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(54) **GROWING UP MILKS CONTAINING PROBIOTIC MICRO-ORGANISMS**

WACHSTUMSMILCH MIT PROBIOTISCHEN MIKROORGANISMEN

LAITS DE CROISSANCE CONTENANT DES MICRO-ORGANISMES PROBIOTIQUES

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- **DATABASE GNPD [Online] Intel; April 2009 (2009-04), anonymus: "Follow-up formula", XP002610821, Database accession no. 1069681**

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Description

[0001] The present invention relates to the field of nutrition for infants and young children. In particular, the present invention relates to growing-up milks comprising probiotic micro-organisms to be administered to infants and young children older than 10 months. These probiotic micro-organisms may be non-replicating probiotic micro-organisms such as bioactive heat treated probiotic micro-organisms, for example.

[0002] Breast milk is the ideal food for healthy growth and development of babies. In 2001 the World Health Organization (WHO) changed its recommended duration of exclusive breastfeeding from 4 to 6 months to 6 months, therefore breastfeeding should be encouraged and promoted accordingly.

[0003] Beginning from the 6th month onwards the behaviour of infants changes. They sit up for the first time or grab a toy with only one hand. An infant's nutritional requirements change as the infant develops. Nutrients like iron and calcium become even more important.

[0004] From the age of one onwards, drinks based on cow's milk become an important item in the baby's diet. But cow's milk itself is not adapted to the nutritional requirements of young children. In particular, cow's milk contains too much protein, too many minerals, too little iron, too much saturated fatty acids and not enough unsaturated fats.

[0005] Growing up milks (GUM) were developed to assist in bridging this step. GUM ensures a steady transition from the lower protein level of breast milk to the high protein level present in cow's milk and normal dairy products used by adults.

[0006] Growing Up Milk (GUM) aims at helping Junior Stage toddlers to maximize their potential in terms of physical and mental development. GUM fulfils the toddler's protein requirements without overloading the still immature metabolic system.

[0007] One important function of early nutrition of infants and young children is to generate a healthy gut flora and to develop a strong immune system.

[0008] A healthy gut flora will contribute to a functional GI tract, which in turn will help to properly digest ingested food and will reduce stomach ache in infants and young children.

[0009] Infants and young children typically have around 10 colds per year. Such colds are uncomfortable and may even have more serious consequences.

[0010] It would hence be desirable to further improve the immune boosting effect of growing up milks.

[0011] It would also be desirable to further improve the anti-inflammatory effect of growing up milks.

[0012] Hence, there is a need in the art for a growing up milk that ensures a steady transition from the lower protein level of breast milk to the high protein level present in cow's milk. Such a growing up milk should have an improved immune boosting effect, an anti-inflammatory effect and/or should facilitate digestion. It would be preferred if this was achieved by using natural ingredients that are safe to administer without side effects and that are easy to incorporate into growing up milk compositions using state of the art industrial techniques.

[0013] The present inventors have addressed this need. It was hence the objective of the present invention to improve the state of the art and to provide a GUM composition that satisfies the needs expressed above.

[0014] The present inventors were surprised to see that they could achieve this object by the subject matter of the independent claim. The dependant claims further develop the idea of the present invention.

[0015] Accordingly, the present inventors propose to provide a growing-up-milk composition comprising probiotics.

[0016] Growing-up-milk compositions are nutritional compositions. Growing-up-milks are in particular specific nutritional compositions having a composition that allows a steady transition from the composition of breast milk to the composition of cow milk. This adjustment may be made stepwise. Several different growing-up-milks may be foreseen to accompany this transition. For example, one growing-up-milk composition may be to be administered beginning at the age of 10 months, a second one growing-up-milk composition may be to be administered beginning at the age of 12 months and a third growing-up-milk composition may be to be administered beginning at the age of 24 months.

[0017] Probiotics were found to be able to provide their health benefits in the framework of growing-up-milks. Additionally, e.g. bifidobacteria, are present in breast milk and are part of what gives breast milk its naturally protective properties.

[0018] Hence, adding probiotic micro-organisms to the nutritional formulas of infants and young children would allow them to more closely resemble breast milk.

[0019] However, as in particular powdered formulas to be reconstituted with water usually have a shelf life that exceeds the shelf life of, e.g., yoghurt drinks comprising probiotics, probiotics are usually not added to such formulas, because of uncertainties that the viability of the probiotics can be ensured during an extended shelf life, for example.

[0020] The present inventors were now able to show that even non-replicating probiotics can provide the health benefits of probiotics and may even have improved benefits.

[0021] Consequently, the present invention relates to a growing-up-milk composition comprising probiotic micro-organisms to be administered to infants or young children starting from the age of 10 months.

[0022] Growing up Milk provides the right amount of lipids with a balanced fatty acid composition. Lipids are the best energy provider (9 kcal/g), needed for the increasing activity of the toddler. But fatty acids are also required to build cell

membranes, and are important precursors for some physiologically active factors (e.g. hormones, coagulation factors etc.).

[0023] FDA regulations define infants as persons not more than 12 months old (Title 21, Code of Federal Regulations 21 CFR 105.3(e)).

[0024] The growing up milk composition of the present invention comprises a balanced protein content.

[0025] On the one hand, protein is needed, especially during growth periods, e.g. to build body tissues such as muscles. On the other hand, high amounts of protein burden the toddler's still immature kidneys' function. If kidney function has not matured, a high protein intake can compromise renal function. Research suggests that children over one year of age tend to have a protein intake which is higher than paediatric recommendations. A high protein intake has also been implicated in obesity in later life.

[0026] Cow's milk has a Sub-optimal fatty acid composition for the nutrition of infants and young children. High in saturated fatty acids and low in unsaturated fatty acids, cow's milk does not provide the optimal and most healthy fatty acid composition. Unsaturated fatty acids are needed for growth, e.g. for building cells in the nervous system, and studies show that many toddlers do not receive the recommended levels. Brain, for instance, is extremely rich in DHA (n-3 unsaturated fatty acid). Therefore, the growing up milk composition of the present invention may have a good balance between the two families of essential fatty acids, with an approximate 4-10 ratio between n-6 fatty acids like linolenic acid and n-3 fatty acids like alpha-linolenic acid.

[0027] If provided as a dried composition it is preferred that the GUM composition has a water activity of below 0.2, preferably below 0.15 to further increase shelf stability. Most bacteria, for example, do not grow at water activities below 0.91, and most molds cease to grow at water activities below 0.80.

[0028] Water activity (a_w) is a measurement of the energy status of the water in a system. It is defined as the vapour pressure of the water divided by that of pure water. Consequently, distilled water has a water pressure of 1.

[0029] The growing up milk composition of the present invention has a caloric density in the range of 70-80 kcal/100ml, and comprises a protein source in an amount of 2-3 g/100kcal, a carbohydrate source in an amount of 12-15 g/100kcal, and a lipid source in an amount of 3-4.6 g/100kcal.

[0030] A growing up milk composition of the present invention to be administered to infants at the age of 10-12 months has a caloric density in the range of about 70 kcal/100ml, and comprises a protein source in an amount of 2.2-2.3 g/100kcal, a carbohydrate source in an amount of about 13 g/100kcal, and a lipid source in an amount of 4.3 to 4.4 g/100kcal.

[0031] A growing up milk composition of the present invention to be administered to children at the age of 12-24 months has a caloric density in the range of about 70-80 kcal/100ml, and comprises a protein source in an amount of 2.2-2.9 g/100kcal, a carbohydrate source in an amount of about 11.9-13.9 g/100kcal, and a lipid source in an amount of 3.9 to 4.6 g/100kcal.

[0032] A growing up milk composition of the present invention to be administered to children at the age of above 24 months has a caloric density of about 70 kcal/100ml, and comprises a protein source in an amount of about 2.5 g/100kcal, a carbohydrate source in an amount of about 14.6 g/100kcal, and a lipid source in an amount of 3.9 to 4.6 g/100kcal.

[0033] The protein source may consist of whey proteins and casein. For example a ratio of whey to casein in the range of about 20:80 to 80:20, e.g., 40:60 to 60:40 may be used.

[0034] The carbohydrate source may consist essentially of Lactose. However, also ratios of Lactose and Maltodextrin in the range of 3:1 to 1:1 may be used, for example.

[0035] The feeding formula of the present invention may comprise 0.2-0.3 g LC-PUFA/100g fatty acids. The LC-PUFA may be selected from ARA, DHA or combinations thereof. For example, the LC-PUFA may comprise a combination of ARA and DHA. Formulas containing DHA and ARA have been shown to provide visual and mental development similar to that of the breastfed infant.

[0036] The feeding formula of the present invention may also contain 1.5-2.5 mg nucleotides per 100 mL formula. Nucleotides and their bases are not considered 'essential' because they can be synthesised by the infant body from simpler compounds. At certain times, however, the processes of synthesis may not be able to meet demand, for example, during periods of rapid cell turnover as in normal growth or in gut disease. At these times, the body relies more heavily on dietary sources of nucleotides.

[0037] The composition comprises probiotic micro-organisms wherein the probiotic micro-organisms comprise non-replicating probiotic micro-organisms, and at least 90 % of said probiotics micro-organisms are non-replicating probiotic micro-organisms, wherein said non-replicating probiotic micro-organisms is Bifidobacterium breve NCC 2950.

[0038] The inventors were surprised to see that, e.g., in terms of an immune boosting effect and/or in terms of an anti-inflammatory effect non-replicating probiotic microorganisms may even be more effective than replicating probiotic microorganisms.

[0039] This is surprising since probiotics are often defined as "live micro-organisms that when administered in adequate amounts confer health benefits to the host" (FAO/WHO Guidelines). The vast majority of published literature deals with live probiotics. In addition, several studies investigated the health benefits delivered by non-replicating bacteria and most

of them indicated that inactivation of probiotics, e.g. by heat treatment, leads to a loss of their purported health benefit (Rachmilewitz, D., et al., 2004, Gastroenterology 126:520-528; Castagliuolo, et al., 2005, FEMS Immunol.Med.Microbiol. 43:197-204; Gill, H. S. and K. J. Rutherfurd, 2001, Br.J.Nutr. 86:285-289; Kaila, M., et al., 1995, Arch.Dis.Child 72:51-53.). Some studies showed that killed probiotics may retain some health effects (Rachmilewitz, D., et al., 2004, Gastroenterology 126:520-528; Gill, H. S. and K. J. Rutherfurd, 2001, Br.J.Nutr. 86:285-289), but clearly, living probiotics were regarded in the art so far as more performing.

[0040] The composition according to the present invention may comprise probiotic micro-organisms in any effective amount, for example in an amount corresponding to about 10^6 to 10^{12} cfu/g dry weight.

[0041] The probiotic micro-organisms are non-replicating probiotic micro-organisms.

[0042] "Non-replicating" probiotic micro-organisms include probiotic bacteria which have been heat treated. This includes micro-organisms that are inactivated, dead, non-viable and/or present as fragments such as DNA, metabolites, cytoplasmic compounds, and/or cell wall materials.

[0043] "Non-replicating" means that no viable cells and/or colony forming units can be detected by classical plating methods. Such classical plating methods are summarized in the microbiology book: James Monroe Jay, Martin J. Loessner, David A. Golden. 2005. Modern food microbiology. 7th edition, Springer Science, New York, N.Y. 790 p. Typically, the absence of viable cells can be shown as follows: no visible colony on agar plates or no increasing turbidity in liquid growth medium after inoculation with different concentrations of bacterial preparations ('non replicating' samples) and incubation under appropriate conditions (aerobic and/or anaerobic atmosphere for at least 24h).

[0044] Probiotics are defined for the purpose of the present invention as "Microbial cell preparations or components of microbial cells with a beneficial effect on the health or well-being of the host." (Salminen S, Ouwehand A, Benno Y. et al "Probiotics: how should they be defined" Trends Food Sci. Technol. 1999:10 107-10).

[0045] The possibility to use non-replicating probiotic micro-organisms offers several advantages. In severely immunocompromised children, the use of live probiotics may be limited in exceptional cases due to a potential risk to develop bacteremia. Non-replicating probiotics may be used without any problem.

[0046] Additionally, the provision of non-replicating probiotic micro-organisms allows the hot reconstitution while retaining health benefit.

[0047] The compositions of the present invention comprise non-replicating probiotic micro-organisms in an amount sufficient to at least partially produce a health benefit. An amount adequate to accomplish this is defined as "a therapeutically effective dose". Amounts effective for this purpose will depend on a number of factors known to those of skill in the art such as the weight and general health state of the child, and on the effect of the food matrix.

[0048] In prophylactic applications, compositions according to the invention are administered to a consumer susceptible to or otherwise at risk of a disorder in an amount that is sufficient to at least partially reduce the risk of developing that disorder. Such an amount is defined to be "a prophylactic effective dose". Again, the precise amounts depend on a number of factors such as the child's state of health and weight, and on the effect of the food matrix.

[0049] Those skilled in the art will be able to adjust the therapeutically effective dose and/or the prophylactic effective dose appropriately.

[0050] In general the composition of the present invention contains non-replicating probiotic micro-organisms in a therapeutically effective dose and/or in a prophylactic effective dose.

[0051] Typically, the therapeutically effective dose and/or the prophylactic effective dose is in the range of about 0,005 mg - 1000 mg probiotic micro-organisms and/or non-replicating, probiotic micro-organisms per daily dose.

[0052] In terms of numerical amounts, the "short-time high temperature" treated non-replicating micro-organisms may be present in the composition in an amount corresponding to between 10^4 and 10^{12} equivalent cfu/g of the dry composition. Obviously, non-replicating micro-organisms do not form colonies, consequently, this term is to be understood as the amount of non replicating micro-organisms that is obtained from 10^4 and 10^{12} cfu/g replicating bacteria. This includes micro-organisms that are inactivated, non-viable or dead or present as fragments such as DNA or cell wall or cytoplasmic compounds. In other words, the quantity of micro-organisms which the composition contains is expressed in terms of the colony forming ability (cfu) of that quantity of micro-organisms as if all the micro-organisms were alive irrespective of whether they are, in fact, non replicating, such as inactivated or dead, fragmented or a mixture of any or all of these states.

[0053] Preferably the non-replicating micro-organisms are present in an amount equivalent to between 10^4 to 10^9 cfu/g of dry composition, even more preferably in an amount equivalent to between 10^5 and 10^9 cfu/g of dry composition.

[0054] The probiotics may be rendered non-replicating by any method that is known in the art.

[0055] The technologies available today to render probiotic strains non-replicating are usually heat-treatment, γ -irradiation, UV light or the use of chemical agents (formalin, paraformaldehyde).

[0056] It would be preferred to use a technique to render probiotics non-replicating that is relatively easy to apply under industrial circumstances in the food industry.

[0057] Most products on the market today that contain probiotics are heat treated during their production. It would hence be convenient, to be able to heat treat probiotics either together with the produced product or at least in a similar

way, while the probiotics retain or improve their beneficial properties or even gain a new beneficial property for the consumer.

[0058] However, inactivation of probiotic micro-organisms by heat treatments is associated in the literature generally with an at least partial loss of probiotic activity.

[0059] The present inventors have now surprisingly found, that rendering probiotic micro-organisms non-replicating, e.g., by heat treatment, does not result in the loss of probiotic health benefits, but - to the contrary - may enhance existing health benefits and even generate new health benefits.

[0060] Hence, one embodiment of the present invention is a composition wherein the non-replicating probiotic micro-organisms were rendered non-replicating by a heat-treatment.

[0061] Such a heat treatment may be carried out at at least 71.5 °C for at least 1 second.

[0062] Long-term heat treatments or short-term heat treatments may be used.

[0063] In industrial scales today usually short term heat treatments, such as UHT-like heat treatments are preferred. This kind of heat treatment reduces bacterial loads, and reduces the processing time, thereby reducing the spoiling of nutrients.

[0064] The inventors demonstrate for the first time that probiotics micro-organisms, heat treated at high temperatures for short times exhibit anti-inflammatory immune profiles regardless of their initial properties. In particular either a new anti-inflammatory profile is developed or an existing anti-inflammatory profile is enhanced by this heat treatment.

[0065] It is therefore now possible to generate non replicating probiotic micro-organisms with anti-inflammatory immune profiles by using specific heat treatment parameters that correspond to typical industrially applicable heat treatments, even if live counterparts are not anti-inflammatory strains.

[0066] Hence, for example, the heat treatment may be a high temperature treatment at about 71.5-150 °C for about 1-120 seconds. The high temperature treatment may be a high temperature/short time (HTST) treatment or a ultra-high temperature (UHT) treatment.

[0067] The probiotic micro-organisms may be subjected to a high temperature treatment at about 71.5-150 °C for a short term of about 1-120 seconds.

[0068] More preferred the micro-organisms may be subjected to a high temperature treatment at about 90 - 140°C, for example 90°- 120°C, for a short term of about 1-30 seconds.

[0069] This high temperature treatment renders the micro-organisms at least in part non-replicating.

[0070] The high temperature treatment may be carried out at normal atmospheric pressure but may be also carried out under high pressure. Typical pressure ranges are from 1 to 50 bar, preferably from 1-10 bar, even more preferred from 2 to 5 bar. Obviously, it is preferred if the probiotics are heat treated in a medium that is either liquid or solid, when the heat is applied. An ideal pressure to be applied will therefore depend on the nature of the composition which the micro-organisms are provided in and on the temperature used.

[0071] The high temperature treatment may be carried out in the temperature range of about 71.5-150 °C, preferably of about 90-120 °C, even more preferred of about 120-140 °C.

[0072] The high temperature treatment may be carried out for a short term of about 1-120 seconds, preferably, of about 1-30 seconds, even more preferred for about 5-15 seconds.

[0073] This given time frame refers to the time the probiotic micro-organisms are subjected to the given temperature. Note, that depending on the nature and amount of the composition the micro-organisms are provided in and depending on the architecture of the heating apparatus used, the time of heat application may differ.

[0074] Typically, however, the composition of the present invention and/or the micro-organisms are treated by a high temperature short time (HTST) treatment, flash pasteurization or a ultra high temperature (UHT) treatment.

[0075] A UHT treatment is Ultra-high temperature processing or a ultra-heat treatment (both abbreviated UHT) involving the at least partial sterilization of a composition by heating it for a short time, around 1-10 seconds, at a temperature exceeding 135°C (275°F), which is the temperature required to kill bacterial spores in milk. For example, processing milk in this way using temperatures exceeding 135° C permits a decrease of bacterial load in the necessary holding time (to 2-5 s) enabling a continuous flow operation.

[0076] There are two main types of UHT systems: the direct and indirect systems. In the direct system, products are treated by steam injection or steam infusion, whereas in the indirect system, products are heat treated using plate heat exchanger, tubular heat exchanger or scraped surface heat exchanger. Combinations of UHT systems may be applied at any step or at multiple steps in the process of product preparation.

[0077] A HTST treatment is defined as follows (High Temperature/Short Time): Pasteurization method designed to achieve a 5-log reduction, killing 99,9999% of the number of viable micro-organisms in milk. This is considered adequate for destroying almost all yeasts, molds and common spoilage bacteria and also ensure adequate destruction of common pathogenic heat resistant organisms. In the HTST process milk is heated to 71.7oC (161oF) for 15-20 seconds.

[0078] Flash pasteurization is a method of heat pasteurization of perishable beverages like fruit and vegetable juices, beer and dairy products. It is done prior to filling into containers in order to kill spoilage micro-organisms, to make the products safer and extend their shelf life. The liquid moves in controlled continuous flow while subjected to temperatures

of 71.5oC (160oF) to 74oC (165oF) for about 15 to 30 seconds.

[0079] For the purpose of the present invention the term "short time high temperature treatment" shall include high-temperature short time (HTST) treatments, UHT treatments, and flash pasteurization, for example.

[0080] Since such a heat treatment provides non-replicating probiotics with an improved anti-inflammatory profile, the composition of the present invention may be for use in the prevention or treatment of inflammatory disorders.

[0081] The inflammatory disorders that can be treated or prevented by the composition of the present invention are not particularly limited. For example, they may be selected from the group consisting of acute inflammations such as sepsis; burns; and chronic inflammation, such as inflammatory bowel disease, e.g., Crohn's disease, ulcerative colitis, pouchitis; necrotizing enterocolitis; skin inflammation, such as UV or chemical-induced skin inflammation, eczema, reactive skin; irritable bowel syndrome; eye inflammation; allergy, asthma; and combinations thereof.

[0082] If long term heat treatments are used to render the probiotic micro-organisms non-replicating, such a heat treatment may be carried out in the temperature range of about 70-150 °C for about 3 minutes - 2 hours, preferably in the range of 80-140°C from 5 minutes - 40 minutes.

[0083] While the prior art generally teaches that bacteria rendered non-replicating by long-term heat-treatments are usually less efficient than live cells in terms of exerting their probiotic properties, the present inventors were able to demonstrate that heat-treated probiotics are superior in stimulating the immune system compared to their live counterparts.

[0084] The present invention relates also to an composition comprising probiotic micro-organisms that were rendered non-replicating by a heat treatment at at least about 70 °C for at least about 3 minutes.

[0085] The immune boosting effects of non-replicating probiotics were confirmed by *in vitro* immunoprofiling. The *in vitro* model used uses cytokine profiling from human Peripheral Blood Mononuclear Cells (PBMCs) and is well accepted in the art as standard model for tests of immunomodulating compounds (Schultz et al., 2003, Journal of Dairy Research 70, 165-173; Taylor et al., 2006, Clinical and Experimental Allergy, 36, 1227-1235; Kekkonen et al., 2008, World Journal of Gastroenterology, 14, 1192-1203)

[0086] The *in vitro* PBMC assay has been used by several authors/research teams for example to classify probiotics according to their immune profile, i.e. their anti- or pro-inflammatory characteristics (Kekkonen et al., 2008, World Journal of Gastroenterology, 14, 1192-1203). For example, this assay has been shown to allow prediction of an anti-inflammatory effect of probiotic candidates in mouse models of intestinal colitis (Foligne, B., et al., 2007, World J.Gastroenterol. 13:236-243). Moreover, this assay is regularly used as read-out in clinical trials and was shown to lead to results coherent with the clinical outcomes (Schultz et al., 2003, Journal of Dairy Research 70, 165-173; Taylor et al., 2006, Clinical and Experimental Allergy, 36, 1227-1235).

[0087] Allergic diseases have steadily increased over the past decades and they are currently considered as epidemics by WHO. In a general way, allergy is considered to result from an imbalance between the Th1 and Th2 responses of the immune system leading to a strong bias towards the production of Th2 mediators. Therefore, allergy can be mitigated, down-regulated or prevented by restoring an appropriate balance between the Th1 and Th2 arms of the immune system. This implies the necessity to reduce the Th2 responses or to enhance, at least transiently, the Th1 responses. The latter would be characteristic of an immune boost response, often accompanied by for example higher levels of IFN γ , TNF- α and IL-12. (Kekkonen et al., 2008, World Journal of Gastroenterology, 14, 1192-1203; Viljanen M. et al., 2005, Allergy, 60, 494-500)

[0088] The composition of the present invention allows it hence to treat or prevent disorders that are related to a compromised immune defence.

[0089] Consequently, the disorders linked to a compromised immune defence that can be treated or prevented by the composition of the present invention are not particularly limited.

[0090] For example, they may be selected from the group consisting of infections, in particular bacterial, viral, fungal and/or parasite infections; phagocyte deficiencies; low to severe immunodepression levels such as those induced by stress or immunodepressive drugs, chemotherapy or radiotherapy; natural states of less immunocompetent immune systems such as those of the neonates; allergies; and combinations thereof.

[0091] The composition described in the present invention allows it also to enhance a child's response to vaccines, in particular to oral vaccines.

[0092] Any amount of non-replicating micro-organisms will be effective. However, it is generally preferred, if at least 90 %, preferably, at least 95 %, more preferably at least 98 %, most preferably at least 99 %, ideally at least 99.9 %, most ideally all of the probiotics are non-replicating.

[0093] In one embodiment of the present invention all micro-organisms are non-replicating.

[0094] Consequently, in the composition of the present invention at least 90 %, preferably, at least 95 %, more preferably at least 98 %, most preferably at least 99 %, ideally at least 99.9 %, most ideally all of the probiotics may be non-replicating.

[0095] All probiotic micro-organisms may be used for the purpose of the present invention.

[0096] For example, the probiotic micro-organisms may be selected from the group consisting of bifidobacteria, lactobacilli, propionibacteria, or combinations thereof, for example *Bifidobacterium longum*, *Bifidobacterium lactis*, *Bifidobac-*

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terium animalis, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium adolescentis*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus salivarius*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus johnsonii*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactococcus lactis*, *Streptococcus thermophilus*, *Lactococcus lactis*, *Lactococcus diacetylactis*, *Lactococcus cremoris*, *Lactobacillus bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii*, *Escherichia coli* and/or mixtures thereof.

[0097] The composition in accordance with the present invention comprise non-replicating *Bifidobacterium breve* NCC 2950.

[0098] All these strains were either deposited under the Budapest treaty and/or are commercially available.

[0099] The strains have been deposited under the Budapest treaty as follows:

Bifidobacterium longum NCC 3001:	ATCC BAA-999
Bifidobacterium longum NCC 2705:	CNCM I-2618
Bifidobacterium breve NCC 2950	CNCM I-3865
Bifidobacterium lactis NCC 2818:	CNCM I-3446
Lactobacillus paracasei NCC 2461:	CNCM I-2116
Lactobacillus rhamnosus NCC 4007:	CGMCC 1.3724
Streptococcus thermophilus NCC 2019:	CNCM I-1422
Streptococcus thermophilus NCC 2059:	CNCM I-4153
Lactococcus lactis NCC 2287:	CNCM I-4154
Lactobacillus casei NCC 4006:	CNCM I-1518
Lactobacillus casei NCC 1825:	ACA-DC 6002
Lactobacillus acidophilus NCC 3009:	ATCC 700396
Lactobacillus bulgaricus NCC 15:	CNCM I-1198
Lactobacillus johnsonii La1	CNCM I-1225
Lactobacillus reuteri DSM17938	DSM17938
Lactobacillus reuteri ATCC55730	ATCC55730
Escherichia coli Nissle 1917:	DSM 6601

[0100] Those skilled in the art will understand that they can freely combine all features of the present invention described herein, without departing from the scope of the invention as disclosed.

[0101] Further advantages and features of the present invention are apparent from the following Examples and Figures.

[0102] Figures 1 A and B show the enhancement of the anti-inflammatory immune profiles of probiotics treated with "short-time high temperatures".

[0103] Figure 2 shows non anti-inflammatory probiotic strains that become anti-inflammatory, i.e. that exhibit pronounced anti-inflammatory immune profiles *in vitro* after being treated with "short-time high temperatures".

[0104] Figures 3 A and B show probiotic strains in use in commercially available products that exhibit enhanced or new anti-inflammatory immune profiles *in vitro* after being treated with "short-time high temperatures".

[0105] Figures 4 A and B show dairy starter strains (i.e. Lc1 starter strains) that exhibits enhanced or new anti-inflammatory immune profiles *in vitro* upon heat treatment at high temperatures.

[0106] Figure 5 shows a non anti-inflammatory probiotic strain that exhibits anti-inflammatory immune profiles *in vitro* after being treated with HTST treatments.

[0107] Figure 6: Principal Component Analysis on PBMC data (IL-12p40, IFN- γ , TNF- α , IL-10) generated with probiotic and dairy starter strains in their live and heat treated (140°C for 15 second) forms. Each dot represents one strain either live or heat treated identified by its NCC number or name.

[0108] Figure 7 shows IL-12p40 / IL-10 ratios of live and heat treated (85°C, 20min) strains. Overall, heat treatment at 85°C for 20 min leads to an increase of IL-12p40 / IL-10 ratios as opposed to "short-time high temperature" treatments of the present invention (Figures 1, 2, 3, 4 and 5).

[0109] Figure 8 shows the enhancement of *in vitro* cytokine secretion from human PBMCs stimulated with heat treated bacteria.

[0110] Figure 9 shows the percentage of diarrhea intensity observed in OVA-sensitized mice challenged with saline (negative control), OVA-sensitized mice challenged with OVA (positive control) and OVA-sensitized mice challenged with OVA and treated with heat-treated or live *Bifidobacterium breve* NCC2950. Results are displayed as the percentage of diarrhea intensity (Mean \pm SEM calculated from 4 independent experiments) with 100 % of diarrhea intensity corresponding to the symptoms developed in the positive control (sensitized and challenged by the allergen) group.

Example 1:

Methodology

5 Bacterial preparations:

[0111] The health benefits delivered by live probiotics on the host immune system are generally considered to be strain specific. Probiotics inducing high levels of IL-10 and/or inducing low levels of pro-inflammatory cytokines *in vitro* (PBMC assay) have been shown to be potent anti-inflammatory strains *in vivo* (Foligné, B., et al., 2007, World J. Gastroenterol. 13:236-243).

10 [0112] Several probiotic strains were used to investigate the anti-inflammatory properties of heat treated probiotics. These were *Bifidobacterium longum* NCC 3001, *Bifidobacterium longum* NCC 2705, *Bifidobacterium breve* NCC 2950, *Bifidobacterium lactis* NCC 2818, *Lactobacillus paracasei* NCC 2461, *Lactobacillus rhamnosus* NCC 4007, *Lactobacillus casei* NCC 4006, *Lactobacillus acidophilus* NCC 3009, *Lactobacillus casei* ACA-DC 6002 (NCC 1825), and *Escherichia coli* Nissle. Several starter culture strains including some strains commercially used to produce Nestle Lc1 fermented products were also tested: *Streptococcus thermophilus* NCC 2019, *Streptococcus thermophilus* NCC 2059, *Lactobacillus bulgaricus* NCC 15 and *Lactococcus lactis* NCC 2287.

15 [0113] Bacterial cells were cultivated in conditions optimized for each strain in 5-15L bioreactors. All typical bacterial growth media are usable. Such media are known to those skilled in the art. When pH was adjusted to 5.5, 30% base solution (either NaOH or Ca(OH)₂) was added continuously. When adequate, anaerobic conditions were maintained by gassing headspace with CO₂. *E. coli* was cultivated under standard aerobic conditions.

20 [0114] Bacterial cells were collected by centrifugation (5,000 x g, 4°C) and re-suspended in phosphate buffer saline (PBS) in adequate volumes in order to reach a final concentration of around 10⁹ -10¹⁰ cfu/ml. Part of the preparation was frozen at -80°C with 15% glycerol. Another part of the cells was heat treated by:

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- Ultra High Temperature: 140°C for 15 sec; by indirect steam injection.
 - High Temperature Short Time (HTST): 74°C, 90°C and 120°C for 15 sec by indirect steam injection
 - 30 - Long Time Low Temperature (85°C, 20 min) in water bath

[0115] Upon heat treatment, samples were kept frozen at -80°C until use.

In vitro immunoprofiling of bacterial preparations:

35 [0116] The immune profiles of live and heat treated bacterial preparations (i.e. the capacity to induce secretion of specific cytokines from human blood cells *in vitro*) were assessed. Human peripheral blood mononuclear cells (PBMCs) were isolated from blood filters. After separation by cell density gradient, mononuclear cells were collected and washed twice with Hank's balanced salt solution. Cells were then resuspended in Iscove's Modified Dulbecco's Medium (IMDM, Sigma) supplemented with 10% foetal calf serum (Bioconcept, Paris, France), 1% L-glutamine (Sigma), 1% penicillin/streptomycin (Sigma) and 0.1% gentamycin (Sigma). PBMCs (7x10⁵ cells/well) were then incubated with live and heat treated bacteria (equivalent 7x10⁶ cfu/well) in 48 well plates for 36h. The effects of live and heat treated bacteria were tested on PBMCs from 8 individual donors splitted into two separated experiments. After 36h incubation, culture plates were frozen and kept at -20°C until cytokine measurement. Cytokine profiling was performed in parallel (i.e. in the same experiment on the same batch of PBMCs) for live bacteria and their heat-treated counterparts.

40 [0117] Levels of cytokines (IFN- γ , IL-12p40, TNF- α and IL-10) in cell culture supernatants after 36h incubation were determined by ELISA (R&D DuoSet Human IL-10, BD OptEIA Human IL12p40, BD OptEIA Human TNF α , BD OptEIA Human IFN- γ) following manufacturer's instructions. IFN- γ , IL-12p40 and TNF- α are pro-inflammatory cytokines, whereas IL-10 is a potent anti-inflammatory mediator. Results are expressed as means (pg/ml) +/- SEM of 4 individual donors and are representative of two individual experiments performed with 4 donors each. The ratio IL-12p40/IL-10 is calculated for each strain as a predictive value of *in vivo* anti-inflammatory effect (Foligné, B., et al., 2007, World J. Gastroenterol. 13:236-243).

45 [0118] Numerical cytokine values (pg/ml) determined by ELISA (see above) for each strain were transferred into BioNumerics v5.10 software (Applied Maths, Sint-Martens-Latem, Belgium). A Principal Component Analysis (PCA, dimensioning technique) was performed on this set of data. Subtraction of the averages over the characters and division by the variances over the characters were included in this analysis.

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Results

Anti-inflammatory profiles generated by Ultra High Temperature (UHT) / High Temperature Short Time (HTST)-like treatments

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[0119] The probiotic strains under investigation were submitted to a series of heat treatments (Ultra High Temperature (UHT), High Temperature Short Time (HTST) and 85°C for 20 min) and their immune profiles were compared to those of live cells *in vitro*. Live micro-organisms (probiotics and/or dairy starter cultures) induced different levels of cytokine production when incubated with human PBMC (Figures 1, 2, 3, 4 and 5). Heat treatment of these micro-organisms modified the levels of cytokines produced by PBMC in a temperature dependent manner. "Short-time high temperature" treatments (120°C or 140°C for 15") generated non replicating bacteria with anti-inflammatory immune profiles (Figures 1, 2, 3 and 4). Indeed, UHT-like treated strains (140°C, 15 sec) induced less pro-inflammatory cytokines (TNF- α , IFN- γ , IL-12p40) while maintaining or inducing additional IL-10 production (compared to live counterparts). The resulting IL-12p40 / IL-10 ratios were lower for any UHT-like treated strains compared to live cells (Figures 1, 2, 3 and 4). This observation was also valid for bacteria treated by HTST-like treatments, i.e. submitted to 120°C for 15 sec (Figures 1, 2, 3 and 4), or 74°C and 90°C for 15 sec (Figure 5). Heat treatments (UHT-like or HTST-like treatments) had a similar effect on *in vitro* immune profiles of probiotic strains (Figures 1, 2, 3 and 5) and dairy starter cultures (Figure 4). Principal Component Analysis on PBMC data generated with live and heat treated (140°C, 15") probiotic and dairy starter strains revealed that live strains are spread all along the x axis, illustrating that strains exhibit very different immune profiles *in vitro*, from low (left side) to high (right side) inducers of pro-inflammatory cytokines. Heat treated strains cluster on the left side of the graph, showing that pro-inflammatory cytokines are much less induced by heat treated strains (Figure 6). By contrast, bacteria heat treated at 85°C for 20 min induced more pro-inflammatory cytokines and less IL-10 than live cells resulting in higher IL-12p40 / IL-10 ratios (Figure 7).

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[0120] Anti-inflammatory profiles are enhanced or generated by UHT-like and HTST-like treatments.

[0121] UHT and HTST treated strains exhibit anti-inflammatory profiles regardless of their respective initial immune profiles (live cells). Probiotic strains known to be anti-inflammatory *in vivo* and exhibiting anti-inflammatory profiles *in vitro* (*B. longum* NCC 3001, *B. longum* NCC 2705, *B. breve* NCC 2950, *B. lactis* NCC 2818) were shown to exhibit enhanced anti-inflammatory profiles *in vitro* after "short-time high temperature" treatments. As shown in Figure 1, the IL-12p40 / IL-10 ratios of UHT-like treated *Bifidobacterium* strains were lower than those from the live counterparts, thus showing improved anti-inflammatory profiles of UHT-like treated samples. More strikingly, the generation of anti-inflammatory profiles by UHT-like and HTST-like treatments was also confirmed for non anti-inflammatory live strains. Both live *L. rhamnosus* NCC 4007 and *L. paracasei* NCC 2461 exhibit high IL-12p40 / IL-10 ratios *in vitro* (Figures 2 and 5). The two live strains were shown to be not protective against TNBS-induced colitis in mice. The IL-12p40 / IL-10 ratios induced by *L. rhamnosus* NCC 4007 and *L. paracasei* NCC 2461 were dramatically reduced after "short-time high temperature" treatments (UHT or HTST) reaching levels as low as those obtained with *Bifidobacterium* strains. These low IL-12p40 / IL-10 ratios are due to low levels of IL-12p40 production combined with no change (*L. rhamnosus* NCC 4007) or a dramatic induction of IL-10 secretion (*L. paracasei* NCC 2461) (Figure 2).

[0122] As a consequence:

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- Anti-inflammatory profiles of live micro-organisms can be enhanced by UHT-like and HTST-like heat treatments (for instance *B. longum* NCC 2705, *B. longum* NCC 3001, *B. breve* NCC 2950, *B. lactis* NCC 2818)

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- Anti-inflammatory profiles can be generated from non anti-inflammatory live micro-organisms (for example *L. rhamnosus* NCC 4007, *L. paracasei* NCC 2461, dairy starters *S. thermophilus* NCC 2019) by UHT-like and HTST-like heat treatments.

- Anti-inflammatory profiles were also demonstrated for strains isolated from commercially available products (Figures 3 A & B) including a probiotic *E. coli* strain.

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[0123] The impact of UHT/HTST-like treatments was similar for all tested probiotics and dairy starters, for example lactobacilli, bifidobacteria and streptococci.

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[0124] UHT/HTST-like treatments were applied to several lactobacilli, bifidobacteria and streptococci exhibiting different *in vitro* immune profiles. All the strains induced less pro-inflammatory cytokines after UHT/HTST-like treatments than their live counterparts (Figures 1, 2, 3, 4, 5 and 6) demonstrating that the effect of UHT/HTST-like treatments on the immune properties of the resulting non replicating bacteria can be generalized to all probiotics, in particular to lactobacilli and bifidobacteria and specific *E. coli* strains and to all dairy starter cultures in particular to streptococci, lactococci and lactobacilli.

Example 2:

Methodology

5 *Bacterial preparations:*

[0125] Five probiotic strains were used to investigate the immune boosting properties of non-replicating probiotics: 3 bifidobacteria (*B. longum* NCC3001, *B. lactis* NCC2818, *B. breve* NCC2950) and 2 lactobacilli (*L. paracasei* NCC2461, *L. rhamnosus* NCC4007).

10 **[0126]** Bacterial cells were grown on MRS in batch fermentation at 37°C for 16-18h without pH control. Bacterial cells were spun down (5,000 x g, 4°C) and resuspended in phosphate buffer saline prior to be diluted in saline water in order to reach a final concentration of around 10E10 cfu/ml. *B. longum* NCC3001, *B. lactis* NCC2818, *L. paracasei* NCC2461, *L. rhamnosus* NCC4007 were heat treated at 85°C for 20 min in a water bath. *B. breve* NCC2950 was heat treated at 90°C for 30 minutes in a water bath. Heat treated bacterial suspensions were aliquoted and kept frozen at -80°C until use. Live bacteria were stored at - 80°C in PBS-glycerol 15% until use.

In vitro immunoprofiling of bacterial preparations

20 **[0127]** The immune profiles of live and heat treated bacterial preparations (i.e. the capacity to induce secretion of specific cytokines from human blood cells *in vitro*) were assessed. Human peripheral blood mononuclear cells (PBMCs) were isolated from blood filters. After separation by cell density gradient, mononuclear cells were collected and washed twice with Hank's balanced salt solution. Cells were then resuspended in Iscove's Modified Dulbecco's Medium (IMDM, Sigma) supplemented with 10% foetal calf serum (Bioconcept, Paris, france), 1% L-glutamine (Sigma), 1% penicillin/streptomycin (Sigma) and 0.1% gentamycin (Sigma). PBMCs (7x10⁵ cells/well) were then incubated with live and heat treated bacteria (equivalent 7x10⁶ cfu/well) in 48 well plates for 36h. The effects of live and heat treated bacteria were tested on PBMCs from 8 individual donors splitted into two separate experiments. After 36h incubation, culture plates were frozen and kept at -20°C until cytokine measurement. Cytokine profiling was performed in parallel (i.e. in the same experiment on the same batch of PBMCs) for live bacteria and their heat-treated counterparts.

25 **[0128]** Levels of cytokines (IFN- γ , IL-12p40, TNF- α and IL-10) in cell culture supernatants after 36h incubation were determined by ELISA (R&D DuoSet Human IL-10, BD OptEIA Human IL12p40, BD OptEIA Human TNF, BD OptEIA Human IFN- γ) following manufacturer's instructions. IFN- γ , IL-12p40 and TNF- α are pro-inflammatory cytokines, whereas IL-10 is a potent anti-inflammatory mediator. Results are expressed as means (pg/ml) +/- SEM of 4 individual donors and are representative of two individual experiments performed with 4 donors each.

35 *In vivo effect of live and heat treated Bifidobacterium breve NCC2950 in prevention of allergic diarrhea*

[0129] A mouse model of allergic diarrhea was used to test the Th1 promoting effect of *B. breve* NCC2950 (Brandt E.B et al. JCI 2003; 112(11): 1666-1667). Following sensitization (2 intraperitoneal injections of Ovalbumin (OVA) and aluminium potassium sulphate at an interval of 14 days; days 0 and 14) male Balb/c mice were orally challenged with OVA for 6 times (days 27, 29, 32, 34, 36, 39) resulting in transient clinical symptoms (diarrhea) and changes of immune parameters (plasma concentration of total IgE, OVA specific IgE, mouse mast cell protease 1, i.e MMCP-1). *Bifidobacterium breve* NCC2950 live or heat treated at 90°C for 30min, was administered by gavage 4 days prior to OVA sensitization (days -3, -2, -1, 0 and days 11, 12, 13 and 14) and during the challenge period (days 23 to 39). A daily bacterial dose of around 10⁹ colony forming units (cfu) or equivalent cfu/mouse was used.

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Results

[0130] Induction of secretion of 'pro-inflammatory' cytokines after heat treatment

50 **[0131]** The ability of heat treated bacterial strains to stimulate cytokine secretion by human peripheral blood mononuclear cells (PBMCs) was assessed *in vitro*. The immune profiles based on four cytokines upon stimulation of PBMCs by heat treated bacteria were compared to that induced by live bacterial cells in the same *in vitro* assay.

[0132] The heat treated preparations were plated and assessed for the absence of any viable counts. Heat treated bacterial preparations did not produce colonies after plating.

55 **[0133]** Live probiotics induced different and strain dependent levels of cytokine production when incubated with human PBMCs (Figure 8). Heat treatment of probiotics modified the levels of cytokines produced by PBMCs as compared to their live counterparts. Heat treated bacteria induced more pro-inflammatory cytokines (TNF- α , IFN- γ , IL-12p40) than their live counterparts do. By contrast heat treated bacteria induced similar or lower amounts of IL-10 compared to live cells (Figure 8). These data show that heat treated bacteria are more able to stimulate the immune system than their

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live counterparts and therefore are more able to boost weakened immune defences. In other words the *in vitro* data illustrate an enhanced immune boost effect of bacterial strains after heat treatment.

[0134] In order to illustrate the enhanced effect of heat-treated *B. breve* NCC2950 (compared to live cells) on the immune system, both live and heat treated *B. breve* NCC2950 (strain A) were tested in an animal model of allergic diarrhea.

[0135] As compared to the positive control group, the intensity of diarrhea was significantly and consistently decreased after treatment with heat treated *B. breve* NCC2950 ($41.1\% \pm 4.8$) whereas the intensity of diarrhea was lowered by only $20 \pm 28.3\%$ after treatment with live *B. breve* NCC2950. These results demonstrate that heat-treated *B. breve* NCC2950 exhibits an enhanced protective effect against allergic diarrhea than its live counterpart (Figure 9).

[0136] As a consequence, the ability of probiotics to enhance the immune defences was shown to be improved after heat treatment.

Further Examples:

[0137] The following growing up milk compositions may be prepared:

	>10 months
Protein (g/100kcal)	2.23
Whey/Casein	50/50
CHO (g/100kcal)	13.0
Lactose (g/100kcal)	7.7
Maltodextrine (g/100kcal)	4.0
Starch (g/100kcal)	1.0
Sucrose (g/100kcal)	
Fat (g/100kcal)	4.31
Prebiotics (g/100kcal)	-
Energy Kcal/100 ml	70

[0138] The growing up milk composition contains 10^9 cfu *Lactobacillus johnsonii* La1 / g dry weight

	>1year					
Protein (g/100kcal)	2.7	2.22	2.23	2.3	2.9	2.26
Whey/Casein	23/77	40/60	40/60	40/60	77/23	60/40
CHO (g/100kcal)	12.2	13.5	13.1	13.0	11.9	13.9
Lactose (g/100kcal)	5.05	6.7	6.1	4.9	4.42	5.33
Maltodextrine (g/100kcal)	4.99	5.8	5.5	4.9	2.31	2.35
Starch (g/100kcal)	-	1.0	1.0	2.9	2.29	3.17
Sucrose (g/100kcal)	1.93				2.66	2.41
Fat (g/100kcal)	4.5	4.14	4.31	4.3	4.53	3.93
Prebiotics (g/100kcal)	-	0.58	0.58	0.52	-	0.49
Energy Kcal/100 ml	70	70	70	79	80	

[0139] The growing up milk compositions contain 10^9 cfu heat treated (75°C,20min) *Bifidobacterium longum* NCC 3001 / g dry weight

	>2 years	
Protein (g/100kcal)	2.5	2.5

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(continued)

	>2 years		
5	Whey/Casein	50/50	40/60
	CHO (g/100kcal)	14.6	14.6
	Lactose (g/100kcal)	8.7	6.9
10	Maltodextrine (g/100kcal)	4.6	6.6
	Starch (g/100kcal)	1.0	1.0
	Sucrose (g/100kcal)		
	Fat (g/100kcal)	3.5	3.5
15	Prebiotics (g/100kcal)	-	0.58
	Energy Kcal/100 ml	70	70

[0140] The growing up milk compositions contain 10^9 cfu heat treated UHT treated *Lactobacillus johnsonii* La1 / g dry weight

Claims

- 25 **1.** Growing-up-milk composition comprising probiotic micro-organisms to be administered to infants or young children starting from the age of 10 months, wherein the probiotic micro-organisms comprise non-replicating probiotic micro-organisms, and at least 90 % of said probiotics micro-organisms are non-replicating probiotic micro-organisms, wherein said non-replicating probiotic micro-organisms is *Bifidobacterium breve* NCC 2950, wherein said composition has a caloric density in the range of 70-80 kcal/100ml, and comprises a protein source in an amount of 2-3 g/100kcal, a carbohydrate source in an amount of 12-15 g/100kcal, and a lipid source in an amount of 3-4.6 g/100kcal.
- 2.** Composition in accordance with claim 1 comprising 0.15-0.25 g LC-PUFA/100g fatty acids, wherein the LC-PUFA may be selected from ARA, DHA or combinations thereof.
- 35 **3.** Composition in accordance with claim 1 or claim 2, comprising 1.5-2.5 mg nucleotides per 100 mL formula.
- 4.** Composition in accordance with one of the preceding claims comprising probiotic micro-organisms in an amount corresponding to about 10^6 to 10^{12} cfu.
- 40 **5.** Composition in accordance with one of claims 3 or 4, wherein the non-replicating probiotic micro-organisms were rendered non-replicating by a heat-treatment, preferably by a high temperature treatment at at least 71.5 °C for at least 1 second.
- 45 **6.** Composition in accordance with claim 5, wherein the heat treatment is a high temperature treatment at 71.5-150 °C for about 1-120 seconds, and preferably is a high temperature/short time (HTST) treatment or a ultra-high temperature (UHT) treatment.
- 7.** Composition in accordance with claim 6 for use in the prevention or treatment of inflammatory disorders.
- 50 **8.** Composition in accordance with claim 5, wherein the heat treatment is carried out in the temperature range of about 70-150 °C for about 3 minutes - 2 hours, preferably in the range of 80-140°C from 5 minutes - 40 minutes.
- 9.** Composition in accordance with claim 8 for use in the prevention or treatment of disorders related to a compromised immune defence.
- 55 **10.** Composition in accordance with one of the preceding claims wherein at least 95 %, more preferably at least 98 %, most preferably at least 99 %, ideally at least 99.9 %, most ideally all of the probiotics are non-replicating.

11. Composition in accordance with one of the preceding claims containing 0,005 mg - 1000 mg non-replicating microorganisms per daily dose.

5 **Patentansprüche**

1. Wachstumsmilchzusammensetzung, die probiotische Mikroorganismen umfasst, zur Verabreichung an Säuglinge oder Kleinkinder ab dem Alter von 10 Monaten, wobei die probiotischen Mikroorganismen nicht-replizierende probiotische Mikroorganismen umfassen und es sich bei mindestens 90 % der probiotischen Mikroorganismen um nicht-replizierende probiotische Mikroorganismen handelt, wobei es sich bei den nicht-replizierenden probiotischen Mikroorganismen um *Bifidobacterium breve* NCC 2950 handelt, wobei die Zusammensetzung eine kalorische Dichte im Bereich von 70-80 kcal/100 ml aufweist und eine Proteinquelle in einer Menge von 2-3 g/100 kcal, eine Kohlenhydratquelle in einer Menge von 12-15 g/100 kcal und eine Lipidquelle in einer Menge von 3-4,6 g/100 kcal umfasst.
2. Zusammensetzung nach Anspruch 1, die 0,15-0,25 g LC-PUFA/100 g Fettsäure umfasst, wobei das LC-PUFA aus ARA, DHA oder Kombinationen davon ausgewählt sein kann.
- 20 3. Zusammensetzung nach Anspruch 1 oder Anspruch 2, die 1,5-2,5 mg Nukleotide je 100 ml Säuglingsmilchnahrung umfasst.
4. Zusammensetzung nach einem der vorstehenden Ansprüche, die probiotische Mikroorganismen in einer Menge umfasst, die etwa 10^6 bis 10^{12} KBE entspricht.
- 25 5. Zusammensetzung nach einem der Ansprüche 3 oder 4, wobei die nicht-replizierenden probiotischen Mikroorganismen mittels einer Wärmebehandlung, vorzugsweise einer Hochtemperaturbehandlung bei mindestens 71,5 °C für mindestens 1 Sekunde, nicht-replizierend gemacht wurden.
- 30 6. Zusammensetzung nach Anspruch 5, wobei es sich bei der Wärmebehandlung um eine Hochtemperaturbehandlung bei 71,5-150 °C für etwa 1-120 Sekunden und vorzugsweise um eine Kurzzeit-Hochtemperaturbehandlung (high temperature/short time, HTST) oder eine Ultrahochtemperaturbehandlung (ultra-high temperature, UHT) handelt.
- 35 7. Zusammensetzung nach Anspruch 6 zur Verwendung bei der Prävention oder Behandlung von entzündlichen Erkrankungen.
8. Zusammensetzung nach Anspruch 5, wobei die Wärmebehandlung in einem Temperaturbereich von etwa 70-150 °C für etwa 3 Minuten - 2 Stunden, vorzugsweise im Bereich von 80-140 °C für 5 Minuten - 40 Minuten, durchgeführt wird.
- 40 9. Zusammensetzung nach Anspruch 8 zur Verwendung bei der Prävention oder Behandlung von Erkrankungen im Zusammenhang mit einer beeinträchtigten Immunabwehr.
- 45 10. Zusammensetzung nach einem der vorstehenden Ansprüche, wobei mindestens 95 %, mehr bevorzugt mindestens 98 %, am meisten bevorzugt mindestens 99 %, idealerweise mindestens 99,9 % und im absoluten Idealfall alle der Probiotika nicht-replizierend sind.
- 50 11. Zusammensetzung nach einem der vorstehenden Ansprüche, die 0,005 mg - 1000 mg nicht-replizierender Mikroorganismen je Tagesdosis enthält.

Revendications

- 55 1. Composition de lait de croissance comprenant des micro-organismes probiotiques à administrer à des nourrissons ou de jeunes enfants à partir de l'âge de 10 mois, dans laquelle les micro-organismes probiotiques comprennent des micro-organismes probiotiques non réplcatifs et au moins 90 % desdits micro-organismes probiotiques sont des micro-organismes probiotiques non réplcatifs, dans laquelle lesdits micro-organismes probiotiques non réplcatifs sont du *Bifidobacterium breve* NCC 2950,

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dans laquelle ladite composition a une densité calorique dans la plage de 70 à 80 kcal/100 ml et comprend une source de protéine en une quantité de 2 à 3 g/100 kcal, une source de glucide en une quantité de 12 à 15 g/100 kcal et une source de lipide en une quantité de 3 à 4,6 g/100 kcal.

- 5 **2.** Composition selon la revendication 1, comprenant 0,15 à 0,25 g d'acides gras polyinsaturés à chaîne longue (LC-PUFA)/100 g d'acides gras, dans laquelle l'acide gras polyinsaturé à chaîne longue peut être choisi parmi l'acide arachidonique (ARA), l'acide docosahexaénoïque (DHA) ou leurs combinaisons.
- 10 **3.** Composition selon la revendication 1 ou la revendication 2, comprenant 1,5 à 2,5 mg de nucléotides pour 100 mL de formulation.
- 15 **4.** Composition selon l'une des revendications précédentes, comprenant des micro-organismes probiotiques en une quantité correspondant à environ 10^6 à 10^{12} ufc.
- 20 **5.** Composition selon l'une des revendications 3 ou 4, dans laquelle les micro-organismes probiotiques non répliatifs ont été rendus non répliatifs par un traitement thermique, de préférence par un traitement à haute température à au moins 71,5 °C pendant au moins 1 seconde.
- 25 **6.** Composition selon la revendication 5, dans laquelle le traitement thermique est un traitement à haute température à 71,5 à 150 °C pendant environ 1 à 120 secondes et est de préférence un traitement à haute température/courte durée (HTST) ou un traitement à ultra-haute température (UHT).
- 30 **7.** Composition selon la revendication 6, destinée à être utilisée dans la prévention ou le traitement de troubles inflammatoires.
- 35 **8.** Composition selon la revendication 5, dans laquelle le traitement thermique est effectué dans la plage de température d'environ 70 à 150 °C pendant environ 3 minutes à 2 heures, de préférence dans la plage de 80 à 140 °C de 5 minutes à 40 minutes.
- 40 **9.** Composition selon la revendication 8, destinée à être utilisée dans la prévention ou le traitement de troubles se rapportant à une défense immunitaire affaiblie.
- 45 **10.** Composition selon l'une des revendications précédentes, dans laquelle au moins 95 %, plus préféablement au moins 98 %, le plus préféablement au moins 99 %, idéalement au moins 99,9 %, de la manière la plus idéale, tous les probiotiques sont non répliatifs.
- 50 **11.** Composition selon l'une des revendications précédentes, contenant 0,005 mg à 1000 mg de micro-organismes non répliatifs par dose journalière.
- 55

Figure 1A

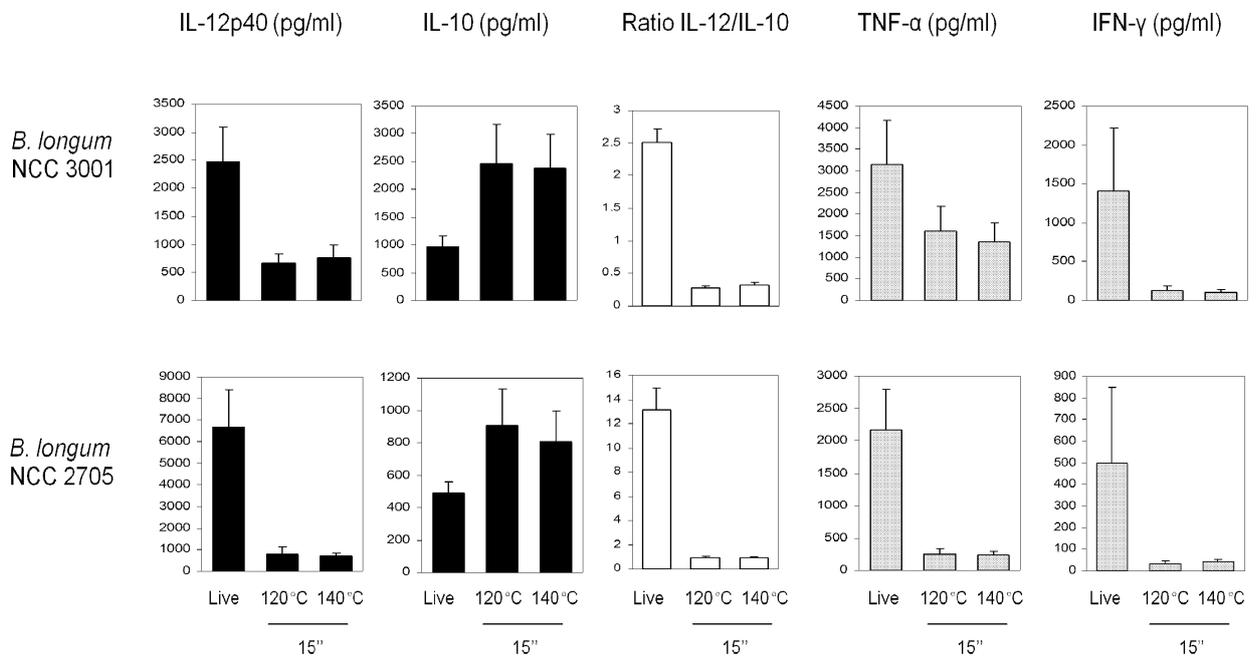


Figure 1B

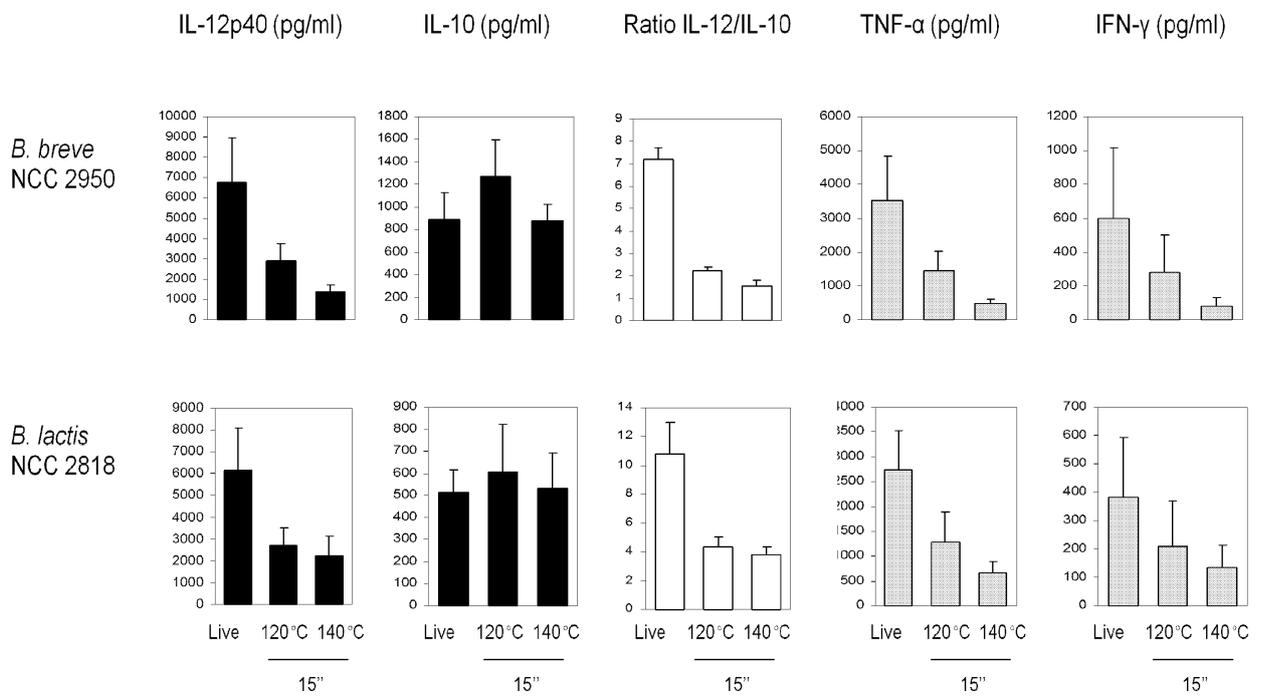


Figure 2

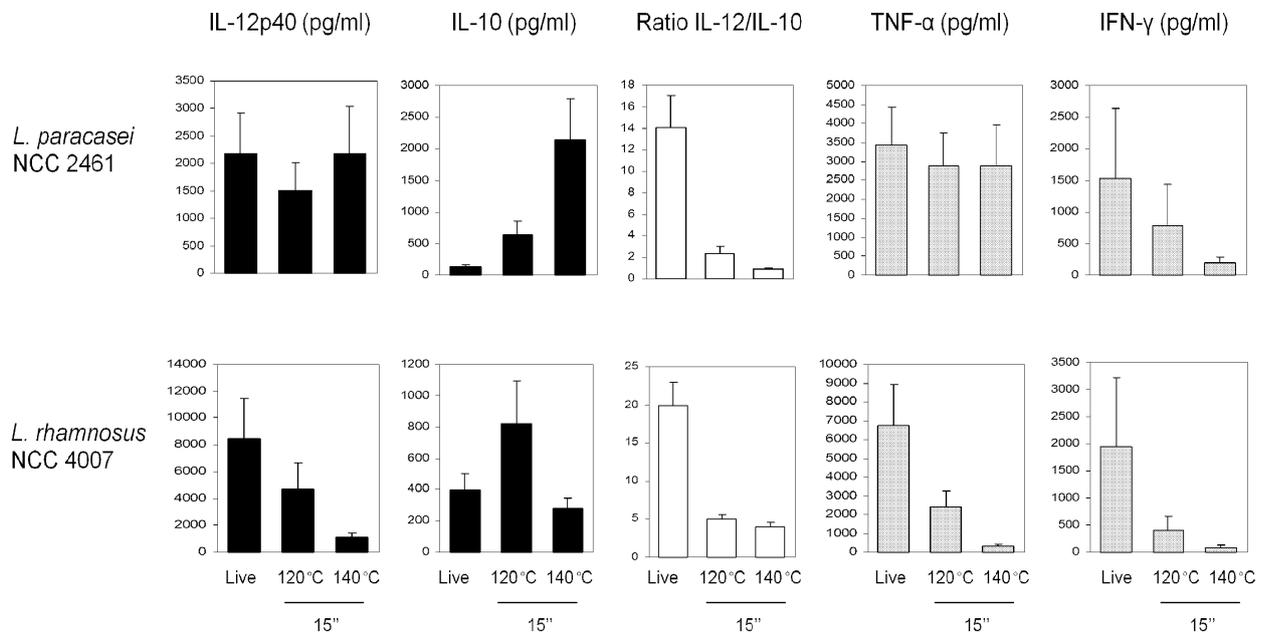


Figure 3A

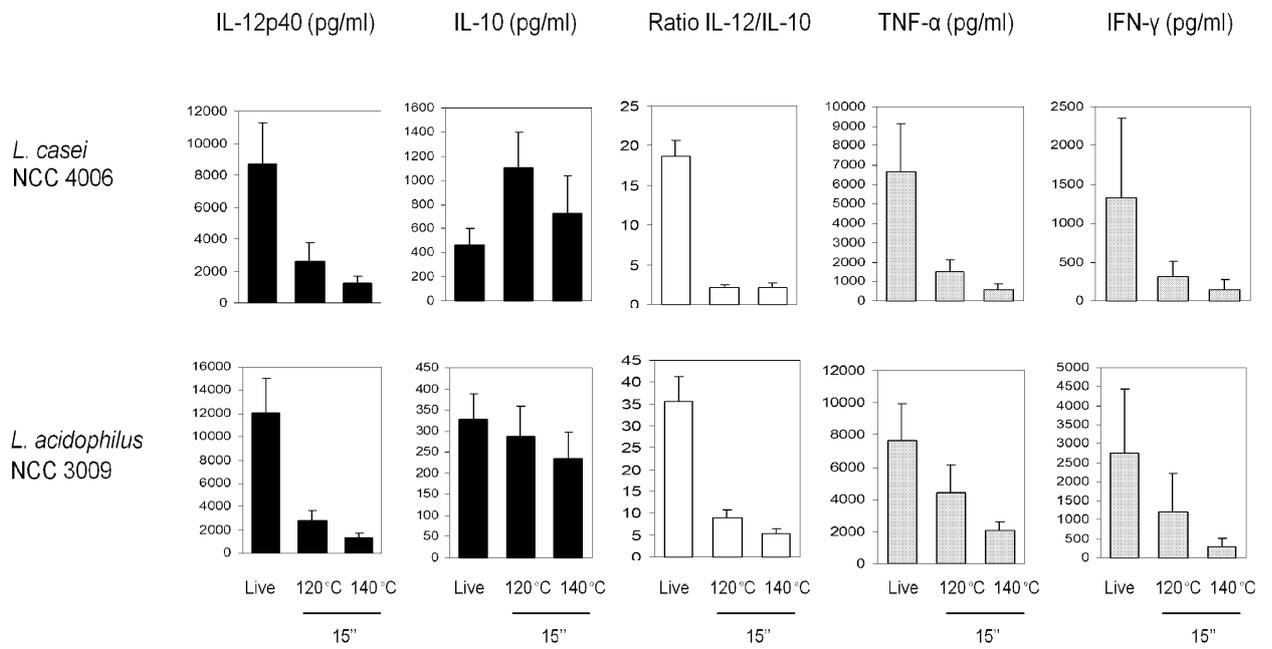


Figure 3B

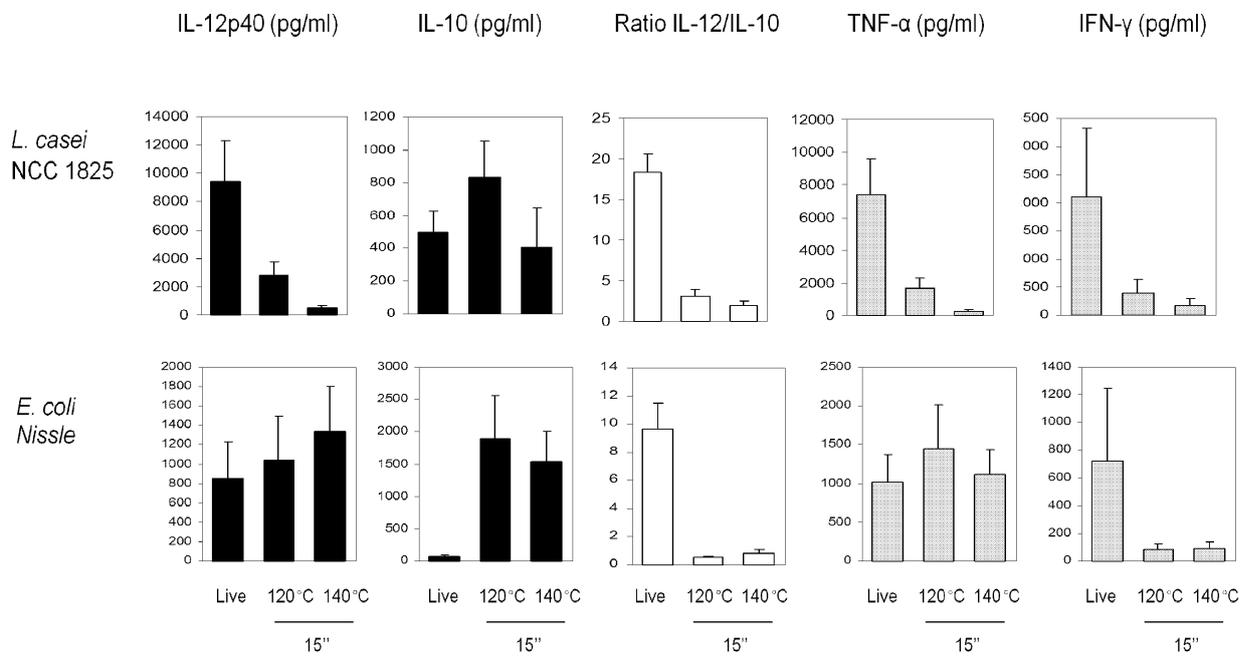


Figure 4A

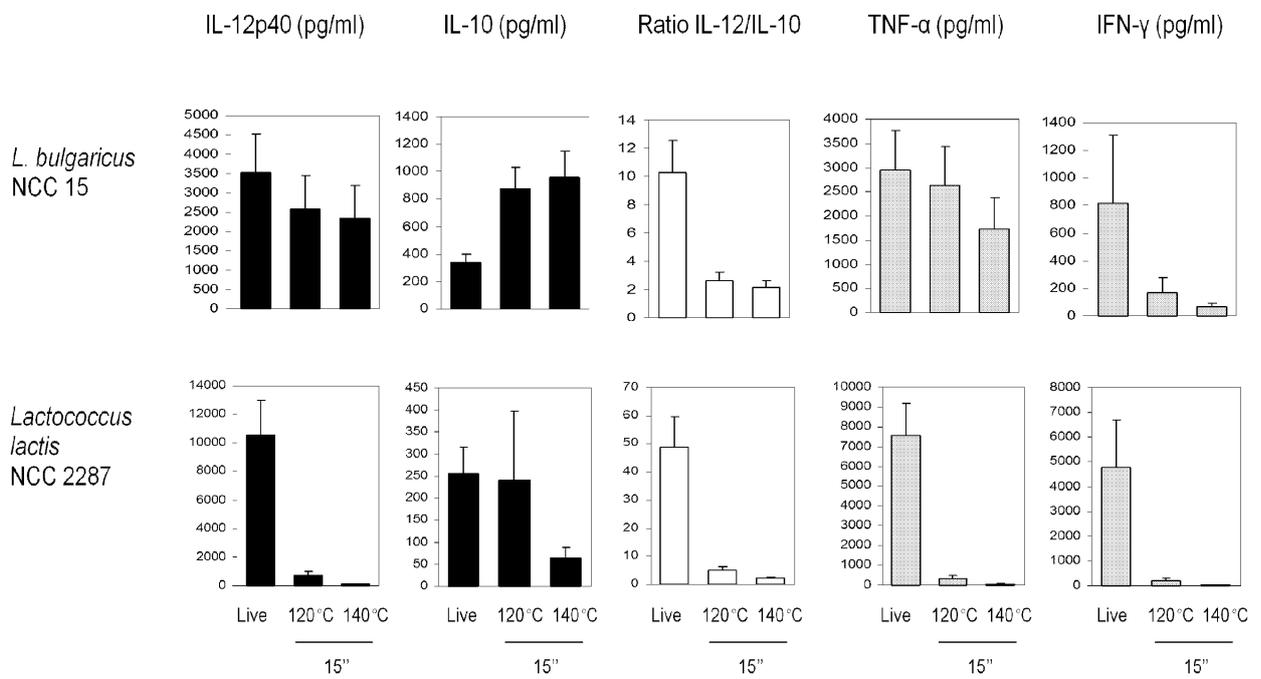


Figure 4B

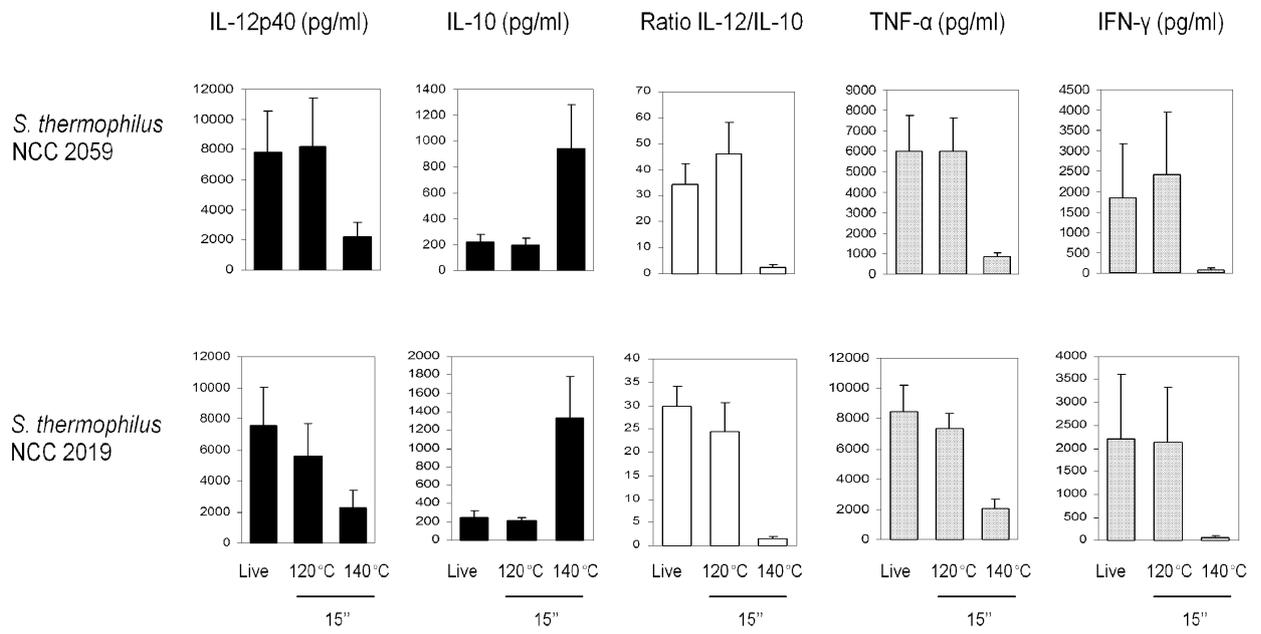


Figure 5

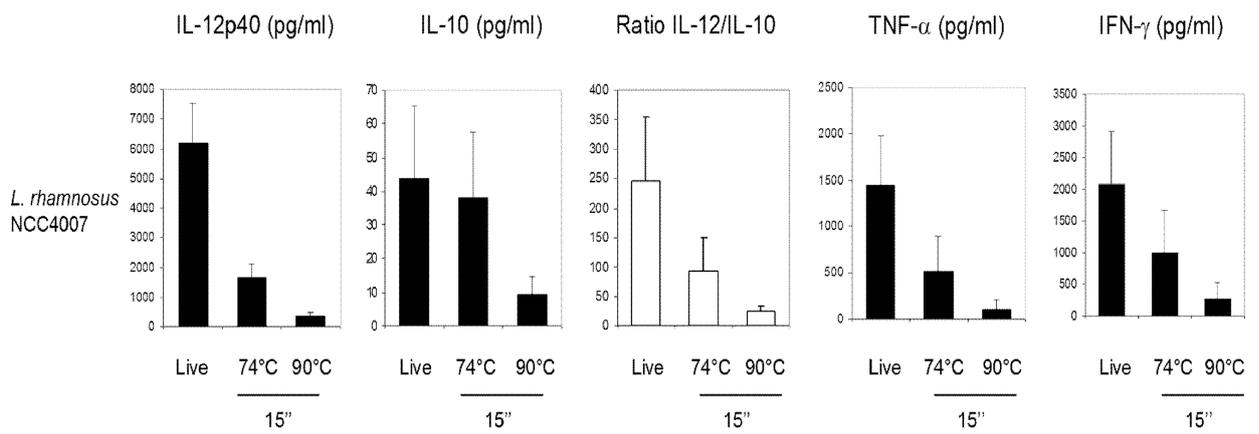


Figure 6

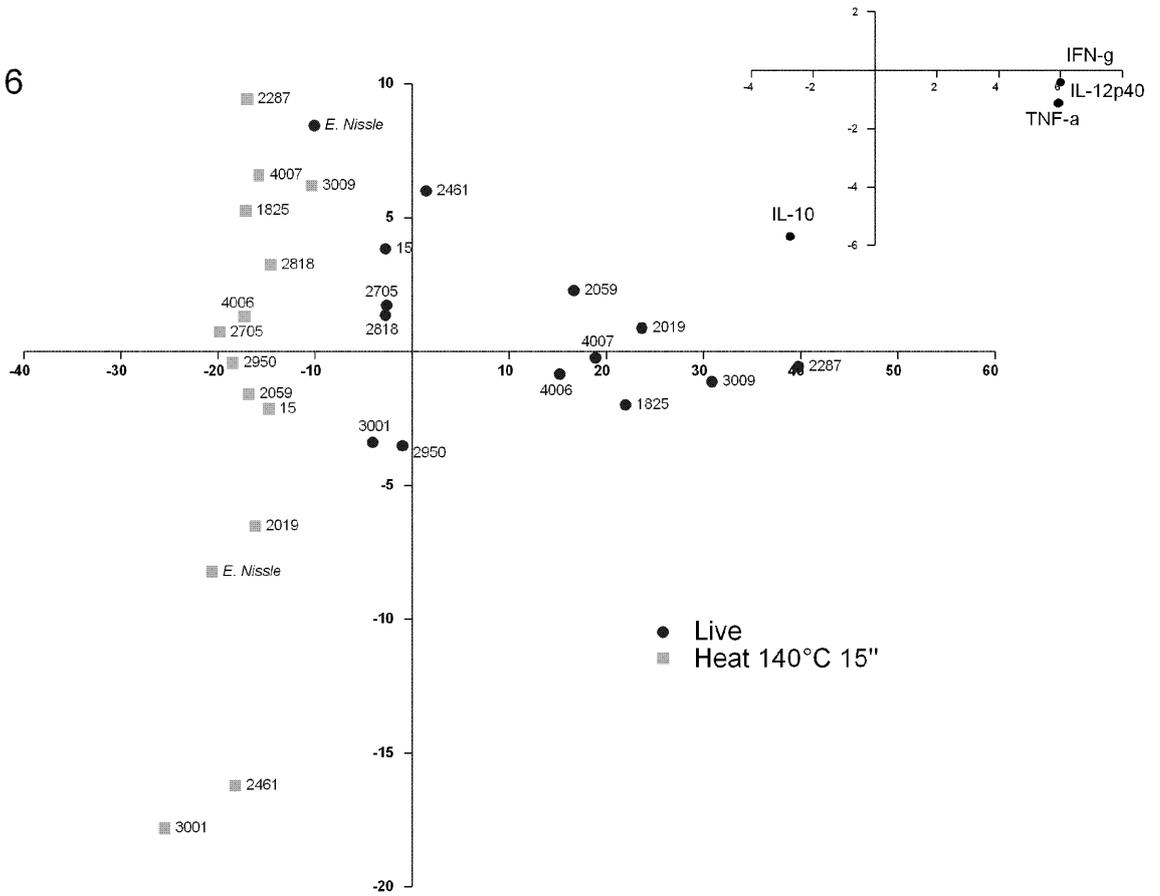


Figure 7

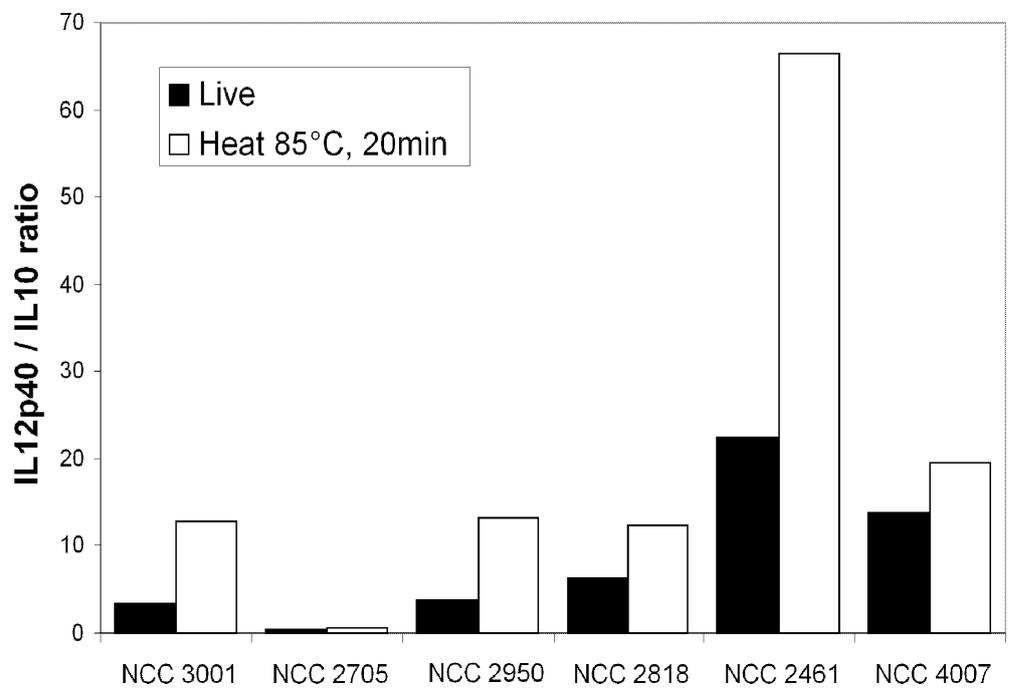


Figure 8:

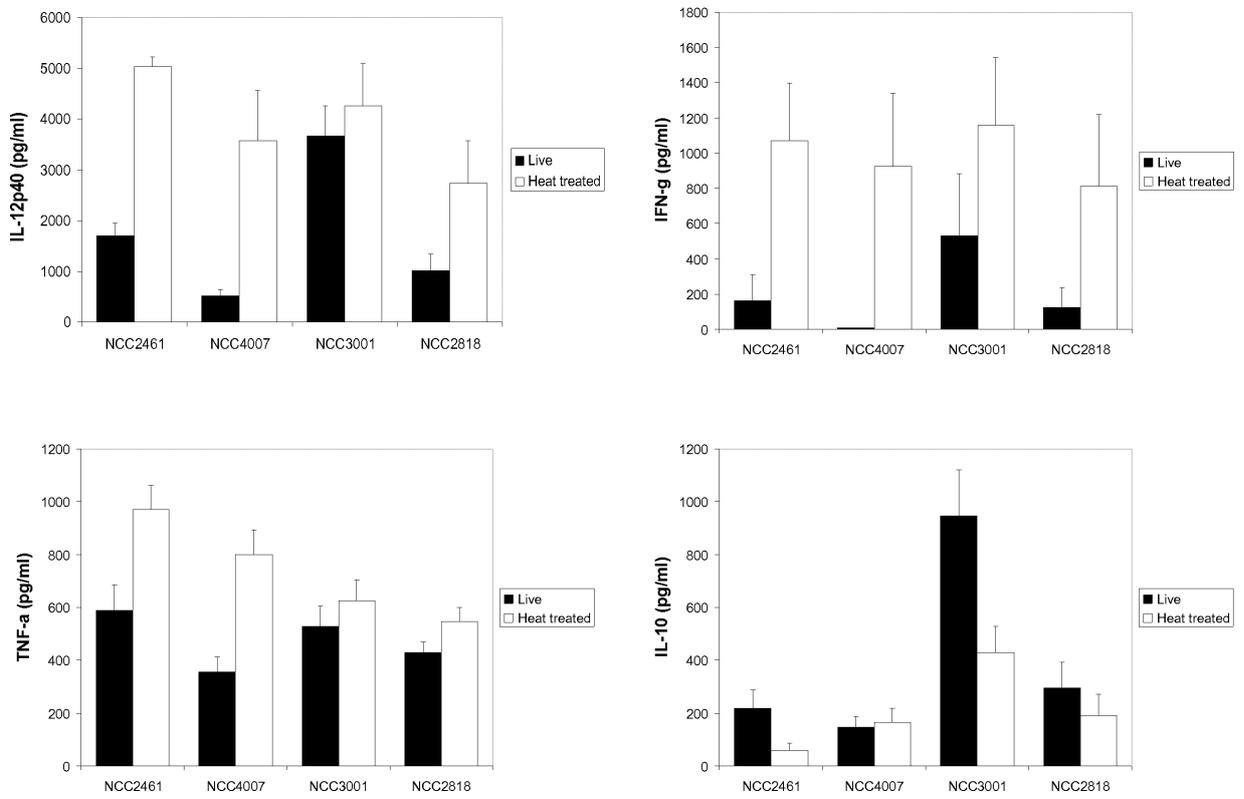
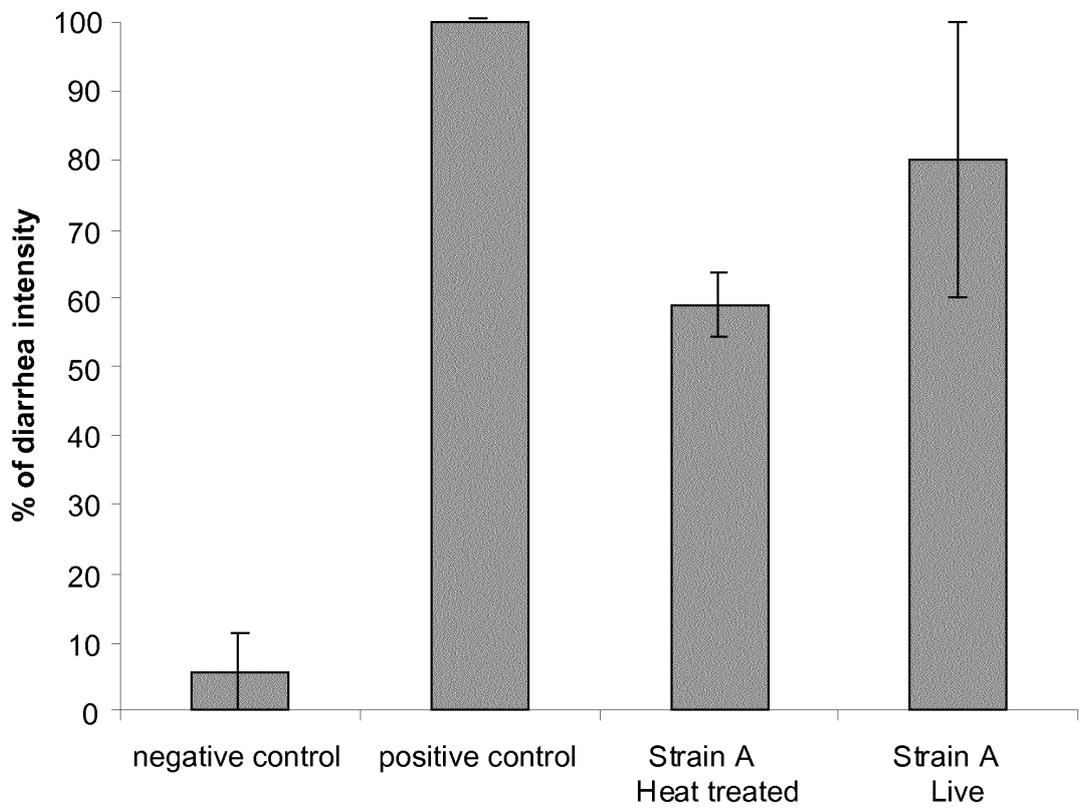


Figure 9:



REFERENCES CITED IN THE DESCRIPTION

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