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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 1/20, A61K 35/74, A23C 9/123

A1

(11) International Publication Number: **WO 98/23727**

(43) International Publication Date: 4 June 1998 (04.06.98)

(21) International Application Number: PCT/CA97/00915

(22) International Filing Date: 28 November 1997 (28.11.97)

(30) Priority Data:
60/032,157 29 November 1996 (29.11.96) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: LACTIC FERMENT COMPRISING A PARTICULAR STRAIN OF *LACTOBACILLUS ACIDOPHILUS* AND USE THEREOF

(57) Abstract

The purified strain of *Lactobacillus acidophilus* CNCM/I–1492 (L.a. 1492) when administered alone or in combination with another *Lactobacillus acidophilus* (L.a.) strain and *Lactobacillus casei* (L.c.) strain, has a beneficial effect on the cholesterol blood level in mammals. It also strenghtens the immune system, facilitates the absorption of nutrients and stimulates the intestinal flora. Such strains also neutralize side effects caused by antibiotics. The invention concerns the specific strain L.a. I–1492, a ferment comprising L.a. I–1492, L.a. and L.c. strains, a dairy product obtained by this ferment and a method of manufacturing the dairy product.

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LACTIC FERMENT COMPRISING A PARTICULAR STRAIN OF LACTOBACILLUS ACIDOPHILUS AND USE THEREOF

FIELD OF THE INVENTION

The present invention concerns a purified strain *Lactobacillus acidophilus* identified as CNCM/I-1492 (hereinafter called L.a. I-1492), the microorganism from this strain, a lactic ferment comprising this strain, a process for making dairy products using this ferment, and the dairy products obtained by this process and containing at least 500 million per gram of *Lactobacillus acidophilus* (including the L.a. I-1492 strain) or 380 million/gram of L.a. I-1492.

The invention also concerns the use of a ferment comprising the strain *Lactobacillus acidophilus* CNCM/I-1492 and of a dairy product containing the same in the pharmaceutical field for reducing the level of cholesterol in the blood of a mammal.

BACKGROUND

Yogurts are fermented dairy products obtained by fermentation of different lactic bacterias with milk. The most widely used milk is cow's milk. These lactic bacterias (mainly *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) are foreign bodies to the human intestinal flora and are not implanted in the digestive system during consumption of these dairy products.

Other lactic bacterias are present in their natural state in the human digestive system. It seems that the presence of such bacterias are beneficial and helpful in particular to improve the absorption of food while maintaining the

equilibrium in the intestinal flora. Such other bacterias in particular are *Lactobacillus casei* and *Lactobacillus acidophilus.*

The dairy products containing such bacterias are of particular interest inasmuch as they allow better digestion and provide an easier absorption of calcium when the latter is in the presence of moderate amounts of lactose. Indeed, during fermentation, milk protein and sugar are hydrolyzed, thereby falicitating hydrolisation of minerals such as calcium, iron essential to their absorption.

It has also been demonstrated that the ingestion of a fermented milk containing Lb Acidophilus alone increase the phagocytes activities thereby stimulating non specific defense of the host.

Furthermore, the consumption of fermented milk *Lb casei* and milk *Lb acidophilus* has been found effective to rapidly reduce acute diarrhea in humans. *Lb acidophilus* has also been found to have a healing effect on other gastric-intestinal related problems such as diverticulitis, food poisining, abnormal fermentation.

However, perfecting such products is extremely difficult inasmuch as these bacterias naturally present in humans are not easily given to rapid production of dairy products having a pleasant taste and texture.

Furthermore, it is known that the performance of combined strains in a same ferment is unpredictable because of the interactions between the strains. Hence the performance and properties of a ferment can be significantly different from the performance and properties of the pure strains of which the ferment is composed.

It has now been, surprisingly, discovered that the *Lactobacillus acidophilus* I- 1492 strain, or L.a. I-1492, has not only a beneficial effect on digestion and the equilibrium of the intestinal tract, but also has a major pharmaceutical effect allowing the reduction of the cholesterol level in the blood of mammals, and more particularly humans.

This effect is particularly remarkable when the strain L.a. I-1492 is combined with some lactic bacterias in a lactic ferment. A thorough study of these products has revealed, in a totally surprising and unexpected way, that fermented dairy products obtained from such ferment display therapeutic properties which reduce the amount of cholesterol in the blood. The main factor seems to evidently be due to the presence of a high concentration of the strain L.a. I-1492 since such an effect has not been established for other yogurts and/or lactic bacterias. The combination of lactic bacterias which compose the ferment according to the invention, allows an increase and acceleration of the development of the strain L.a. I-1492 during production of the fermented dairy product. Furthermore, it is very likely that this ferment creates a synergy and thereby achieves the production of a fermented dairy product having improved therapeutic results as compared to the dairy products obtained with the L.a. I-1492 strain alone.

BRIEF DESCRIPTION OF THE INVENTION

The present invention concerns a purified strain *Lactobacillus acidophilus* I-1492 (L.a. I-1492) and the microorganism from this strain.

This strain can used in a lactic ferment, which is also an object of the invention, comprising a mixture of the following lactic strains:

- *L.a. I-1492*
- at least one other *Lactobacillus acidophilus* strain; and
- at least one *Lactobacillus casei* strain.

The latter two strains may be of commercial origin and can be purchased from manufacturers of lactic ferments. For example, the commercial Lactobacillus acidophilus strain that is used in the ferment of the present invention may be the one sold under the trademark RP Texel by Rhône-Poulenc or the one sold under the trademark L.a. RO-52 by Rosell. The commercial Lactobacillus casei strain may be the one sold under the trademark E2AL by Rhône-Poulenc.

Another object of the invention is a fermented lactic product of the yogurt type obtained by fermentation of the strain or of the lactic ferment according to the invention, with a dairy compound.

A further object of the invention is a method of manufacturing a fermented dairy product by the fermentation of the ferment according to the invention, said product having the texture of a yogurt.

A further object of the invention is the use of such fermented dairy product in the stimulation of the immune system for curing sore throat, flu, sinusitis and urinary infection. This same fermented dairy product has also been found to be useful in treating gatric-intestinal related diseases and infections.

Still a further object of the invention is the use of the above dairy products to reduce the cholesterol blood level in mammals, especially humans, and a method of medical treatment comprising the ingestion of a dairy product according to the invention.

The invention as well as its advantages will be better understood upon reading the following non-restrictive description, and by referring to the enclosed drawings.

DETAILED DESCRIPTION OF THE INVENTION

The purified *Lactobacillus acidophilus* strain I-1492 according to the invention was filed on November 15, 1994 in the National Collection of Microorganism Cultures (25, Rue du Dr. ROUX, F-75724 Paris Cedex 15) according to the provisions of the Budapest Treaty.

The physiological, chemical and biochemical characteristics of the *acidophilus* strains that may be used in the ferment according to the invention are shown in tables 1 and 2. It has been found that the L.a. I-1492 strain present chemical, biochemical and physiological characteristics similar to those of the other L.a. strains. As a matter of fact, the L.a. I-1492 strain differs from the others only in that it degrades cholesterol more efficiently.

Table 1

Lactobacillus type (Group I); physiological characteristics

Species	Mobility	Growth at/in						Optimal Growth temperature	Maximum Growth temperature
		15°C	45°C	pH 3,3	pH 0,7	4% NaCl	10% NaCl		
L.b.acetotolerens	-	-	-	+	-				<40°C
L.b.acidophilus	-	-	+	-	+			45°C	52°C
L.b.amylophilus	-	+						30°C	<45°C

Table 2

Lactobacillus type (Group I): chemical and biochemical characteristics

Species	G & C %	Type of Murein	Nature of the teichoïc acid	lactic acid Isomer	électrophoretic mobility of		Production of:			
					L-LDH	D-LDH	LDH ALLOSTERIC	ARGININE DEHYDROLASE	GAS FROM D-GLUCOSE	GAS FROM D-GLUCOSATE
L.b acetotolerens	35-36	Lys D-Asp		DL				-	-	-
L.b.acidophilus	34-37	Lys D-Asp	Glycerol	DL	1,35-1,5	1,3	-	-	-	-

The fermenting profile for the L.a. strains which include the L.a. I-1492 strain is shown in table 3.

Table 3

Lactobacillus type (Group I): fermenting profile

SPECIES	ESCULIN HYDROLYSIS	ACID FERMENTATION FROM:												
		ADONITOL	STARCH	AMYGDALINE	ARABINOSE	CELLOBIOSE	FRUCTOSE	GALACTOSE	GLUCONATE	GLUCOSE	GLYCEROL	INOSITOL	INULIN	LACTOSE	MALTOSE
Lb acetotolerens group I	+		-	-	-	-	+	-	-	+				-	+
Lb acetotolerens group II	+		-	-	-	+	+	-	-	+				-	<
Lb acidophilus	+		>	+	-	+	>	+	-	+				+	+

Table 3 (continued)

Lactobacillus type (Group I): fermenting profile

SPECIES	ESCULIN HYDROLYSIS	ACID FERMENTATION FROM:												
		MANNITOL	MANNOSE	MELEZITOSE	MELIBIOSE	RAFFINOSE	RHAMNOSE	RIBOSE	SACCHAROSE	SALICIN	SORBITOL	L-SORBOSE	TREHALOSE	XYLOSE	DEXTRINS
Lb acetotolerens group I	±	+	+	-	-	-	-	+	-	+	-	-	+	-	
Lb acetotolerens group II	+	-	+	-	-	-	-	-	-	+	-	-	+	-	
Lb acidophilus	+	-	+	-	v	v	-	-	+	+	-	-	v	-	-

The chemical, biochemical and physiological characteristics of the *casei* strain which can be used in the ferment according to the invention in combination with the L.a. I-1492 strain, are compiled in tables 4 and 5.

Table 4

Lactobacillus type (Groupe II) Chemical and biochemical characteristics

Species	G & C %	Type of murein	Nature of teichoic acid	Lactic acid isomer	electrophoretic mobility of		Production of:			
					L-L DH	D-LDH	LDH allosteric	Arginine dehydrogenase	Gas from D-Glucose	Gas from D-Glucosate
Lb.casei	45-47	Lys D-Asp	-	L (+)	1,22	0,93	+	-		

Table 5

Lactobacillus type (Group II): physiological characteristics

Species	Mobility	growth at/in:						Optimal Growth temperature	Maximum Growth temperature
		15°C	45°C	pH3.3	pH7,0	6,5% NaCl	10% NaCl		
Lb.casei	-	+	v					30 à 37° C	< 45°C

The fermenting profile of the casei strain is shown in table 6.

Table 6

Lactobacillus type (Group III): fermenting profile

Species	Esculin Hydrolysis	Acid Production by fermentation of:														
		N-Acetyl Glucosamine	Adonitol	Starch	Amygdaline	Arabinose	D-Arabitol	L-Arabitol	Arbutin	Cellobiose	2 Céto-gluconate	5 Céto-gluconate	Dextrine	Dulcitol	Erythritol	
Lb.casei	+		-	-	+	-			+	+			v	-		

Table 6 (continued)

Lactobacillus type (Group III): fermenting profile

Species	Esculin Hydrolysis	ACID PRODUCTION BY FERMENTATION OF:														
		D-Fucose	L-Fucose	Fructose	Galactose	Gentobiose	Gluconate	Glucose	Glycerol	Glycogen	Inositol	Inulie	Lactose	D-Lyscose	Maltose	Mannitol	Mannose
Lb.casei	+	-	v	+	+	-	+	+	-		-	-	-	-	v	+	

Table 6 (continued)

Lactobacillus type (Group II): fermenting profile

Species	ESCULIN HYDROLYSIS	ACID PRODUCTION BY FERMENTATION OF:																			
		MELEZITOSE	MELIBIOSE	α-METHYL-D-GLUCOSIDE	α-METHYL-D-MANNOSIDE	β-METHYL-XYLOSIDE	RAFFINOSE	RHAMNOSE	RIBOSE	SACCHAROSE	SALICIN	SORBITOL	L-SORBOSE	D-TAGATOSE	THREALOSE	D-TURANOSE	XYLITOL	XYLOSE			
Lb.casei	+	+		+				+		+	+		+		+	+					

A dairy product according to the present invention can be obtained by fermenting the ferment of the invention in a milk-based medium. For this purpose, the following process may be used.

Firstly, the L.a. I-1492, *acidophilus* and *casei* strains are incubated in a MRS type fermentation medium under 10% of CO_2 according to a standard program comprising several steps. The recombined lacteal base which is partially lactose-free and degassed is pasteurized for 1,5 minutes at 95°C and inoculated at 10%. Finally, it is incubated according to the following program:

10

1) the *L.a.I-1492* strain: 2 hours at 37°C under 10% CO_2;
2) the *acidophilus* strain: 2 hours at 37°C; and
3) the *casei* strain: 1 hour at 37°C.

The product is then co-fermented in an anaerobic atmosphere and medium for 15 hours at 37°C (degassing under CO_2).

20 In order to realize the invention, any *acidophilus* and *casei* strains may be used as long as they present no health risk. Preferably, the following acidophilus strain should be used: RP Texel ™ by Rhône-Poulenc and L.a. RO-52 ™by Rosell. The commercial casei E2AL™ by Rhône-Poulenc is preferably used. The

proportions of L.a. I-1492, *acidophilus* and *casei* cultured strains, are respectively about 70/20/10. These proportions may evidently be somewhat varied. Nevertheless, the total concentration of *Lactobacilli acidophilus* (including those obtained from L.a.I-1492 strains) which is present in the dairy product once fermented, must be at least equal to $500*10^6$/g and the concentration of L.a. I-1492 must be at least $380*10^6$/g.

Although total amino acid content is similar to milk, free amino acid are significantly higher. The level of peptides comprised in the fermented dairy product, having a molecular weight between 1000 and 5000 Da. is around 30% and the level of small peptides having less than 10 residues is approximately 15%. It is known that such levels of peptides fortify, in a suprising way, the immune and digestive systems.

In the fermented dairy product thus obtained, the dairy proteins are disintegrated into peptides whose size may be more or less large, allowing for an easier digestion of the product. Indeed, subject allergic to milk proteins were able to digest the fermented dairy product thus obtained. Once the fermented dairy product is manufactured, it is thereafter refrigerated and must be consumed before it deteriorates. Generally, the consumption of the same must take place within a period of 60 to 120 days.

A dairy product according to the invention has been commercialized by the applicant Bio-K PLUS International Inc. (whose address is: 635 Victoria, Westmount, H3Y 2R9, CANADA) since June 2, 1996, under the trade-mark BioK+. However, the ferment of the present invention, which comprises the L.a. I-1492, acidophilus and casei strains has never been disclosed. Nor have the specific purified strain L.a. I-1492 and method of manufacturing the commercialized Bio K + product been disclosed. As it may be appreciated, a man skilled in the art would not , with the commercialized Bio K + product at hand, be able to reproduce the same product.

The applicant has also suprisingly found that the fermented dairy product according to the invention has a stimulation effect on the immune system and is thereby effective for treating various inflammatory reactions and/or infectious diseases such as colds, sinusitis, urinary infections. The recommanded treatment is a dose of 100g per day of the fermented dairy product for a period of 10 to 60 days depending on the case. Furthermore the fermented dairy product neutralizes side effects caused by antibiotics.

The fermented dairy product of the invention has also been found to have significant effect in treating diseases and infections related to the intestinal tract such as diarrhea, diverticulitis, mega-colon, Crohn disease. Indeed, the applicant has found that daily consumption of the dairy product of the invention eliminates most cases of the above-mentioned diseases.

Furthermore, the fermented dairy product of the present invention contains a fair amount of folic acid and vitamin B12 which was produced during fermentation of the lactic culture. As a result, consumption of such product resulted in fast recuperation time in subjects whom regularly exercise. This observation is especially true for older individuals. As a matter of fact, 8 out of 10 people consuming the product on a daily basis said they felt better.

The fermented dairy product according to the invention may also be used for the treatment of high blood levels of cholesterol. The recommended treatment consists of a dose of 100g per day for a period of 30 to 60 days.

The effect of this dairy product on the level of cholesterol in the blood was tested on a group of 14 patients to whom was prescribed a dose of 100g per day for a period of, depending on the case, 30 to 60 days. The results of these tests are shown in the following charts where the total cholesterol blood level (CHO total) present in the blood is indicated. Hence, these tests reveal that upon

regular intake during a period of 60 days, the total cholesterol level falled from between 7 to 22% and that the "low density level" (LDL) falled from between 9 to 25%. Furthermore, the level of triglycerides (TRIGLY) drops from between 28 to 43%. It can be seen that after 60 days of treatment, certain individuals recover a normal level of CHO/HDL (High density level) ratio, that is, a level comprised between 3.2 to 4.4 or of LDL/HDL ratio (that is, inferior to 3.8 for people aged 30 and over).

Of course, the above-mentioned example is solely for the purpose of illustrating the invention and is given only as a representative means. It must not be used to limit the scope of the invention which may extend to any obvious variations. For example, one may consider administering the ferment of the invention or powder on a dehydrated support.

BIOK + CHOLESTEROL STUDY

RD Case #1 (Med)

	T=0	T=30	T=60	REDUCTION
CHO TOTAL	6,64	6,06	5,16	22% after 60 days
HDL	0,72	0,62	0,66	
LDL	not available			
TRIGLY	4,64	6,64	4,59	
CHO/HDL	9,22	9,77	7,82	
LDL/HDL	not available			

RM Case #2 (Med)

	T=0	T=30	T=60	REDUCTION
CHO TOTAL	5,90	5,05	4,65	21% after 60 days
HDL	1,36	1,44	1,14	
LDL	4,13	3,25	3,09	25% after 60 days
TRIGLY	0,91	0,80	0,93	
CHO/HDL	4,33	4,43	4,07	
LDL/HDL	3	2,3	2,7	

EC Case #3 (Med)

	T=0	T=30	REDUCTION
CHO TOTAL	7,94	6,49	18% after 30 days
HDL	0,98	0,84	
LDL	5,86	4,88	17% after 30 days
TRIGLY	2,41	1,70	29% after 30 days
CHO/HDL	8,10	7,72	
LDL/HDL	6	5,8	

MB Case #4 (Med)

	T=0	T=30	T=60	REDUCTION
CHO TOTAL	7,75	6,49	6,58	15% after 60 days
HDL	1,15	1,00	1,06	
LDL	6,05	4,89	5,00	17% after 60 days
TRIGLY	1,22	1,31	1,15	
CHO/HDL	6,74	6,49	6,20	
LDL/HDL	5,3	4,9	4,7	

IM Case #5 (Med)

	T=0	T=30	T=60	REDUCTION
CHO TOTAL	7,06	6,74	6,59	6,7% after 60 days
HDL	1,26	1,29	1,16	
LDL	5,08	4,70	4,56	10% after 60 days
TRIGLY	1,59	1,65	1,92	
CHO/HDL				
LDL/HDL	3,6		3,9	

YC Case #6 (Med)

	T=0	T=30	T=60	REDUCTION
CHO TOTAL	7,38	7,03	7,06	4,3% after 60 days
HDL	1,49	1,65	1,66	
LDL	5,45	4,84	4,91	11.2% after 30 days
TRIGLY	0,97	1,18	1,07	
CHO/HDL				
LDL/HDL	3,7	2,9	3,0	19% after 60 days

ES Case #7 (Med)

	T=0	T=30	T=60	REDUCTION
CHO TOTAL	6,33	6,11	6,16	2,7% after 60 days
HDL	1,26	1,04	1,08	
LDL	4,24	4,24	4,48	
TRIGLY	1,83	1,83	1,31	28,4% after 60 days
CHO/HDL				
LDL/HDL	3,4	4,1	4,2	

RL Case #8 (Med)

	T=0	T=30	T=60	REDUCTION
CHO TOTAL	6,41	6,64	6,84	6,7% after 60 days
HDL	1,28	1,30	1,13	
LDL	4,73	4,85	5,02	
TRIGLY	0,88	1,07	1,52	
CHO/HDL				
LDL/HDL	3,7	3,7	4,4	

JGT Case #9 (Med)

	T=0	T=30	REDUCTION
CHO TOTAL	6,33	6,36	
HDL	1,88	1,90	
LDL	4,07	3,93	
TRIGLY	0,84	1,16	
CHO/HDL			
LDL/HDL	2,2	2,1	TB

CC Case #10 (Med)

	T=0	T=60	REDUCTION
CHO TOTAL	7,64	6,99	
HDL	0,96	1,03	
LDL	5,18	5,10	
TRIGLY	3,31	1,89	43% after 60 days
CHO/HDL	7,9	6,8	
LDL/HDL	5,4	5,0	

XB Case #11 (Med)

	T=0	T=30	REDUCTION
CHO TOTAL	7,03	6,31	10% after 30 days
HDL	1,23	1,18	
LDL	4,97	4,45	10,5% after 30 days
TRIGLY			
CHO/HDL	5,7	5,3	
LDL/HDL	4,04	3,77	Return to normal

XX Case #12 (Med)

	T=0	T=30	T=60	REDUCTION
CHO TOTAL	6,84	6,25	6,03	12% after 60 days
HDL	1,21	1,17	1,13	
LDL	4,93	4,38	4,44	9% after 60 days
TRIGLY				
CHO/HDL				
LDL/HDL	4,07	3,74	3,93	

CV Case #13 (MR)

	T=0	T=120 days	REDUCTION
CHO TOTAL	6,60	5,18	21% after 4 months
HDL	1,28	1,30	
LDL	3,51	2,79	20,5% NORMAL
TRIGLY	3,96	2,39	39%
CHO/HDL	5,12	3,98	22% return to NORMAL
LDL/HDL	2,74	2,14	22% return to NORMAL

ITC Case #14 (HCLM)

	T=0	T=30	T=60	REDUCTION
CHO TOTAL	9,41	8,34	7,76	17,5%
HDL	1,27	1,11	1,10	
LDL	7,35	6,31	5,89	20%
TRIGLY	2,13	2,48	1,68	21% return to NORMAL
CHO/HDL	7,4	7,5	7,05	
LDL/HDL	5,78	5,68	5,4	

HCLM - Charles Le Moine Hospital

MED - Medicis

REFERENCES

CHO TOTAL 4,2-5, HDL 0-0,9 LDL <3,4 TRIGLY 0,4-2,1CHO/HDL 3,2-4,4 LDL/HDL <3,8 for >30 years old.

CLAIMS:

1. The microorganism Lactobacillus acidophilus CNCM I-1492.

2. A biologically pure culture of the microorganism Lactobacillus acidophilus CNCM I-1492.

3. A lactic ferment comprising a purified strain of Lactobacillus acidophilus CNCM I-1492.

4. A lactic ferment of claim 3, further comprising at least one other Lactobacillus acidophilus strain and at least one Lactobacillus casei strain.

5. A method for producing a fermented dairy product by fermentation of the ferment of claim 4 in a milk-based medium.

6. A fermented dairy product obtained by the method of claim 5, wherein it comprises at least 500 millions per gram of microorganisms of the Lactobacillus acidophilus strain, where at least 380 millions per gram are microorganisms of the Lactobacillus acidophilus CNCM/I-1492 strain.

7. A method for reducing blood cholesterol level in a mammal, comprising the step of administrating through ingestion 100 gram per day of the fermented dairy product as claimed in claim 6, for a period of 30 to 60 days.

8. A method for treating infectious diseases, comprising the step of administrating through ingestion 100 gram per day of the fermented dairy product as claimed in claim 6, for a period of 10 to 60 days.

9. A method of claim 8, wherein the infectious diseases consist of colds, sinusitis and urinary infections.

10. A method for treating gastric-intestinal related diseases and infections comprising the step of administering through ingestion 100 gram per day of the fermented dairy product of claim 6 for a minimum period of 10 to 60 days.

11. A method of claim 10, wherein the gastric-intestinal diseases and infections consist of food poisining, diarrhea, diverticulitis, mega-colon and Crohn disease.

12. Use of the fermented dairy product of claim 6, for reducing blood cholesterol level in a mammal.

13. Use of the lactic ferment of claim 4, for reducing blood cholesterol level in a mammal.

14. Use of the Lactobacilus acidophilus CNCM I-1492 of claim 2, for reducing the blood cholesterol level in a mammal.

15. Use of the fermented dairy product of claim 6, for treating infectious diseases comprising colds, sinusitis and urinary infections

16. Use of the lactic ferment of claim 4, for treating infectious diseases comprising colds, sinusitis and urinary infections.

17. Use of the Lactobacilus acidophilus CNCM I-1492 of claim 2, for treating infectious diseases comprising colds, sinusitis and urinary infections.

18. Use of the fermented dairy product of claim 6, for treating gastric-intestinal related diseases and infections comprising diarrhea, diverticulitis, mega-colon and Crohn disease.

19. Use of the fermented dairy product of claim 4, for treating gastric-intestinal related diseases and infections comprising diarrhea, diverticulitis, mega-colon and Crohn disease.

20. Use of the fermented dairy product of claim 2, for treating gastric-intestinal related diseases and infections comprising diarrhea, diverticulitis, mega-colon and Crohn disease.

21. Use of the feremented dairy product of claim 6 for neutralizing side effects caused by antibiotics.

INTERNATIONAL SEARCH REPORT

Intern: ial Application No
PCT/CA 97/00915

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N1/20 A61K35/74 A23C9/123

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)
IPC 6 C12N

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 181 170 A (ADVANCE KAIHATSU KENKYUSHO) 14 May 1986 see page 1, line 22 - page 3, line 9 see page 12 - page 15; example 1 ---	1-3,14
X	US 5 516 684 A (SAITO YOSHIO ET AL) 14 May 1996 see column 2, line 46 - column 4, line 20 --- -/--	1-3,14

[X] Further documents are listed in the continuation of box C.

[X] 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

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Date of the actual completion of the international search	Date of mailing of the international search report
6 March 1998	26. 03. 1998

Name and mailing address of the ISA	Authorized officer
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	Sitch, W

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE MEDLINE FILE SERVER STN KARLSRUHE ABSTRACT 96114484, MITAL ET AL: "ANTICARCINOGENIC, HYPOCHOLESTEROLEMIC, AND ANTAGONISTIC ACTIVITIES OF LACTOBACILLUS ACIDOPHILUS" XP002057947 see abstract & CRITICAL REVIEWS IN MICROBIOLOGY, vol. 21, no. 3, 1995, pages 175-214, ---	1-3,14
A	GONZALEZ ET AL: "PREVENTION OF INFANTILE DIARRHOEA BY FERMENTED MILK" MICROBIOLOGIE-ALIMENTS-NUTRITION, vol. 8, 1990, pages 349-354, XP002057945 see page 349 see abstract ---	
A	NADER DE MARCIAS ET AL: "INHIBITION OF SHIGELLA SONNEI BY LACTOBACILLUS CASEI AND LACT. ACIDOPHILUS" JOURNAL OF APPLIED BACTERIOLOGY, vol. 73, 1992, pages 407-411, XP002057946 see page 407 see abstract ---	
A	DATABASE FSTA INTERNATIONAL FOOD INFORMATION SERVICE (IFIS), FRANFURT/MAIN, DE ABSTRACT 90-1-10-P0121 FSTA, PERDIGON ET AL: "PREVENTION OF GASTROINTESTINAL INFECTION USING IMMUNOBIOLOGICAL METHODS WITH MILK FERMENTED WITH LACTOBACILLUS CASEI AND LACTOBACILLUS ACIDOPHILUS" XP002057948 see abstract & JOURNAL OF DAIRY RESEARCH, vol. 57, 1990, pages 255-264, ---	
A	EP 0 199 535 A (NEW ENGLAND MEDICAL CENTER INC) 29 October 1986 see page 1, line 3 - page 2, line 23 see page 10, line 6 - page 11, line 12 ---	
1 A 1	EP 0 577 903 A (NESTLE SA) 12 January 1994 see claims 1-10 ---	
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INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/CA 97/00915

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 577 904 A (NESTLE SA) 12 January 1994 see page 2, line 16 - line 43 ---	
A	GB 2 261 372 A (REID GREGOR ;WATT BRUCE ANDREW (GB)) 19 May 1993 see page 4, line 21 - page 6, line 3 -----	

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 97/00915

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

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 II Observations where unity of invention is lacking (Continuation of item 2 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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 7-21 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.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte .onal Application No
PCT/CA 97/00915

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr nal Application No
PCT/CA 97/00915

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