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(54) Title: **LACTOBACILLUS** FOR PREVENTING AND/OR TREATING CHRONIQUE FATIGUE SYNDROME AND ASSOCIATED DISEASES

(57) Abstract: The present invention describes a probiotic product comprising as active ingredient a *Lactobacillus acidophilus* strain and/or a *Lactobacillus helveticus* strain having the following properties of: - restoring immunological competence and/or, - limiting the pro-inflammatory response and/or, - reducing abnormal shedding of an herpes type virus in human, and hence reducing the clinical outcomes of fatigue, cognitive defects, and impaired performance. Preferably the probiotic product comprises the *Lactobacillus acidophilus* strain CBS 116411, which can be used as a medicament for preventing and/or treating CFS and associated diseases in a human being in a need thereof. This strain can also be formulated in a food product. The invention further relates to the use of said strain for manufacturing said medicament or said food product.

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LACTOBACILLUS FOR PREVENTING AND/OR TREATING CHRONIQUE FATIGUE SYNDROME AND ASSOCIATED DISEASES

Field of the invention

5 The present invention relates to the field of chronique fatigue syndrome (CFS) and associated diseases, and in particular to *Lactobacillus* strains, which have the capacity to prevent and/or treat CFS and associated diseases.

Background of the invention

10

 Many people are affected by CFS. CFS is characterized by a chronic, debilitating fatigue, which lasts for longer than 6 months. Frequent and recurrent findings include fewer, pharyngitis, difficulties in cognition and disorders of the mood. In a majority of patients, the illness is initiated suddenly with an acute "flue like" illness. Abnormalities like lymphocystosis, atypical lymphocystosis, monocytosis, elevation of hepatocellular enzymes, low levels of antinuclear antibodies, varying levels of antithyroid antibodies, elevated CD4:CD8 ratio, decreased cystolytic activity of natural killer cells and low levels of immune complexes. Clinical and serological studies suggest an association of CFS with all of the herpesviruses, particularly Epstein-Barr virus (EBV) (Komaroff & Goldenberg 1989, The chronic fatigue syndrome: definition, current studies and lessons for fibromyalgia research. Journal of Rheumatology - Supplement. 10:23-27). In addition, other infectious agents such as Hepatitis, Ross River virus, Q fever could also in some cases be associated with CFS after illness or stress. It is likely that, depending both on the genetic and environment background of each person, several combinations of factors can result in CFS.

25

 US 2003/0180260 alleged that a probiotic composition, which could comprise any probiotic could be used in human for immunotherapy and/or for preventing or treating bacterial or viral infections.

 There is still a need for improved probiotic formulation having an established clinical effect on CFS and associated diseases in human.

30

Detailed description of the invention

For the first time, the efficacy of a probiotic product has been directly established on
5 human for preventing and/or treating CFS and associated diseases.

The present invention relates to a probiotic product comprising as active ingredient a
Lactobacillus acidophilus strain and/or a *Lactobacillus helveticus* strain, which has the
capacity to prevent and/or treat CFS and associated diseases in a human being in a
need thereof.

10 According to a preferred embodiment, the probiotic product of the invention comprises as
active ingredient any *Lactobacillus acidophilus* strain or *Lactobacillus helveticus* strain
having the following properties of:

- restoring immunological competence and/or,
- limiting the pro-inflammatory response in response to a stress and/or
- 15 - reducing abnormal shedding of an herpes type virus in human, and hence reducing
the clinical outcomes of fatigue, cognitive defects, and impaired performance,

These effects (reducing, restoring, limiting) are preferably measured in humans suffering
from CFS after daily oral administration of at least 10^8 cfu (colony forming units) per day
during at least 1 day. More preferably, during at least 5 days, even more preferably during
20 at least 10 days, even more preferably at least 30 days, even more preferably at least 60
days, even more preferably at least four month and most preferably during at least one
year. The number of cfu is preferably estimated by enumeration of *Lactobacillus* colony
formed on Mann Ragosa Sharp (MRS) (Oxoid, UK) agar, after incubation of inoculated
agar plates at 37°C for at least 48 hours under anaerobic conditions.

25 Humans suffering from CFS and which can be used to assess the efficacy of probiotic
strains are preferably individuals that have been subjected to physical stress, e.g.
athletes or other individuals that are susceptible to EBV or other viral infections that
cause CFS or related diseases linked to fatigue and impaired physical performance.

Restoring immunological competence means restoring normal level of mucosal
30 immunity. This is preferably evaluated by measuring the level of interferon gamma (IFN- γ)
and/or of salivary immunoglobuline A (IgA) and/or of interleukine 12 (IL12) in CFS subject
before and after probiotic treatment and compared to the corresponding level found in a
normal subject. Normal subject in this context is a subject not suffering from CFS. The

level of IgA and IFN γ is preferably measured as described in the example. According to a preferred embodiment, IFN γ is used as the indicator of restoration of immunological competence. We expect that when normal subjects are treated as described in the example (after daily oral administration of at least 10^8 cfu (colony forming units) of the composition given in the example during one month), their IFN γ level may increase from about 20% to about 150%. We further expect that when humans suffering from CFS are given the same treatment, their IFN γ level may increase from about 150% to about 400%. According to a preferred embodiment, restoring immunological competence in human suffering from CFS means that after at least ten days of daily administration of at least 10^8 cfu (colony forming units) of CBS 116411, the level of IFN γ as detected using the assay used in the example is increased of at least 150%. Even more preferably, the level of IFN γ as detected using the assay used in the example is increased of at least 200%, even more preferably at least 300%, and most preferably at least 400%. More preferably, the treatment is continued during at least 30 days or one month to obtain this effect, even more preferably during at least two months, even more preferably during at least four months, even more preferably at least six months, and most preferably during at least one year.

Limiting the pro-inflammatory response after a stress means having an as low as possible pro-inflammatory response after a stress (such as maximal exercise as exemplified in the example), by comparison to the absence of a pro-inflammatory response in a normal subject. A normal subject in this context is a subject not suffering from CFS. Preferably, the pro-inflammatory response is evaluated by measuring the level of NO formed. This is preferably performed using the method described in the example. According to a preferred embodiment, limiting the pro-inflammatory response after a stress in human means that after treatment after daily oral administration of at least 10^8 cfu (colony forming units) per day during at least 5 day, a normal level of NO is detected using the assay used in the example. More preferably, the treatment is continued during at least 10 days to obtain this effect, even more preferably during at least 30 days, even more preferably at least 60 days, even more preferably at least four month and most preferably during at least one year.

Reducing abnormal shedding of a herpes type virus in human is preferably determined with EBV as herpes type virus. More preferably, the presence of EBV is detected by PCR as described in the example. According to a preferred embodiment, reducing abnormal

shedding of EBV in human means that after treatment after daily oral administration of at least 10^8 cfu (colony forming units) per day during at least 5 day, no shedding of EBV is detected using PCR. More preferably, the treatment is continued during at least 10 days to obtain this effect, even more preferably during at least 30 days, even more preferably at least 60 days, even more preferably at least four month and most preferably during at least one year. According to another preferred embodiment, the herpes type virus is a herpes type 1 and type 2 virus or a cytomegalovirus.

According to another preferred embodiment, the probiotic product of the invention comprises as active ingredient a *Lactobacillus acidophilus* strain and/or a *Lactobacillus helveticus* strain, which has the capacity to prevent CFS and associated diseases in a human being in a need thereof. In this case, the probiotic product is able to prevent:

- the deterioration of the immunological competence and/or,
- the induction of an pro-inflammatory response in response to a stress and/or
- the abnormal shedding of an herpes type virus in human, and hence preventing the clinical outcomes of fatigue, cognitive defects, and impaired performance.

To check whether or not a new identified *Lactobacillus* strain could be used in the probiotic product of the invention, this strain is preferably tested in the trial presented in the example.

Lactobacillus acidophilus and *Lactobacillus helveticus* are defined by their ability to utilize range of carbohydrates, as described in table 1. The ability of strain CBS 116411 is also given. In addition, the strain should have at least 70% DNA-DNA homology with *L. helveticus* DSM 20075^T as determined by spectroscopic DNA-DNA hybridisation as described briefly in the section experimental before the examples. More preferably, the strain should have at least 75% DNA-DNA homology with said strain using the same program, most preferably at least 79% DNA-DNA homology. All strains of *Lactobacillus acidophilus* or *Lactobacillus helveticus* having these properties are encompassed by the present invention.

Table 1. Carbohydrate utilization of *Lactobacillus* strains

	<i>L. acidophilus</i>	LAFTI L10	<i>L.helveticus</i>
Acid from:			
Galactose	++	++	++
Glucose	++	++	++
Fructose	++	++	V
Mannose	++	++	++
NAG	V	++	++
Amygdalin	V	++	-
Arbutin	V	-	-
Esculin	++	++	-
Salicin	++	++	-
Cellobiose	++	++	-
Maltose	++	++	-
Lactose	++	++	++
Melibiose	V(-)	-	-
Saccharose	++	++	-
Trehalose	V(+)	++	-
Raffinose	++(v)	-	-
Amidon	V(-)	+(w)	-
Gentibiose	++	+	-
Lactic Acid	DL	DL	DL
Growth at 15 °C	-	-	-
Growth at 45 °C	+	+	+

According to an even more preferred embodiment, the probiotic product of the invention comprises as active ingredient at least the following probiotic: *Lactobacillus acidophilus* strain LAFTI® L10 deposited at the CBS under accession number CBS 116411 on 15/10/04.

According to a preferred embodiment, the probiotic product of the invention consists of a biologically pure culture of said deposited strain. According to a more preferred embodiment, the product consists of a biologically pure culture of said deposited strain in combination with any other valuable probiotic strain. A probiotic strain is defined herein as a live microbial strain, which beneficially affects the human host cell

by improving its microbial balance. Preferred probiotica are isolated strains of *Bifidobacterium*, *Lactobacillus*, *Propionibacterium*, and *Enterococcus*. More preferred probiotic species are *Bifidobacterium infantis*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus paracasei* and
5 *Lactobacillus helveticus*.

In the context of the invention, CFS is defined as follows: any type of fatigue associated with a viral infection.

10 In the context of the invention, associated disease is defined as being preferably a herpes type viral disease. Preferably, the associated disease is: EBV infections, herpes type 1 and type 2 infections, cytomegalovirus infections.

In the context of the invention, a human being in the need of the probiotic product of the
15 invention is defined as follows: human subjects that experience some form of fatigue and/or stress. Stress and fatigue can have any origin but has preferably a professional origin: shiftwork, international travel, academic stress in students, over-training in sport (athletes). Alternatively and/or in combination with stress having a professional origin, stress has preferably a domestic origin: family stress, stress caused by children.

20 Alternatively and/or in combination with previous origin of stress, stress is caused by mid-life crisis and/or diet-related stress. More preferably, the stress and/or fatigue causes impaired physical performance in human over-training in sport such as athletes.

For the first time, it has been directly demonstrated that the administration of a probiotic
25 product (*Lactobacillus acidophilus* strain CBS 116411) has the capacity to prevent and/or treat CFS and associated diseases in human. This effect has been demonstrated by the fact that probiotic treatment leads to a

- restoration of immunological competence and
- limitation of the pro-inflammatory response and
- 30 - reduction of abnormal shedding of EBV in human, and hence a diminution of the clinical outcomes of fatigue, cognitive defects, and impaired performance,

This probiotic product reduces shedding of EBV in saliva. This effect was associated with a normalisation of mucosal level of INF- γ as well as a reduced mucosal level of Nitric

Oxide, which demonstrates that oral administration of the probiotic product leads to a restoration of mucosal immunity and a limitation of mucosal inflammation. Inhibition of reactivation of EBV by the probiotic product, should reduce fatigue in athletes with impaired performance.

5

In a preferred embodiment, the probiotic product of the invention is a medicament or a food product.

The invention also relates to the use of a *Lactobacillus acidophilus* strain and/or a *Lactobacillus helveticus* strain, preferably the *Lactobacillus acidophilus* strain is

10

CBS116411, having the properties of:

- restoring immunological competence and/or,
- limiting the pro-inflammatory response and/or,
- reducing abnormal shedding of an herpes type virus in human, and hence reducing the clinical outcomes of fatigue, cognitive defects, and impaired performance,

15

for preventing and/or treating CFS and associated diseases in a human being in a need thereof.

According to a preferred embodiment, said *Lactobacillus* strain is used for the manufacture of said medicament or food product. The medicament of the invention may comprise any physiologically or pharmacologically acceptable excipient and/or diluent.

20

According to another preferred embodiment, the probiotic product is a food product or part thereof. If the probiotic product is part of a food product, the probiotic product can be present as a granule, powder or the like. The food product of the invention comprising the probiotic product of the invention may comprise any physiologically or acceptable excipient and/or diluent. Preferred food products are cultured milk, yoghurt, cheese, milk drink, milk powder, sport drink, coveratures (defined as mixtures of oil, sugar(s) and milk protein (whey)), infant formulas and fermented meat products.

25

Alternatively and according to another preferred aspect of the invention, a *Lactobacillus* strain as defined before, preferably the *Lactobacillus acidophilus* strain CBS 116411 is used in the preparation of a food product for preventing and/or combating CFS and associated diseases in a human being in a need thereof.

30

The product (medicament or food product) of the invention comprises a *Lactobacillus* strain as defined earlier, preferably the *Lactobacillus acidophilus* strain CBS 116411 in an effective amount to prevent and or treat CFS and associated diseases. According to a more preferred embodiment, the food product or medicament comprises
5 at least 10^6 (cfu)/g product, which is consumed to give at least 10^8 cfu as the daily dose, or preferentially from 10^9 to 10^{11} cfu/day.

According to another preferred embodiment, the probiotic product (medicament and food product) of the invention is in the form of tablet, capsule, powder or granule,
10 orally liquid administered liquid preparations, suppositories, dry preparations. More preferably, the probiotic product is in the form of an enteric tablet, capsule, powder or granule, meaning it will survive the stomach and arrive intact in the intestine. All these forms can be prepared by known means, using food grade, respectively pharmaceutically acceptable carriers, excipients, solvents or adjuvants. For the preparation of the
15 medicament, standard ingredients and method of preparation as already described in Remington: The science and practice of pharmacy, 1995, Mack Publishing, Co Easton, PA 18042, USA) can be used. Remington is herewith incorporated by reference.

The invention therefore also relates to a method wherein a *Lactobacillus* strain as
20 defined earlier, preferably the *Lactobacillus acidophilus* strain CBS 116411 is formulated in a food product or in a medicament, and said food product or medicament is administered to a given human being in an amount effective for preventing and/or treating CFS and associated diseases. Therefore, the invention further relates to a method for preventing and/or treating CFS and associated diseases in a given human being. This
25 method comprises the step of administering the medicament or food product of the invention to said human being. It will be appreciated that the exact dosage of the essential active ingredient which is said strain, preferably the *Lactobacillus acidophilus* strain CBS 116411 constituting an amount effective for preventing and/or treating CFS and associated diseases in a human being in a need thereof may vary depending on the
30 specific nature of the clinical conditions being treated, severity of the condition, age of human being treated, weight and condition of the human being treated, mode of administration of the dosage form, and the specific formulation being administered.

Preferably, administrating the product of the invention preferably means ingesting said product to give a daily dose of at least 10^8 cfu per day. More preferably, an amount of the product that corresponds to 10^{10} cfu in said product per day.

5 According to another preferred embodiment, the product of the invention consist of the strain as defined earlier, preferably the strain *Lactobacillus acidophilus* CBS 116411 in combination with at least one of the following ingredients in order to get a synergetic combination on the human being treated. Preferred ingredients are: vitamin A, vitamin C, vitamin D3, vitamin E, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin
10 C, nicotinamide, folic acid, beta carotene, lutein, Zinc, Copper, selenium, polyphenol, and prebiotics. According to a more preferred embodiment, the probiotic product of the invention consist of the strain as defined earlier, preferably the strain *Lactobacillus acidophilus* CBS 116411 in combination with a polyphenol. Even more preferably, the polyphenol is a green tea extract. Even more preferably, the major compound present in
15 the green tea extract is epigallocatechin gallate (EGCG). Most preferably, the probiotic product of the invention consist of the strain as defined earlier, preferably the strain *Lactobacillus acidophilus* CBS 116411 in combination with Teavigo™. According to another preferred embodiment, the prebiotic is at least one of the prebiotic defined in US 6,180,099: inulin, and/or fructoligosaccharides.

20

The invention will now be illustrated by the following example, which should not be construed as limiting the scope of the invention.

Experimental:

25 Briefly, a method is given to determine DNA-DNA homology by spectroscopic DNA-DNA hybridisation. DNA is isolated using a French pressure cell (Thermo Spectronic) and is subsequently purified by chromatography on hydroxyapatite as described by Cashion et al (Cashion et al, (1977), A rapid method for base ratio determination of bacterial DNA. Anal. Biochem. 81, 461-466). DNA-DNA hybridisation is carried out as described by De
30 Ley et al (De Ley et al, (1970) The quantitative measurement of DNA hybridisation from renaturation rate. Eur. J. Biochem. 12, 133-142) with the modification apported by Huss et al (Huss et al, (1983), Studies on the spectrophotometric determination of DNA hybridization from renaturation rate, Syst. Appl. Microbiol., 4, 184-192) and Escara and

Hutton (Escara and Hutton, (1980), Thermal stability and renaturation of DNA in dimethylsulphoxide solutions: acceleration of renaturation rate. Biopolymers, 19:1315-1327), using a model 2600 spectrophotometer equipped with a model 2527-R thermoprogrammer and plotter (Gilford Instrument Laboratories). Renaturation rates are computed with the TRANSFER.BAS program of Jahnke, 1992 (Jahnke K.D. (1992), Basic computer program for evaluation of spectroscopic DNA renaturation data from Gilford System 2600 spectrometer on a PC/XT/AT type personal computer. J. Microbiol. Methods, 15:61-73).

Example

In this example, a clinical trial is reported which demonstrates that *Lactobacillus acidophilus* CBS 116411:

- restores the immunological competence and
- limits the pro-inflammatory host response and
- reduces abnormal shedding of EBV in man suffering from CFS, and hence reduces the clinical outcomes of fatigue, cognitive defects, and impaired performance.

Nine subjects were recruited because of complaints of impaired sporting performance. The clinical characteristics were of reduced energy, cognitive defects (variable) and fall-off in athletic performance. Often this was episodic with episodes initiated by 'sore throats', and exercise and training would exacerbate. This group was selected as a defined selection within the broader context of 'chronic fatigue syndrome (CFS)'. In table 2 below, several parameters are indicated, which were measured in the CFS group as well as in the control group (normal).

Of the nine CFS subjects, eight were seropositive for EBV (a similar proportion to the general public) as tested by Polymerase Chain Reaction (PCR) as already described ("Valtrex therapy for EBV reactivation and upper respiratory symptoms in elite runners". Med Sci Sports Exerc 36 No.7 1104-1110. Cox A, Gleeson M, Pyne D, Saunders P, Clancy R, Fricker P).

	Normal	CFS
Number	18	9
EBV antibody detected (ELISA)	67%	89%
URTI/year (Upper Respiratory Tract Infection)	1.7	3.5
Days lost	5.2	13.4
URTI/intense training	11%	67%
Sleep disturbance	17%	56%
High-moderate stress	39%	78%

Table 2: Characterisation of Normal and CFS groups

The study was planned as follows:

- 5 The eight seropositive persons, so called "CFS" and 18 "normal" subjects were followed. The effect of the probiotica product or of a placebo was tested on both groups before and after a physical exercise (one hour running on a trade mill). Several parameters were measured: EBV shedding, IFN γ , saliva IgA, NO.

10 The placebo used was Avicel100TM (microcrystalline cellulose). The probiotica product tested was a daily dose of 10¹⁰cfu of *Lactobacillus acidophilus* CBS 116411 formulated in Avicel100TM.

1.1. Effect of probiotica on EBV excretion within CFS group

15 The excretion of EBV within the eight seropositive subjects (CFS subjects) was determined by PCR (as defined in the former paragraph) and was followed as explained below. Three samples were taken for each subject (24 samples in total).

EBV excretion was measured four times within the same eight subjects:

- at the beginning of the study: three samples were found positive (3/24)
- after one month of placebo: 3/24
- 20 - after one month of no treatment (washout period): 6/24

- after one month of treatment with the probiotic product: 1/24

The results are given again in table 3 below:

5		<u>0</u>	<u>1 month</u>
	<u>Placebo:</u>	<u>EBV positive by PCR</u> 3/24	3/24
	<u>L.acidophilus:</u>	<u>EBV positive by PCR</u> 6/24	1/24

Table 3: effect of probiotica on EBV excretion within CFS subjects

10

We conclude EBV excretion was reduced from six to one positive sample after treatment with *Lactobacillus acidophilus* CBS 116411, compared to no change (ie 3 & 3) with placebo.

15 1.2. Probiotica restores mucosal immunity towards normal within CFS group

As indicators of mucosal immunity level, saliva immunoglobuline A (IgA) and interferon gamma (IFN- γ) levels were measured within CFS group and normal group before and after treatment with either *Lactobacillus acidophilus* CBS116411 or placebo.

Saliva immunoglobuline A (IgA) was measured as described in the example of

20 WO01/97836. INF- γ from whole blood culture was measured according to the following publication ("Circulating T cell response to Helicobacter pylori infection in chronic gastritis". Helicobacter 5;3:135-141 (2000)).

The results are presented below:

(i) Saliva IgA (median levels mg/l)

25

<u>Normals</u>	<u>Before</u>	probiotic 59mg/l (25-129)
	<u>After</u>	probiotic 51mg/l (27-103)

<u>CFS</u>	<u>Before</u>	probiotic 56mg/l (38-185)
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30

	<u>After</u>	probiotic 70mg/l (20-85)
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(ii) INF- γ (from whole blood culture)

Normals Before probiotic 11.9 pg/ml
 After probiotic 26.8 pg/ml

CFS Before probiotic 7.8 pg/ml
 After probiotic 32.1 pg/ml

P = 0.04

(Before means before treatment with probiotics, after means after treatment with probiotics)

Three samples were taken from each subject at each time point (ie 24 samples in total).

We conclude:

- The placebo did not have any detectable effect on both Normal and CFS subjects before and after physical exercise (data not shown).
- The CFS group is characterized by a lowered IFN- γ level as compared to the one of the normal group ($p < 0.02$), indicating an impaired mucosal immune competence.
- Treatment with *Lactobacillus acidophilus* restores mucosal immunity level towards a normal level: the level of mucosal immunity is comparable within CFS group after probiotic treatment and normal subjects before or after probiotic treatment as indicated by IFN- γ levels.

1.3.Limitation of nitric oxide production

In CFS group, the stress of maximal exercise (ie VO₂ max) caused an increase and uncontained level of NO (one hour post physical exercise as defined before) as measured in saliva by measuring nitrite accumulation, a stable metabolic product of NO with oxygen. This is determined by the Griess reaction. Briefly, equal volumes of saliva and Griess reagent (0.5% sulfanilamide and 0.05% N-1 naphthylethylenediamide hydrochloride in 2.5% phosphoric acid) were mixed at room temperature. After 5 mins, the absorbance was measured at 550 nm using an ELISA microplate reader. The concentration of nitrite was determined by a standard curve prepared with sodium nitrite (1- 100 mM).

After one month of treatment with *Lactobacillus acidophilus* CBS 116411, the NO response to exercise was contained and 'normalised' to pattern found in normal control group as demonstrated below:

Nitric Oxide (recovery levels one hour after exercise challenge)

Normals Before probiotic 160uM
 After probiotic 175uM

5

CFS Before probiotic 215uM
 After probiotic 145uM

10 The placebo did not have any detectable effect on both Normal and CFS subjects before
and after physical exercise (data not shown).

We conclude probiotica treatment of CFS subject limits the inflammatory response of the
subject, which should lead to a reduction of the symptoms associated with CFS.

15 Therefore for the first time, it is demonstrated that probiotica treatment of CFS subjects
leads to the 'normalisation' of immune marker (IFN- γ) and parameter of inflammation
(NO).

Applicant's or agent's file reference number 24475WO	International application No.
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description Firstly mentioned on page 4 line 17 in the word document	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet Name of depositary institution CENTRAAL BUREAU VOOR SCHIMMELCULTURES Address of depositary institution (including postal code and country) Uppsalaalan 8 P.O. Box 85167 NL-3508 AD Utrecht The Netherlands Date of deposit 15 October 2004 Accession Number CBS 116411	
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet	
We inform you that the availability of the microorganism identified above, referred to Rule 13bis PCT, shall be effected only by issue of a sample to an expert nominated by the requester until the publication of the mention of grant of the national patent or, where applicable, for twenty years from the date of filing if the application has been refused, withdrawn or deemed to be withdrawn.	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
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CLAIMS

1. Probiotic product comprising as active ingredient a *Lactobacillus acidophilus*
5 strain and/or a *Lactobacillus helveticus* strain, which have the capacity to prevent
and/or treat CFS and associated diseases in a human being in a need thereof.
2. Probiotic product according to claim 1 having the properties of:
 - restoring immunological competence and/or,
 - 10 - limiting the pro-inflammatory response and/or,
 - reducing abnormal shedding of an herpes type virus in human, and hence
reducing the clinical outcomes of fatigue, cognitive defects, and impaired
performance,
- 15 3. Probiotic product according to claim 1 or 2, wherein the *Lactobacillus acidophilus*
strain is CBS116411.
4. Probiotic product according to any one of claim 1 to 3, wherein the product is a
medicament or a food product.
- 20 5. Probiotic product according to claim 4, wherein the product is for preventing
and/or treating CFS and associated diseases in a human being in a need thereof.
6. Probiotic product according to claim 5, wherein the associated disease is a
25 herpes type viral disease selected from the group consisting of: EBV infections,
herpes type 1 and type 2 infections, cytomegalovirus infections.
7. Probiotic product according to claim 5 or 6, wherein the human being in a need of
the medicament is selected from the group consisting of: human subjects that
30 experience some form of fatigue and/or stress.
8. Use of a *Lactobacillus acidophilus* strain and/or a *Lactobacillus helveticus* strain,
preferably the *Lactobacillus acidophilus* strain is CBS116411, having the
properties of:

- restoring immunological competence and/or,
- limiting the pro-inflammatory response and/or
- reducing abnormal shedding of an herpes type virus in human, and hence reducing the clinical outcomes of fatigue, cognitive defects, and impaired performance,

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for preventing and/or treating CFS and associated diseases in a human being in a need thereof.

9. Use of a *Lactobacillus* strain as defined in claim 8 for the preparation of a medicament.

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10. Use of a *Lactobacillus* strain as defined in claim 8 for the preparation of a food product or a medicament for preventing and/or treating CFS and associated diseases in a human being in a need thereof.

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11. Use according to any one of claim 8 to 10, wherein the associated disease is a herpes type viral disease selected from the group consisting of: EBV infections, herpes type 1 and type 2 infections, cytomegalovirus infections.

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12. Use according to any one of claim 8 to 11, wherein the human being in a need of said food product or medicament is selected from the group consisting of: human subjects that experience some form of fatigue and/or stress.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2005/055750

A. CLASSIFICATION OF SUBJECT MATTER A61K35/74 A61P25/00 A61P31/22		
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 A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the International search (name of data base and, where practical, search terms used) EPO-Internal, BIOSIS, EMBASE, MEDLINE, CHEM ABS Data, WPI Data, PAJ		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/180260 A1 (CLANCY ROBERT LLEWELLYN ET AL) 25 September 2003 (2003-09-25) paragraphs '0018! - '0025!, '0041!, '0054!, '0055!; claims; figures ----- -/--	1-12
☒ Further documents are listed in the continuation of box C. ☒ Patent family members are listed in annex.		
° 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 *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family		
Date of the actual completion of the international search 3 January 2006		Date of mailing of the international search report 12/01/2006
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Langer, A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2005/055750

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MAHONEY M ET AL: "The effect of processed meat and meat starter cultures on gastrointestinal colonization and virulence of <i>Listeria monocytogenes</i> in mice." INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, vol. 84, no. 3, 1 August 2003 (2003-08-01), pages 255-261, XP002322052 ISSN: 0168-1605 Abstract page 260, column 1, paragraph 2 - column 2, paragraph 2; figures	1-7,9
Y	UCHIDA S ET AL: "STUDIES ON VIRUS-INHIBITING SUBSTANCES OF BACTERIAL ORIGIN II. INHIBITION OF ANIMAL VIRUS PLAQUE FORMATION BY CULTURE FILTRATES OF LACTIC ACID BACTERIA" KOBE JOURNAL OF MEDICAL SCIENCES, KOBE UNIVERSITY SCHOOL OF MEDICINE, KOBE, JP, vol. 24, no. 2, June 1978 (1978-06), pages 91-98, XP000979543 ISSN: 0023-2513 Abstractfigures	1-12
Y	US 4 983 163 A (WINANS, JR. ET AL) 8 January 1991 (1991-01-08) claims	1-12
A	KOMAROFF A L ET AL: "THE CHRONIC FATIGUE SYNDROME DEFINITION CURRENT STUDIES AND LESSONS FOR FIBROMYALGIA RESEARCH" JOURNAL OF RHEUMATOLOGY, vol. 16, no. SUPPL. 19, 1989, pages 23-27, XP009045555 & PALM SPRINGS FIBROMYALGIA SYNDROME SYMPOSIUM, PALM SPRINGS, CALIFORNIA, USA, MARCH 18-20, 1988. J RH ISSN: 0315-162X cited in the application the whole document	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2005/055750

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 8, 11, 12 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP2005/055750

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2003180260	A1	25-09-2003	WO 0197821 A1 27-12-2001
			CN 1444484 A 24-09-2003
			EP 1296693 A1 02-04-2003
			JP 2003535903 T 02-12-2003
US 4983163	A	08-01-1991	NONE