 16 S rRNA sequence when compared to the type strain *Bifidobacterium infantis* (strain LMG 8811<sup>T</sup> species name: *Bifidobacterium longum* Reuter 1963 AL, ATCC 15697), more preferably at least 97 % identity. A method to determine the percentage identity with the 16 S rRNA is described by Haarman and Knol in Quantitative real-time PCR assays to identify and quantify fecal Bifidobacterium species in infants receiving a prebiotic infant formula. Appl. Environ. Microbiol. 2005;71(5):2318-24. The abovementioned strain with at least 95 %, preferably at least 97%, identity of the 16 S rRNA sequence is herein referred to as *Bifidobacterium infantis* (*B.infantis*).

According to a preferred embodiment, the present composition contains at least one bifidobacterium selected from the group consisting of *B.longum* spp. *infantis* M-63.

The present composition a) preferably contains 10<sup>2</sup> to 10<sup>13</sup> colony forming units (cfu) *B.infantis* per g dry weight of the composition, preferably 10<sup>4</sup> to 10<sup>12</sup>, more preferably 10<sup>5</sup> to 10<sup>10</sup> *B.infantis* per g dry weight of the present composition a). Preferably, the present composition a) contains 10<sup>4</sup> to 10<sup>12</sup>, more preferably from 10<sup>5</sup> to 10<sup>9</sup> colony forming units (cfu) *B.infantis* per g of the total of non-digestible saccharides. The *B.infantis* is preferably administered at a daily dose of 10<sup>2</sup> to 10<sup>13</sup>, more preferably from 10<sup>5</sup> to 10<sup>11</sup> from colony forming units (cfu).

**Non-digestible oligosaccharides**

The present composition a) preferably comprises galactooligosaccharides. The term "galacto-oligosaccharide" as used herein refers to a non-digestible oligosaccharide, wherein at least 30% of the saccharide units are galactose units, preferably at least 50%, more preferably at least 60%. Lactose is considered digestible. The present composition preferably comprises galacto-oligosaccharides with a DP of 2 to 100, more preferably a
DP of 2 to 10. Preferably the saccharides of the galacto-oligosaccharide are β-linked. Many human milk oligosaccharide -core structures are also β-linked.

Preferably the present composition a) comprises a galacto -oligosaccharide selected from the group consisting of (trans)galacto -oligosaccharides, lacto -N-tetraose (LNT) and lacto -N-neotetraose (neo-LNT). In a particularly preferred embodiment the present composition comprises transgalacto -oligosaccharide. Transgalacto -oligosaccharides have the chemical structure of \([\text{galactose}]_n\text{-glucose and/or }[\text{galactose}]_n\text{-glucose }([\text{galactose}])_m\) wherein n and/or m is an integer from 1 up to and including 60, i.e. 2, 3, 4, 5, 6, ..., 59, 60; preferably n is 2, 3, 4, 5, 6, 7, 8, 9 and/or 10. Preferably m is 2, 3, 4, 5, 6, 7, 8, 9 and/or 10. Preferably the present composition comprises \([\text{galactose}]_n\text{-glucose}\) wherein n is an integer from 1 up to and including 60. Preferably n is 2, 3, 4, 5, 6, 7, 8, 9 and/or 10, more preferably n is 2, 3, 4, 5, 6 and/or 7. Transgalacto -oligosaccharides (TOS) are for example sold under the trademark Vivinal ™ (Borculo Domo Ingredients, Netherlands) and Oligomate 55™ (Yakult). Preferably the saccharide units of the galacto -oligosaccharides are mainly β-linked.

The present composition a) preferably comprises 0.01 to 10 g of the galacto -oligosaccharides per 100 g dry weight, preferably between 0.1 and 5 g, more preferably between 0.25 and 2 g.

The present composition a) preferably contains fructooligosaccharides. The term "fructo -oligosaccharide" as used herein refers to a non -digestible polysaccharide carbohydrate comprising a chain of at least 2 β-linked fructose units, with a DP of 2 to 250, preferably 7 to 100, more preferably 20 to 60. Preferably inulin is used. Inulin is available under the tradename Raftilin HP®, (Orafti). The average DP of the present fructo -oligosaccharide is preferably at least 7, more preferably at least 10, preferably below 100. The fructooligosaccharide used preferably has the (majority of) fructose units linked with a \(\beta(2 \rightarrow 1)\) linkage. Other terms for fructooligosaccharides include inulin, fructopolysaccharide, polyfructose, fructans and oligofructose. The present composition preferably comprises fructo -oligosaccharides with a DP of 2 to 100, preferably with a DP of 7 to 60.
The present composition a) preferably comprises 0.01 to 10 g of the fructooligosaccharides per 100 g dry weight, preferably between 0.1 and 5 g, more preferably between 0.25 and 2 g.

The present composition a) thus preferably comprises at least two non-digestible oligosaccharides with different average degrees of polymerization (DP). Preferably the weight ratios are:

i. (non-digestible oligosaccharides with DP 2 to 5) : (non-digestible oligosaccharides with DP 6, 7, 8, and/or 9) > 1; and/or

ii. (non-digestible oligosaccharides with DP 10 to 60) : (non-digestible oligosaccharides with DP 6, 7, 8, and/or 9) > 1

Preferably one or both weight ratios are above 2, even more preferably above 5. These combinations provide optimal production of acetate.

For further improvement, the present non-digestible oligosaccharide preferably has a relatively high content of short chain oligosaccharides, as these strongly stimulate acetate production. Preferably at least 50 wt.%, more preferably at least 75 wt.% of the non-digestible oligosaccharides have a DP of 2 to 9 (i.e. 2, 3, 4, 5, 6, 7, 8, and/or 9).

In the present composition a) preferably at least 75 wt.% of the total galactooligosaccharides have a DP of 2-7 and at least 75 wt.% of the total fructooligosaccharides have a DP of 6-60.

The present composition a) preferably comprises 0.01 to 10 g non-digestible oligosaccharides, preferably between 0.1 and 5 g, more preferably between 0.25 and 2 g. Per 100 g dry weight???

**Method**

The present invention relates to a method for making a food product comprising adding a composition a) to a composition b), to obtain a composition c).
Composition a) preferably comprises *Bifidobacterium infantis* and (ii) galactooligosaccharides and fructooligosaccharides. Composition a) preferably has a weight below 100 grams, most preferably below 10 gram, more preferably below 5 gram. Composition a) is preferably packaged in a container, preferably a sachet. Individual packaging of composition a) enables a precise dosing of the components and prevents overdosing. Composition a) is preferably in powder form. In powdered form the bacteria have an increased shelf life. The water activity of composition a) is preferably limited to increase shelf life of the bacteria. The water activity (a_w) of composition a) is preferably below 0.5, more preferably below 0.3, most preferably below 0.2.

Composition b) preferably has a weight below 100 g and a viscosity of at least 100 mPas at a shear rate of 100 s⁻¹ at 20 °C. Preferably composition b) has a viscosity of at least 200 mPas, more preferably a viscosity of at least 500 mPas at a shear rate of 100 s⁻¹ at 20 °C. Highly viscous compositions are not preferred, particularly not for infants. Hence, preferably composition b) has a viscosity below 10000 mPas, preferably below 5000 mPas at a shear rate of 100 s⁻¹ at 20 °C. Composition b) is preferably a fruit puree, a spoonable milk-based cereal composition or a meal suitable for infants in the age of 3 to 24 months. It is preferred composition b) is a ready-to-use food product, preferably composition b) is a commercially available food product.

According to the present method, composition a) and composition b) are combined, to obtain a composition c), e.g. by strewing composition a) onto composition b), and optionally followed by mixing. The method preferably has a minimal influence on the taste and/or consistency of the composition b). Hence the composition a) preferably has a limited volume compared to composition b). Therefore, the weight ratio of composition a) to composition b) is below 0.1, more preferably below 0.05. The weight ratio of composition a) to composition b) is preferably above 0.005, more preferably above 0.01 and is for example about 0.025.

In a further embodiment, the present invention provides a container with multiple individually packaged compositions a). The present invention provides a container
comprising at least 5 packaged powder compositions, wherein each packaged powder composition comprises 0.5 and 10 grams powder, including galactooligosaccharides, fructooligosaccharides and Bifidobacterium infantis. The container preferably contains at least 5 packaged powder compositions, preferably at most 30 packaged powder compositions.

Health benefits
The present invention relates to a method for (i) the treatment and/or prevention of intestinal discomfort due to introduction of weaning foods in the infant's diet and/or (ii) the treatment and/or prevention of constipation and/or (iii) the treatment and/or prevention of intestinal cramps, by administering a composition c) to the infant, wherein composition c) has been prepared by combining a composition a) and a composition b). Composition a) and composition b) are described hereinabove.

The composition c) is preferably administered to an infant with an age of 3 to 24 months, more preferably to an infant with an age of 3-12 months. Preferably the composition c) is administered to the infant at least once per week, for a period of at least 5 weeks. More preferably the composition c) is administered to the infant at least three times per week, for a period of at least 2 weeks. Most preferably the composition c) is administered to the infant at least once per day, for a period of at least 2 weeks. A more frequent administration will result in a more gradual change of intestinal flora from a milk fed infant to a situation wherein the infant also receives solid foods.

EXAMPLES

Example 1: Changes in the intestinal microbiota

Aim
It is well known that the intestinal microbiota of breast fed infants is dominated by bifidobacteria and lactobacilli. It has been suggested that the introduction of solid weaning foods may disturb the intestinal microbiota of breast fed infants. The current
study is aimed at observing the changes in the intestinal microbiota, and its metabolic activity in fully breast fed infants that were introduced to regular solid weaning foods.

Methods

The study was an observational study, in which healthy, fully breast fed infants, aged 4-6 months old, were followed from the first introduction of solid foods until six weeks thereafter. Fecal samples were taken before, and 3 and 6 weeks after the introduction of solids. The fecal samples were analyzed with fluorescent in situ hybridization and quantitative real time PCR for the analysis of percentages of bifidobacteria, *B. infantis*, *B. adolescentis*, lactobacilli, *E. coli*, Clostridia, and *Bacteroides* species. In addition, fecal short-chain fatty acids (SCFAs), and pH levels were analyzed. Furthermore, information on number of stools, stool characteristics and gastrointestinal complaints (possetting, diarrhea, colics, flatulence, constipation, diaper dermatitis) were recorded in daily study diaries. All data were statistically analyzed with a repeated measures ANOVA.

Results

Thirteen infants completed the study. The mean age at study entrance was 5.0 months (s.d. 0.6 months). After six weeks of consumption of solid weaning foods, a significant increase in lactobacilli from 3.3% to 6.0% (p<0.0001), and a significant increase in *B. adolescentis* from 0.8% to 4.1% (p<0.0001) was observed. The percentage of *B. infantis* decreased significantly from 35.6% to 28.5% (p<0.0001). Other bacteria remained stable throughout the study period. The distribution of the various SCFAs changed after the introduction of solid foods. The percentage of fecal acetate decreased significantly with mean percentages of 93% and 83% at baseline and after six weeks respectively (p=0.010). The percentage of propionate significantly increased from 4% at baseline to 15% (p=0.002). No significant changes in fecal pH were observed. The stool consistency significantly increased from 1.7 (SEM 0.4) (watery to soft -pudding -like) to 2.7 (SEM 0.7) (soft-formed to dry formed) within the six -week study period (p<0.001). Stool frequency did not change significantly.
Conclusion
The introduction of solid weaning foods is associated with a change in the microenvironment of fully breast fed infants, as observed in a shift in the percentages of lactobacilli, *B. adolescentis* and *B. infantis*, and as observed in a change in the metabolic activity of the intestinal microbiota, with changes in the pattern of the short-chain fatty acids acetate and propionate.

Example 2: Effect of oligosaccharides on acetate/propionate production

Micro-organisms were obtained from fresh faeces from bottle fed babies. Fresh faecal material from babies ranging 1 to 4 month of age was pooled and put into preservative medium within 2 h. As substrate either prebiotics (TOS; TOS/inulin (HP) mixture in a 9/1 (w/w) ratio; inulin; oligofructose(OS)/inulin mixture in a 1/1 (w/w) ratio, or none (blanc) were used. The transgalactooligosaccharides (TOS) were obtained from Vivinal GOS, Borculo Domo Ingredients, Zwolle, The Netherlands). The inulin (HP) Orafti active food ingredients, Tienen, Belgium, i.e. Raftiline HP®.

The experiment was carried out using the following samples: 1) 85mg TOS 2) 85 mg inulin 3) 85mg TOS/inulin in a ratio of 9/1 (w/w) and 4) 85mg OS/inulin in a ratio of 1/1 (w/w). SCFA (acetate, propionate, butyrate) were quantitated using a Varian 3800 gas chromatograph (GC) (Varian Inc., Walnut Creek, U.S.A.) equipped with a flame ionisation detector.

<table>
<thead>
<tr>
<th>TABEL 1</th>
<th>Week 0</th>
<th>Week 6</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. adolescentis</em></td>
<td>0.8</td>
<td>4.1</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td><em>B. infantis</em></td>
<td>35.6</td>
<td>28.5</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>acetate</td>
<td>93%</td>
<td>83%</td>
<td>p = 0.010</td>
</tr>
<tr>
<td>propionate</td>
<td>4%</td>
<td>15%</td>
<td>p = 0.002</td>
</tr>
</tbody>
</table>
Figure 1 depicts the absolute (Figure IA) and relative SCFA profile (Figure IB) resulting from fermenting the different oligosaccharides. Figure 1A shows that a mixture of two different oligosaccharides (TOS/Inulin), wherein the two distinct oligosaccharides have a different chain length results in a significantly and synergistically increased amount of SCFA (particularly acetate) per gram fiber than single components. Figure 1B shows that the addition of a combination of TOS/Inulin favored a higher proportion of the beneficial acetate (B). [Figures 1A and 1B to be included]

Example 3: Composition

Packaged powder composition in sachet containing 5 g powder, including 1 g galactooligosaccharides (95 wt.% of the galactooligosaccharides have a DP of 2 -7), 0.1 g fructooligosaccharides (95 wt.% of the fructooligosaccharides have a DP of 6 -100) and 10^6 cfu *B.infantis*, and maltodextrin carrier.

The packaged powder composition is opened and the powder is admixed with 100 g apple puree and served to an infant of 6 months in order to prevent constipation as a result of introduction of weaning foods.
CLAIMS

1. Use of a composition comprising *Bifidobacterium infantis* and galactooligosaccharides and fructooligosaccharides for the preparation of a food product c) for treatment and/or prevention of intestinal discomfort in an infant due to introduction of weaning foods in the diet of said infant, wherein said food product c) is prepared by combining 0.5 – 10 g of a composition a) which comprises *Bifidobacterium infantis* and galactooligosaccharides and fructooligosaccharides and a composition b) which is a ready-to-use weaning food where in the weight ratio of composition a) to composition b) is between 0.005 and 0.1.

2. The use according to claim 1, wherein composition b) has a viscosity of at least 100 mPas at a shear rate of 100 s^-1 at 20°C.

3. A method for making a food product comprising adding a composition a) to a composition b), to obtain a food product c) wherein:
   
   composition a) comprises
   
   i. *Bifidobacterium infantis* ; and
   
   ii. galactooligosaccharides and fructooligosaccharides ;

   composition b) is a ready-to-use food product having a viscosity of at least 100 mPas at a shear rate of 100 s^-1 at 20°C; and

   the weight ratio composition a) to composition b) is between 0.005 and 0.1.

4. The use according to claim 1 or 2 or the method according to claim 3, wherein composition b) is a fruit puree, a spoonable milk based cereal composition or a meal suitable for infants in the age of 3 to 24 months.

5. The use according to claim 1 or 2 or the method according to claim 3 or 4, wherein composition a) is a powder.
6. The use according to claim 1 or 2 or the method according to claim 3 - 5, wherein the food product \( c \) is administered to an infant.

7. The use according to claim 1 or 2 or the method according to claim 3 - 6, wherein composition \( a \) has a caloric content from digestible carbohydrates, fats and proteins below 10 kcal/g, preferably below 5 kcal/g, more preferably below 1 kcal/g.

8. Packaged powder composition comprising 0.5 and 10 g powder, including galactooligosaccharides, fructooligosaccharides and \textit{Bifidobacterium infantis} and/or \textit{Bifidobacteria m}.

9. A container comprising at least 5 packaged compositions according to claim 7.

10. A composition \( c \) for treatment and/or prevention of intestinal discomfort in an infant due to introduction of weaning foods in the diet of said infant, wherein composition \( c \) is prepared by combining 0.5 g to 10 g of a composition \( a \) with a composition \( b \) wherein:

composition \( a \) comprises

i. \textit{Bifidobacterium infantis} ; and

ii. galactooligosaccharides and fructooligosaccharides;

composition \( b \) is a food product having a viscosity of at least 100 mPas at a shear rate of 100 \( \text{s}^{-1} \) at 20\(^{\circ}\)C; and

the weight ratio of composition \( a \) to composition \( b \) is between 0.005 and 0.1.
A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/70 C12N1/20 A61P1/00 A61K35/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. RELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, FSTA, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>WO 2007/046699 A (NUTRICIA NV [NL]; SCHMIDT JOACHIM [DE]; BOEHM GUNTER [DE]; BEERMMANN C) 26 April 2007 (2007-04-26) page 1 paragraph 3</td>
<td>1-10</td>
</tr>
<tr>
<td>Y</td>
<td>EP 1 597 978 A (NUTRICIA NV [NL]) 23 November 2005 (2005-11-23) example 1, figures 1 and 2</td>
<td>1-10</td>
</tr>
<tr>
<td>Y</td>
<td>EP 1 714 660 A (NUTRICIA NV [NL]) 25 October 2006 (2006-10-25) example 1</td>
<td>1-10</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C

See patent family annex

Additional information:

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the international filing date

'L' document which may throw doubts on novelty claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

'S' document member of the same patent family

Date of the actual completion of the international search: 21 May 2008

Date of mailing of the international search report: 09/06/2008

Name and mailing address of the ISA:
European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040, Tx 31651 epi nl
Fax (+31-70) 340-3016

Authorized officer:
Habedanck, Robert

Form PCT/ISA/210 (second sheet) (April 2005)
### DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>KNOL JAN ET AL: &quot;Increase of faecal bifidobacteria due to dietary oligosaccharides induces a reduction of clinically relevant pathogen germs in the faeces of formula-fed preterm infants&quot; ACTA PAEDIATRICA, UNIVERSITETSFORLAGET, OSLO, NO, vol. 94, no. Suppl.449, 1 October 2005 (2005-10-01), pages 31-33, XP009096903 ISSN: 0803-5253</td>
<td>1-10</td>
</tr>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 2006303477 AI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2007045502 AI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2007046697 AI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2007046698 AI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2007046699 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 2006303477 AI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2007045502 AI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2007046697 AI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2007046698 AI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2007046699 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 1964638 AI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2007536067 T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2005110121 AI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2006112714 A2</td>
</tr>
</tbody>
</table>