Title: BIFIDOBACTERIUM LONGUM NCC2705 (CNCM 1-2618) AND IMMUNE DISORDERS

Abstract: The present invention generally relates to the field of preventing and/or treating inflammatory and infectious disorders, in particular by boosting the endogenous antimicrobial defences. One embodiment of the present invention is the use of B. longum NCC2705 (deposit number CNCM 1-2618) for use in the treatment or prevention of disorders related to the immune system including infections.
Bifidobacterium longum NCC2705 (CNCM 1-2618) and immune disorders

The present invention generally relates to the field of preventing and/or treating inflammatory and infectious disorders, in particular by boosting the endogenous antimicrobial defences. One embodiment of the present invention is the use of B. longum NCC2705 (deposit number CNCM 1-2618) for use in the treatment or prevention of disorders related to the immune system including infections.

Our environment is contaminated by a vast array of potentially pathogenic microorganisms. Skin keratinocytes, epithelial cells lining the gastrointestinal tract, respiratory tract, genitourinary tract all provide a physical barrier that protect against microbial intrusion into the body.

In addition, these epithelia contribute to the host defences by producing and secreting antimicrobials to limit access of bacteria and other microorganisms. These antimicrobial molecules constitute key components of the basic defence line of the innate immunity.

Defensins are one of the most important classes of antimicrobial peptides in humans. Defensins are produced by epithelial cells of the lung, skin, oral cavity, genitourinary, respiratory and gastrointestinal tract. Among these, there is the family of β-defensins including the defensin 1 (hBD1) and 2 (hBD2).

HBD1 is expressed in various mucosal surfaces such as oral mucosa, salivary gland, stomach, small intestine, colon, liver and pancreas. HBD2 is also present in epithelial cells at multiple mucosal surfaces including that of gastrointestinal
tract. Moreover, these two defensins are also present in saliva and airway surface fluid (Cunliffe, R.N. and Mahida, Y.R. 2004, J Leukoc.Biol. 75:49-58).

HBD2 is present at very low levels in normal tissues, and its expression is up-regulated by bacteria and pro-inflammatory cytokines. Contrary to hBD2, HBD1 is constitutively expressed. HBD1 has never been shown to be consistently up-regulated by bacteria or inflammation (Ou, G., et al., 2009, Scand.J Immunol 69:150-161).

Probiotics are well known to be able to reinforce the various lines of gut defence: immune exclusion, immune elimination, and immune regulation. Probiotics are also known stimulate non-specific host resistance to microbial pathogens and thereby aid in their eradication.

However, despite this, the expression of the constitutive hBD1 has been reported as unaffected by probiotic bacteria (O'Neil, D.A. et al., J Immunol 163:6718-6724) and as very mildly upregulated by commensal (Escherichia coli) and pathogenic (Salmonella typhimurium) strains (Ou, G., et al., 2009, Scand.J Immunol 69:150-161).

The application of probiotics currently lies in reducing the risk of diseases associated with gut barrier dysfunction (E. Isolauri, et al, 2002, Gut 2002;50:iii 54-iii 59). Probiotics are thought to be effective through survival in the gut, acid and bile stability, and temporal colonisation of the mucosal surfaces in the intestinal tract.

Therefore, the vast majority of published literature deals with live probiotics. However, several studies investigated the health benefits delivered by non-replicating bacteria and most of them indicated that inactivation of probiotics, e.g.

However, working with viable bacteria in food products today has several disadvantages. Viable bacteria are usually not very stress resistant and are consequently difficult to handle in industrial scales while maintaining viability. Furthermore, for some product categories it may not be optimal to add viable micro-organisms to the formulation due to safety concerns.

On the other hand, the provision of non-replicating probiotic micro-organisms allows the hot reconstitution, e.g., of powdered nutritional compositions while retaining health benefit for the consumer patient.

Based thereon it may be desirable to work with non-replicating bacteria instead of their live counterparts, but the studies available in this respect are not encouraging.

The use of live probiotics as a strategy to treat or prevent inflammatory bowel diseases has been reported in the literature and recently reviewed by Dotan et al. (Dotan, I. and D. Rachmilewit z. 2005; Curr.Opin.Gastroenterol . 21:426-430). For, example, a highly concentrated cocktail of eight live probiotic bacteria (VSL#3) has been shown to be effective in prevention (Gionchetti, P., et al., 2003, Gastroenterology 124:1202-1209) and treatment of recurrent or refractory pouchitis in humans (Gionchetti, P., et al., 2000, Gastroenterology 119:305-309; Mimura, T., et al., 2004, Gut 53:108-114). Interestingly using a murine model of DSS-induced
colitis, Rachmilewitz et al. (Rachmilewitz, D., et al., 2004, Gastroenterology 126:520-528) reported that treatments with viable and γ-irradiated VSL#3 but not heat-killed VSL#3 protect against colitis. Similarly, heat-killed L. crispatus failed to protect against DSS-induced colitis while its viable counterpart clearly reduced the loss of body weight and the MPO activity in the gut (Castagliuolo, et al., 2005, FEMS Immunol. Med. Microbiol. 43:197-204). These studies suggest that probiotics are more effective alive in the context of gut inflammation than their non-replicating counterparts.

Inactivated L. reuteri (heat-killed and γ-irradiated) was found not to be able to decrease the TNFα-induced IL-8 production by T84 cells while its live counterpart exhibited a significant beneficial effect (Ma, D., et al., 2004, Infect. Immun. 72:5308-5314).

Hence, there is a need in the art for natural compositions that are easy to handle under industrial conditions, that are safe and easy to administer and that allow preventing and/or treating inflammatory and infectious disorders, in particular by boosting the endogenous antimicrobial defences.

Ideally the natural composition should be prepared from probiotic cultures, in particular from a probiotic microorganism that is well accepted today and recognized by consumers for delivering health benefits. Advantageously, the composition should contain non-replicating bacteria and should be more effective than their live counterpart.

The present inventors have addressed this need.

Hence, it was the object of the present invention to improve the state of the art and to provide a natural composition, that allows preventing and/or treating inflammatory and
infectious disorders, in particular by boosting the endogenous antimicrobial defences and that fulfils the requirements listed above.

The inventors were surprised to see that they could achieve the object of the present invention by the subject matter of the independent claims. The dependant claims further define preferred embodiments of the present invention.

The subject matter of the present invention strengthens the mammalian endogenous antimicrobial defences by administering a product containing micro-organisms, such as non-replicating micro-organism, for example heat-treated microorganisms.

The inventors describe that *B. longum* NCC 2705 (deposit number CNCM 1-2618) has superior effects on the induction of antimicrobial peptide expression than those previously identified and described in the literature.

It was found, for example, that:

- *B. longum* NCC 2705 (deposit number CNCM 1-2618) induces hBD2 expression more strongly than other micro-organisms and also than the combination of 8 bacteria (Mondel, M., et al., 2009, Mucosal Immunol 2:166-172; Schlee, M., et al., 2007, Infect. Immun. 75:2399-2407), and

- heat-treated *B. longum* NCC 2705 (deposit number CNCM 1-2618) up-regulates hBD2 more strongly than its live counterpart and, in addition, upregulates hBD1.

HBD1 and hBD2 display antibacterial activity against a broad spectrum of bacteria including *E. coli* and *Pseudomonas aeruginosa*, *H. pylori* (Nuding, S., et al., 2009, Microbes Infect., 11:384-393) and also against yeasts such as *Candida albicans*

More and more evidence indicate that the levels of defensins are reduced in certain pathophysiological conditions and that this is a risk factor in the pathogenesis and complications of infectious and inflammatory diseases such as (Doss, M. et al., 2010, J Leukoc.Biol 87:79-92; Rivas-Santiago, B. et al., 2009, Infect. Immun. 77:4690-4695):

- In the respiratory tract:
cystic fibrosis, reactive airways disease, lung infections and tobacco smoking, asthma, pneumonia, rhinitis, otitis, sinusitis, tuberculosis

- In the gastrointestinal tract:
Crohn's disease (colon and ileum), ulcerative colitis, gastritis and gastric ulcer induced by Helicobacter pylori infection, infectious diarrhea, necrotising enterocolitis, antibiotic-associated diarrhea, intestinal immaturity.

- In the genitourinary tract:
Bacterial vaginosis, HIV, Herpes simplex virus, urinary infection

- In the skin:
Atopic dermatitis, chronic ulcer, carcinoma, atopic eczema, burn injury
In the oral cavity:
HIV patients, tonsillitis, gingivitis, dental caries
Keratitis in eyes

The results presented herein indicate that *B. longum* NCC 2705 (CNCM 1-2618) has a stronger capacity to boost the endogenous antimicrobial defence than previously identified probiotic bacteria, and thus may be more efficient in the prevention and treatment of SIBO (Small intestinal Bacterial Overgrowth), inflammatory and infectious disorders. In addition, the inventors data indicate - contrary to what would be expected from the literature - that heat treatment does not decrease, but further increases the strong antimicrobial effect of *B. longum* NCC 2705 (deposit number CNCM 1-2618).

Hence, one embodiment of the present invention is a composition comprising *B. longum* NCC 2705 (deposit number CNCM 1-2618) for use in the treatment or prevention of disorders related to the immune system including infections.

According to the present invention the disorders linked to the immune system may be treated or prevented by increasing endogenous hBD1 and/or hBD2 expression.

The present invention also relates to a composition comprising *B. longum* NCC 2705 (deposit number CNCM 1-2618) for use in the treatment or prevention of disorders linked to a decreased hBD1 expression, such as microbial infections, for example.

The present invention also concerns the use of *B. longum* NCC 2705 (deposit number CNCM 1-2618) in the preparation of a composition for the treatment or prevention of disorders linked to the immune system.
Non-replicating *B. longum* NCC 2705 (deposit number CNCM 1-2618) may be used at least partially. Non-replicating, in particular heat treated, *B. longum* NCC 2705 (deposit number CNCM 1-2618) have the advantage of being even more effective than their live counterpart.

The use of non-replicating microorganisms, such as heat-treated *B. longum* NCC 2705 (deposit number CNCM 1-2618), instead of their live counterparts, has further the advantages to:

- reduce the potential risk of live probiotic-associated sepsis in the sensitive targeted populations,
- represent a safe alternative to immunocompromised patients, and
- lower processing hurdles, can be integrated in shelf stable liquid products with an long shelf life.

Hence, in one embodiment of the present invention at least 90%, for example at least 95 % preferably at least 98 %, most preferably at least 99 %, ideally at least 99.9 %, or all of the *B. longum* NCC 2705 (deposit number CNCM 1-2618) are non-replicating.

The present invention also relates to a composition comprising *B. longum* NCC 2705 (deposit number CNCM 1-2618), wherein at least 95 % preferably at least 98 %, most preferably at least 99 %, ideally at least 99.9 %, or 100 % of the *B. longum* NCC 2705 (deposit number CNCM 1-2618) are non-replicating.

Thus, the present invention also relates to bioactive, non-replicating, e.g., heat treated, *B. longum* NCC 2705 (deposit number CNCM 1-2618).
"Non-replicating" \textit{B. longum} NCC 2705 include \textit{B. longum} NCC 2705 (deposit number CNCM 1-2618), which have been heat treated. This includes \textit{B. longum} NCC 2705 that are inactivated, dead, non-viable and/or present as fragments such as DNA, metabolites, cytoplasmic compounds, and/or cell wall materials.

"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).

The \textit{B. longum} NCC 2705 may be rendered non-replicating by heat inactivation. Heat inactivation may occur at at least about 70° C.

Any heat treatment may be used to inactivate the probiotics as long as it is carried out long enough to achieve inactivation. For example, such a heat treatment may be carried out for at least 10 seconds.

Typically a high temperature will require a short heating time, while lower temperatures will require longer heating.

For example, the \textit{B. longum} NCC 2705 (deposit number CNCM 1-2618) may be rendered non-replicating at 110° to 140° for 1-30 seconds, e.g. 10-20 seconds or 75° to 95° for 10-30 minutes.
This given time frame refers to the time the *B. longum* NCC 2705 (deposit number CNCM 1-2618) are subjected to the given temperature. Note that depending on the nature and amount of the composition the *B. longum* NCC 2705 (deposit number CNCM 1-2618) are provided in and depending on the architecture of the heating apparatus used, the time of heat application may differ.

The 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. An ideal pressure to be applied will depend on the nature of the composition which the micro-organisms are provided in and on the temperature used.

If the compositions the *B. longum* NCC 2705 (deposit number CNCM 1-2618) are provided in are anyway heat treated, e.g., before they are packaged and distributed, it may be preferable to use this heat treatment step to inactivate *B. longum* NCC 2705 (deposit number CNCM 1-2618).

Typically, compositions containing *B. longum* NCC 2705 (deposit number CNCM 1-2618) may be treated by a high temperature short time (HTST) treatment, flash pasteurization or a ultra high temperature (UHT) treatment.

A UHT treatment is Ultra-high temperature processing or an 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.

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.

A HTST treatment is defined as follows (High Temperature/Short Time): Pasteurization method designed to achieve a 5-log reduction, killing 99,999% of the number of viable microorganisms in milk. This is considered adequate for destroying almost all yeasts, molds and common spoilage bacteria and to also ensure adequate destruction of common pathogenic heat resistant organisms. In the HTST process milk is heated to 71.7°C (161°F) for 15-20 seconds.

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 71.5°C (160°F) to 74°C (165°F) for about 15 to 30 seconds.

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, low temperature long time for example.

The compositions of the present invention may comprise B. longum NCC 2705 (deposit number CNCM 1-2618) in an amount
sufficient to at least partially treat infections and disorders linked to the immune system and/or their complications. 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 severity of the disease and the weight and general health state of the consumer, and on the effect of the food matrix.

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

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

In general the composition of the present invention contains *B. longum* NCC 2705 (deposit number CNCM 1-2618) in a therapeutically effective dose and/or in a prophylactic effective dose.

Typically, the therapeutically effective dose and/or the prophylactic effective dose is in the range of about 0.005 mg - 1000 mg LaI per daily dose.

In terms of numerical amounts, *B. longum* NCC 2705 (deposit number CNCM 1-2618) 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 microorganisms 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.

For example, the composition in accordance with the present invention may contain an amount of *B. longum* NCC 2705 (deposit number CNCM 1-2618) corresponding to about $10^4$ to $10^{12}$ cfu per daily dose.

The composition of the present invention may contain about 0,005 mg - 1000 mg *B. longum* NCC 2705 (deposit number CNCM 1-2618) per daily dose.

The composition of the present invention may be any kind of composition. The composition may be to be administered orally, enterally, parenterally (subcutaneously or intramuscularly), topically or ocularly, by inhalation, intrarectally and intravaginally for example.

Hence, the composition of the present invention may be selected from the group consisting of food compositions, food products including pet foods, drinks, formulas for complete nutrition, nutritional supplements, nutraceuticals, food additives, pharmaceutical compositions, cosmetical compositions, topical compositions and medicaments.
Prebiotics may be added. Prebiotics may support the growth of probiotics before they are rendered non-replicating. Prebiotics may also act synergistically with viable probiotic bacteria that are present in the composition and/or that may be added.

The disorder linked to the immune system may be selected from the group consisting of infections, in particular bacterial, viral, fungal and/or parasite infections; inflammations; phagocyte deficiencies; epithelial barrier dysfunction or immune system immaturity, SIBO and combinations thereof.

In one embodiment of the present invention the composition comprising *B. longum* NCC 2705 (deposit number CNCM 1-2618) may be for use in the treatment or prevention of microbial infections, such as viral, fungal and/or parasite infections.

The disorder linked to the immune system may also be selected from the group of disorders linked to a reduced level of defensins, in particular hBD2.

Additionally, in case non-replicating *B. longum* NCC 2705 (deposit number CNCM 1-2618) are used, the disorder linked to the immune system may also be selected from the group of disorders linked to a reduced level of hBD1.

Such disorders may be selected from the group consisting of cystic fibrosis, reactive airways disease, lung infections from tobacco smoking, asthma, pneumonia, rhinitis, otitis, sinusitis, tuberculosis, Crohn's disease (colon and ileum), ulcerative colitis, intestinal immaturity, gastritis and gastric ulcer induced by *Helicobacter pylori* infection, infectious diarrhea, necrotising enterocolitis, antibiotic-associated diarrhea, bacterial vaginosis, HIV, Herpes simplex virus, urinary infection, atopic dermatitis, chronic ulcer,
carcinoma, atopic eczema, burn injury, tonsillitis, gingivitis, dental caries, keratitis in eyes, and combinations thereof.

The composition of the present invention may be used to boost the endogenous antimicrobial defences.

This may be achieved, for example, by boosting the endogenous hBD2 expression; and - in case non-replicating \( B. longum \) NCC 2705 (deposit number CNCM 1-2618) are used - by additionally boosting the hBD1 expression.

The present inventors have found that \( B. longum \) NCC 2705 (deposit number CNCM 1-2618) strongly induces the constitutive hBD2 expression; that non-replicating, e.g. heat treated, \( B. longum \) NCC 2705 (deposit number CNCM 1-2618) up-regulates hBD2 expression even more than its live counterpart; and that heat treated \( B. longum \) NCC 2705 (deposit number CNCM 1-2618) also up-regulates hBD1 expression.

Consequently, the subject matter of the present invention also embraces a method to increase the effectiveness of \( B. longum \) NCC 2705 (deposit number CNCM 1-2618) in the treatment or prevention of disorders linked to the immune system comprising the step of rendering \( B. longum \) NCC 2705 non-replicating, e.g., by heat treatment.

The disorder linked to the immune system may be one of the disorders listed above, for example.

In particular, the disorder may be a disorder linked to a reduced level of \( \beta \)-defensins.

In one embodiment of the present invention the method comprises a heat treatment step at at least about 70 °C for at least about 10 seconds.
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. In particular, features described for the compositions of the present invention may be applied to the uses and/or to the method of the present invention and vice versa.

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

Figure 1 shows that heat treated B. longum (deposit number CNCM 1-2618) at 120°C - 15 sec induces hBD2 mRNA in intestinal epithelial cells in vitro more strongly than other heat-treated strains. T84 cells were incubated for 4h the heat-treated strains. Gene expression of hBD2 was analyzed by real-time PCR. The data represent the means ± sem normalized to basal expression of non stimulated cells.

Figure 2 shows that heat-treated B. longum (deposit number CNCM 1-2618) at 85°C - 20 min exhibits the strongest induction of hBD2 (A) and hBD1 (B). T84 cells were stimulated for 4h with the live and the heat-treated B. longum (deposit number CNCM 1-2618) at 120°C - 15 sec or 85°C - 20 min. Gene expression of hBD2 was analyzed by real-time PCR. The data represent the means ± sem normalized to basal expression of non stimulated cells.

Examples:

Experimental protocol:

T84 cells were used from passage 30-40 and cultured in Dulbecco's modified essential medium/F-12 (Sigma D 6421) containing 5% of foetal calf serum (FCS) (Amined BioConcept) and 2mM glutamine. Cells were seeded at a concentration of 2 x
10^6 cell/well in 6-well culture plates and grown as monolayers at 37°C in a 5% CO2 - 95% air atmosphere. Cells grown to 1 week after confluence were incubated with serum and antibiotic-free medium for at least 12H. This step was necessary to eliminate serum-induced defensin expression and prevent any influence of antibiotics on the probiotics and on the cell immune response. Cells were further incubated with probiotics or heat-treated strains for 4H. At the end of the incubation time, cells were washed with PBS and harvested with TriPure™ isolation reagent according to the supplier's protocol. Human hBD1 and hBD2 gene expression in the so-treated cells was assessed by quantitative PCR.

Bacterial strains used in this experiment are B. longum NCC2705 (deposit number CNCM 1-2618), B. lactis (NCC 2818, deposit number CNCM 1-3446), L. johnsonii (LaI, NCC 533, deposit number CNCM 1-1225), L. paracasei (STII, NCC 2461, deposit number CNCM 1-2116). These strains were tested live or heat-treated at either 120°C - 15 sec or 85°C - 20 min.

Results:

The expression of hBD2 mRNA was upregulated by all the studied heat-treated (120°C - 15 sec) strains, but B. longum (NCC2705) (deposit number CNCM 1-2618) induced a much stronger effect than other heat-treated strains (Figure 1).

In addition, the presented data show that the heat-treatment of B. longum NCC2705 (deposit number CNCM 1-2618) improves its effect on hBD2 mRNA expression. Indeed, heat-inactivated B. longum NCC2705 (deposit number CNCM 1-2618) at 120°C - 15 sec or at 85°C - 20 min is more efficient than the live strain (Figure 2).
Furthermore, inactivation of *B. longum* NCC2705 (deposit number CNCM 1-2618) at a low temperature (85°C) and during a long time (20 min) strongly increases not only hBD2 but also hBD1 mRNA expression (Figure 2 A and B).
0-1 Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis) Prepared Using PCT Online Filing Version 3.5.000.204 MT/FOP 20020701/0.20.5.9

0-2 International Application No.

0-3 Applicant's or agent's file reference NO 10868

1 The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:

1-1 page 17

1-2 line 17

1-3 Identification of deposit
1-3-1 Name of depositary institution CNCM Collection nationale de cultures de micro-organismes

1-3-2 Address of depositary institution Institut Pasteur, 28, rue du Dr Roux, 75724 Paris Cedex 15, France

1-3-3 Date of deposit 02 July 1992 (02.07.1992)

1-3-4 Accession Number CNCM I-1225

1-5 Designated States for Which Indications Are Made all designations

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2-3-2 Address of depositary institution Institut Pasteur, 28, rue du Dr Roux, 75724 Paris Cedex 15, France

2-3-3 Date of deposit 12 February 1999 (12.02.1999)

2-3-4 Accession Number CNCM I-2116

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Claims:

1.) Composition comprising *B. longum* NCC 2705 (deposit number CNCM 1-2618) for use in the treatment or prevention of infections and disorders related to the immune system including infections.

2.) Composition in accordance with claim 1, wherein the *B. longum* NCC 2705 (deposit number CNCM 1-2618) are rendered at least in part non-replicating by heat inactivation, preferably by a treatment at at least about 70 °C.

3.) Composition in accordance with claim 2, wherein the heat treatment is carried out for at least 10 seconds.

4.) Composition in accordance with one of the preceding claims wherein *B. longum* NCC 2705 (deposit number CNCM 1-2618) are rendered non-replicating at 110° to 140 °C for 10-20 seconds or at 75° to 95°C for 10-30 minutes.

5.) Composition in accordance with one of the preceding claims, wherein the composition contains an amount of non-replicating *B. longum* NCC 2705 (deposit number CNCM 1-2618) corresponding to about $10^4$ to $10^{12}$ cfu per daily dose.

6.) Composition in accordance with one of the preceding claims, wherein the composition contains about 0,005 mg - 1000 mg non-replicating *B. longum* NCC 2705 (deposit number CNCM 1-2618) per daily dose.

7.) Composition in accordance with one of the preceding claims, wherein at least 95 % of the *B. longum* NCC 2705 (deposit number CNCM 1-2618) are non-replicating,
preferably all _B. longum_ NCC 2705 (deposit number CNCM I-2618) are non-replicating.

8.) Composition in accordance with one of the preceding claims, wherein the composition is selected from the group consisting of food compositions, food products including pet foods, drinks, nutritional formulas, feeding formulas, nutraceuticals, food additives, pharmaceutical compositions, cosmetical compositions, topical compositions and medicaments.

9.) Composition in accordance with one of the preceding claims, wherein the disorder related to the immune system is selected from the group consisting of infections, in particular bacterial, viral, fungal and/or parasite infections; phagocyte deficiencies; epithelial barrier defect or immune system immaturity, small intestinal bacterial overgrowth (SIBO) and combinations thereof.

10.) Composition in accordance with one of the preceding claims, wherein the disorder related to the immune system is selected from the group of disorders linked to a reduced level of defensins, in particular hBD2.

11.) Composition in accordance with one of the preceding claims, wherein the disorder linked to a reduced level of hBD2 is selected from the group consisting of cystic fibrosis, reactive airways disease, lung infections and tobacco smoking, asthma, pneumonia, rhinitis, otitis, sinusitis, tuberculosis, Crohn's disease (colon and ileum), ulcerative colitis, intestinal immaturity, gastritis and gastric ulcer induced by *Helicobacter pylori* infection, infectious diarrhea, necrotising enterocolitis, antibiotic-associated diarrhea, bacterial vaginosis, HIV, Herpes simplex virus, urinary infection, atopic dermatitis,
chronic ulcer, carcinoma, atopic eczema, burn injury, tonsillitis, gingivitis, dental caries, keratitis in eyes.

12.) Composition in accordance with one of the preceding claims, to be used to boost the endogenous antimicrobial defenses, and/or the endogenous hBD2 and hBD1 expression.

13.) Method to increase the effectiveness of B. longum NCC 2705 (deposit number CNCM 1-2618) in the treatment or prevention of disorders related to the immune system comprising the step of rendering B. longum NCC2705 (deposit number CNCM 1-2618) non-replicating.

14.) Method in accordance with claim 13, wherein B. longum NCC 2705 (deposit number CNCM 1-2618) are rendered non-replicating by a heat treatment step at least about 75 °C for at least about 10 seconds.

15.) Composition comprising B. longum NCC 2705 (deposit number CNCM 1-2618), wherein at least 95 % preferably at least 98 %, most preferably at least 99 %, ideally at least 99.9 %, or 100% of the B. longum NCC 2705 (deposit number CNCM 1-2618) are non-replicating.
FIG. 1

HBD2 mRNA

Fold change

NS  L. paracasei  La1  B. lactis  B. longum
NCC2461  NCC533  NCC2818  NCC2705
FIG. 2

HBD2 mRNA

Fold change

N S L i v e 8 5° C 1 2 0° C

HBD1 mRNA

Fold change

N S L i v e 8 5° C 1 2 0° C
A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K35/74 A61P37/04 A61P31/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

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, WPI Data, COMPENDEX, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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| X        | EP 1 227 152 A1 (NESTLE SA [CH])
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         | page 13, line 4 - page 14, line 16; figure 5
         | 13-15                            |
| X        |                                                                            |                     |

Special categories of cited documents

- 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
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- 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

Further documents are listed in the continuation of Box C

Date of the actual completion of the international search: 7 September 2010

Date of mailing of the international search report: 15/09/2010

Name and mailing address of the ISA:
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Fax (+31-70) 340-3016

Authorized officer: Bochelen, Damien
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