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(72) Feltaláló(k):
MÖLLSTAM, Bo, S-443 21 LERUM (SE)
CONNOLLY, Eamonn, S-181 30 Lidingö (SE)

(73) Jogosult(ak):
BIOGAIA AB, 404 26 Gothenburg (SE)

(74) Képviselő:
Olle Bäcklund, Spanga

(54) **Tejsavtermelő baktériumok szelekciója és alkalmazása helicobacter által okozott gyulladások csökkentésére**

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(54) **SELECTION AND USE OF LACTIC ACID BACTERIA FOR REDUCING INFLAMMATION CAUSED BY HELICOBACTER**

AUSWAHL UND VERWENDUNG VON MILCHSÄUREBAKTERIEN ZUR VERMINDERUNG VON DURCH HELICOBACTER AUSGELÖSTE ENTZÖNDUNGEN

SELECTION ET UTILISATION DE LA BACTERIE DE L'ACIDE LACTIQUE POUR REDUIRE L'INFLAMMATION DUE A L'HELICOBACTER

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(73) Proprietor: **BIOGAIA AB**
404 26 Gothenburg (SE)

(72) Inventors:
- **MÖLLSTAM, Bo**
  S-443 21 LERUM (SE)
- **CONNOLLY, Eamonn**
  S-181 30 LIDINGÖ (SE)

(74) Representative: **Owen, Deborah Jane et al**
Dehns
St Bride's House
10 Salisbury Square
London, EC4Y 8JD (GB)

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Description

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] This invention relates to use of a method for screening nonpathogenic anti-inflammatory bacterial strains, and products and methods using such strains for treatment and prophylaxis of inflammation caused by gastrointestinal bacteria such as *Helicobacter pylori,* other species of *Helicobacter,* and other inflammation-causing gastrointestinal pathogens.

Description of the Related Art

[0002] *Helicobacter pylori* is a spiral-shaped bacterium that colonizes the stomach by, among other things, its ability to produce urease to neutralize the acids in the stomach. Urease converts urea, of which there is an abundant supply in the stomach, to bicarbonate and ammonia, which are strong bases. This results in a cloud of acid-neutralizing bases around the *H. pylori* cells, protecting them from the acid in the stomach. The *H. pylori* cells penetrate and traverse the gastric mucus layer and attach to epithelial cells in the lining of the stomach. At least some strains of *H. pylori* have the ability to produce toxins. Infection with *H. pylori* activates the host immune system, which sends white blood cells, killer T cells and other infection-fighting agents to the area, but the body's immune system is not effective in reversing the effects of *H. pylori* in the mucus lining of the stomach. The *H.* pylori cells remain in the lining, and the immune system escalates its response to the cells, creating an inflammation if there are not sufficient anti-inflammatory mechanisms available. During the infection with *H. pylori,* cytokine intercellular signal proteins generated by the host epithelium dendritic cells, natural killer cells, T-cells and other immune defense cells propagate the immune response to the invading pathogen. Consequently, host neutrophils are attracted to and infiltrate the stomach epithelium and persist there throughout the infection. These cells generate, among other factors, reactive oxygen products, such as superoxide radicals, which lead to oxidation in the epithelial cells and consequent epithelial cell death, ulcer formation and ultimately carcinogenesis. *H. pylori* also induces leakage of nutrients from the host over the stomach epithelium providing a nutrient source to sustain the *H. pylori* cells and exacerbate the infection and its consequences. *H. pylori* is able to evade the human immune system and survive in the stomach despite the immune response of the host and the mechanisms of this evasion are the subject of current research.

[0003] Current therapy is based on eradicating *H. pylori* through antibiotics and proton pump inhibitors rather than attempting to eliminate the effects of excessive immune response of the host to the infection, such as making sufficient anti-inflammatory mechanisms available, which is the purpose of the present invention.

[0004] Thus, infection with *H. pylori* causes an increased risk of developing gastritis, gastric and duodenal ulcers, including peptic ulcer, gastric cancer, and gastric mucosaassociated lymphoid tissue lymphoma. These problems are not caused directly by the *H. pylori* cells, but by the inflammation of the stomach lining in response to the *H. pylori.* Various treatments have been used to ameliorate the symptoms of gastric and duodenal ulcers, such as treatments that reduce acid production in the stomach, combined with antibiotics. Novel vaccines against *H. pylori* has also been tried but with limited success. It is also known that other species of *Helicobacter,* as well as other gastrointestinal pathogens, can cause gastrointestinal inflammation.

[0005] In a recent research article, researchers studying *H. pylori* infections concluded that infection by *H. pylori* elicits gastric mucosal sialylation as part of the chronic inflammatory response and that many virulent strains are thus better able to attach to the inflamed site (Science 18:573-578, 2002).

[0006] Inflammation in the stomach and gastrointestinal tract is mediated by intercellular signal proteins known as cytokines which are produced by macrophages and dendritic cells in the epithelium in response to an antigenic stimulus such as that produced by *H. pylori* or other pathogens. Upon contact between the epithelium and the antigen such as *H. pylori* or endotoxins produced by it, such as LPS, antigen presenting cells (including dendritic cells) in the epithelium propagate the signal to naive macrophages which then respond in a so-called Th-1 type response where pro-inflammatory cytokines including TNFα, IL-1, IL-6, IL-12 are produced by the macrophages. These cytokines in turn stimulate natural killer cells, T-cells and other cells to produce interferon γ (IFNγ), which is the key mediator of inflammation. IFNγ leads to an escalation of the inflammatory response and the reactions described above that lead to cytotoxicity. Naive macrophages can also respond to antigens with a Th-2 type response. This response is suppressed by IFNγ. These Th-2 type cells produce anti-inflammatory cytokines such as IL-4, IL-5, IL-9 and IL-10.

[0007] IL-10 is known to inhibit the production of IFNγ and thus dampen the immune response. The balance between Th-1 and Th-2 type cells and their respective cytokine production defines the extent of the inflammation response to a given antigen. Th-2 type cells can also stimulate the production of immunoglobulins via the immune system. Anti-inflammatory activity in the gastrointestinal tract, where there is a reduced TNFα level, correlates with enhanced epithelial cells (gut wall lining integrity) and thus to a reduction in the negative effects caused by gastrointestinal pathogens and toxins.

[0008] The results of a number of research studies indicate that DNA can exert an anti-inflammatory action on intestinal epithelial cells, or can stimulate the immune system. (Madsen et al. and Rachmilewitz et al, respectively, presentations at Digestive Disease Week, May

19-22, 2002, The Moscone Center, San Francisco).

[0009] Mice spontaneously develop chronic colitis, which does not occur in germfree animals. Mouse colitis is similar to human Crohn's disease, a chronic serious inflammatory disease of the gastrointestinal tract. Crohn's disease usually occurs in the intestines, but may occur anywhere in the gastrointestinal tract. These conditions require the presence of enteric bacteria and are both Th1-mediated-IL-12-dependent forms of colitis. Because of the similarities of the causes and symptoms, mouse models of colitis and other mouse models are used to study components of the inflammatory response directly, and are, as the same mechanisms apply in man, accepted to be used to develop treatments for human gastrointestinal disease.

[0010] WO 00/41707 discloses that *Lactobacillus salivarius* can be useful in the prophylaxis or treatment of undesirable inflammatory activity, especially gastrointestinal inflammatory activity such as inflammatory bowel disease or irritable bowel syndrome.

[0011] US 5,578,302 discloses that stomach ulcers can be treated by administering orally to a human in need thereof an *anti-Helicobacter pylori* effective amount of a composition containing, in combination with an ingestible support, a culture of *Lactobacillus johnson ii* strain CNCM I-1225 or a supernatant phase isolated from a culture of *Lactobacillus johnsonii* strain CNCM I-1225.

[0012] JP 2001258549 discloses a lactic acid bacteria having antimicrobial activity against *Helicobacter pylori* KS 51 strain separated from the stomach ulcer of a patient. The lactic acid bacteria are selected from the genus *Lactobacillus* separated from human and preserved, and also from the genus *Bifidobacterium* and *Enterococcus.* Lactic acid bacteria having urease activity control the ability of *Helicobacter pylori* and control the fixation of *Helicobacter pylori* in the stomach.

[0013] In addition, Coconnier et al., Applied and Environmental Microbiology, Nov. 1998, p. 4573-4580 discloses antagonistic activity against *Helicobacter* infection in *vitro* and *in vivo* by the human *Lactobacillus acidophilus* strain LB.

[0014] *Lactobacillus reuteri* is one of the naturally occurring inhabitants of the gastrointestinal tract of animals and is routinely found in the intestines or healthy animals and despite the low pH, occasionally also in the human stomach. It is known to have antibacterial activity. See, for example U.S. Patent Nos. 5,439,678, 5,458,875, 5,534,253, 5,837,238, and 5,849,289. When *L. reuteri* cells are grown under anaerobic conditions in the presence of glycerol, they produce the antimicrobial substance known as reuterin (β-hydroxy-propionaldehyde). The aforementioned U.S patent No. 5,837,238 discloses a therapeutic method of treating diarrhea of a patient, such as that caused by rotavirus, in which a liquid suspension of one or more strains of *Lactobacillus reuteri* is administered to the patient.

[0015] *L. coryniformis* is a less well-known species of *Lactobacillus* which is a rather common inhabitant of the human oral cavity. It can also be found in soil, manure and plant material. It has been found in silage and as a beer spoiler, and good lactic acid production has been reported as well as antifungal activity. The *L. coryniformis* MM7 isolate (ATCC PTA-4660) used herein was found in human mother's milk.

[0016] Immunomodulating activity has also been associated with various lactobacilli. While the possibility of effective antibacterial activity by several lactobacilli is known, it was not previously known that substantial differences existed between strains in their ability to reduce gastrointestinal inflammation, nor that such strains could be selected.

[0017] It is therefore an object of the invention to provide strains of *Lactobacillus* which have been selected for their capability of reducing gastrointestinal inflammation, such as that due to *Helicobacter pylori.* It is a further object of the invention to provide products containing said strains, including agents for treatment or prophylaxis of inflammation associated with *Helicobacter pylori* for administration to humans, including conditioned media in which said strains have grown and protein-containing extracts thereof.

[0018] Other objects and advantages will be more fully apparent from the following disclosure and appended claims.

SUMMARY OF THE INVENTION

[0019] The invention herein provides certain *Lactobacillus* strains which have been selected for their capability of reducing gastrointestinal inflammation, such as that due to *Helicobacter pylori,* and products derived from said strains, including agents for treatment or prophylaxis of inflammation associated with *Helicobacter pylori* for administration to humans, and include conditioned media in which said strains have grown and protein-containing extracts of the conditioned media.

[0020] Other objects and features of the inventions will be more fully apparent from the following disclosure and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021]

Figure 1 is a bar graph showing the effect of *Lactobacillus*-conditioned media on TNFα production by LPS-activated macrophages. Forty-five *Lactobacillus* strains were tested.

Figure 2 is a bar graph showing the fold change in macrophage TNFα expression in the presence of conditioned media from various *Lactobacillus* strains and LPS compared to macrophages with LPS alone.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED

EMBODIMENTS THEREOF

[0022] The present invention provides a biologically pure culture of *Lactobacillus coryniformis* strain MM7, ATCC PTA-4660.

[0023] In a further embodiment, the present invention also provides a biologically pure culture of *Lactobacillus reuteri* strain MM2-3, ATCC PTA-4659.

[0024] The present invention also provides a cell-free culture supernatant isolated from a biologically pure culture of *Lactobacillus reuteri* strain MM2-3, ATCC PTA-4659.

[0025] The present invention also provides a cell-free culture supernatant isolated from a biologically pure culture of *Lactobacillus coryniformis* strain MM7, ATCC PTA-4660.

[0026] The present invention also provides the biologically pure cultures and cell free supernatants of the invention for use in reducing gastrointestinal inflammation associated with *Helicobacter pylori* infection in the gastrointestinal tract in mammals.

[0027] The present invention also provides a product comprising cells of a *Lactobacillus* strain of the invention or a cell-free supernatant of the invention.

[0028] The present invention also provides a food composition comprising an ingestible support and an *H. pylori* associated inflammation-reducing component derived from a strain of *Lactobacillus* selected from the group consisting of *Lactobacillus coryniformis* strain MM7, ATCC PTA-4660 and *Lactobacillus reuteri* strain MM2-3, ATCC PTA-4659, wherein said component comprises cells of a biologically pure culture of a strain of *Lactobacillus* of the invention, or wherein said component comprises a cell-free culture supernatant of the invention.

[0029] The present invention also provides a pharmaceutical composition comprising a pharmaceutical carrier and an *H. pylori* associated inflammation-reducing component derived from a strain of *Lactobacillus* selected from the group consisting of *Lactobacillus coryniformis* strain MM7, ATCC PTA-4660 and *Lactobacillus reuteri* strain MM2-3, ATCC PTA-4659, wherein said component comprises cells of a biologically pure culture of a strain of *Lactobacillus* of the invention, or wherein said component comprises a cell-free culture supernatant of the invention.

[0030] The present invention also provides a nutritional supplement comprising an ingestible support and an *H. pylori* associated inflammation-reducing component derived from a strain of *Lactobacillus* selected from the group consisting of *Lactobacillus coryniformis* strain MM7, ATCC PTA-4660 and *Lactobacillus reuteri* strain MM2-3, ATCC PTA-4659, wherein said component comprises cells of a biologically pure culture of a strain of *Lactobacillus* of the invention, or wherein said component comprises a cell-free culture supernatant of the invention.

[0031] Thus, the present invention herein provides strains of *Lactobacillus* which have been selected for their capability of reducing gastrointestinal inflammation, such as that due to *Helicobacter pylori,* which are *Lactobacillus coryniformis MM7,* ATCC PTA-4660 and *Lactobacillus reuteri* MM2-3, ATCC PTA-4659. Products such as foods, nutritional additives and formulations, pharmaceuticals or medical devices containing whole cells or components derived from these strains may be formulated as is known in the art, and generally include an ingestible support as known plus the *Lactobacillus*-strain, or its derived component. Previously known strains, now identified to have good TNFα reducing capacity, such as *L.rhamnosus* GG ATCC 53103, *L.reuteri* ATCC 55730 and others, can also be used in above formulations. These products are agents for treatment or prophylaxis of inflammation associated with *Helicobacter pylori* for administration to mammals.

[0032] Model systems using the appropriate cytokines are used to determine factors that reduce or increase inflammation. In the examples provided herein, a mouse macrophage assay, using the RAW 264.7 macrophage cells (ATCC, Rockville, MD, ATCC # TIB-71), is used to screen strains of bacteria, primarily lactobacilli, for their effect on the inflammatory pathway. IL-10 is used in this assay as a positive control, with treatments with IL-10 showing inhibition of pro-inflammatory cytokines such as TNFα (tumor necrosis factor alpha). After individual growth of the Lactobacillus strains to be screened in laboratory media, the live bacterial cells are removed by filtration and the supernatant fluid (also called the "conditioned-medium" herein) is tested in the macrophage assay. The macrophages are first stimulated with the pro-inflammatory antigen for example, purified LPS (*E coli* derived lipopolysaccharide), *S. aureus* derived lipoteichoic acid (LTA) or cell free *E. coli* or *Helicobacter* conditioned media, to produce the pro-inflammatory cytokines including TNFα. The conditioned medium from the *Lactobacillus* strain, containing the putative immunomodulating substances derived from the bacteria to be screened, is co-incubated with the antigen-activated macrophages. The capacity of the conditioned medium to modulate the immune response of the macrophages is monitored by the change in TNFα production by the cells. The TNFα profile from the assay enables a selection of the strains most effective in reducing the production of TNFα by the macrophages. Control experiments with pH adjustment in the assay system eliminates the possibility that a changed pH could cause the observed effect.

[0033] Surprisingly, apparently similar bacterial isolates and strains of *Lactobacillus,* even coming from very similar human sources show varying and widely different abilities to influence the production of TNFα by macrophages in response to a pro-inflammatory antigen. These strains cannot be identified even by genetic fingerprinting since they can be up to 98% similar genetically but still show very different effects on the immune cells. The

strains thus screened and found to have a strong inhibitory effect against stimulated, pro-inflammatory cytokine production by macrophages are especially effective in the treatment of inflammation in the gastrointestinal tract of man, including *H. pylori* caused inflammation in the stomach.

[0034] The features of the present invention will be more clearly understood by reference to the following examples, which are not to be construed as limiting the invention.

Example 1. Selection of anti-inflammatory strains.

[0035] *Lactobacillus* spp. (including for example *L. rhamnosus* GG ATCC 53103, *L. johnsonii* ATCC 33200, *L. reuteri MM2-3 ATCC PTA-4659, L. coryniformis, MM7, ATCC PTA-4660*) and *E. coli* Nissle were grown in de Man, Rogosa, Sharpe (MRS) and Luria-Bertani (LB) media (Difco, Sparks, MD), respectively. Overnight cultures of lactobacilli were diluted to an OD_{600} of 1.0 (representing approximately 10^9 cells/ml) and further diluted 1:10 and grown for an additional 4, 8 and 24 h. *Helicobacter pylori,* (Sydney strain SS1) and *Helicobacter hepaticus* 3B1(ATCC 51449) were cultured for 48 h in Brucella broth (Difco) supplemented with 10% fetal bovine serum (FBS). Cultures were diluted 1:10 and grown for another 24 and 48 h. Bacterial cell-free conditioned medium was collected by centrifugation at 8500 rpm for 10 min at 4°C. Conditioned medium was separated from the cell pellet and then filtered through a 0.22 μm pore filter unit (Millipore, Bedford, Mass.).

[0036] Mouse monocyte/macrophage cell lines, RAW 264.7 (ATCC TIB-71) and RAW 264.7 gamma NO(-) (ATCC CRL-2278), were used as a reporter cells for studying the inflammatory response pathway. RAW 264.7 cells were grown in either Dulbecco's Modified Eagle Medium (wild-type) or RPMI Medium 1640 (gamma NO-) (Gibco-Invitrogen, Carlsbad, CA) supplemented with 10% FBS and 2% antibiotic (5000 units/ml Penicillin and 5 mg/ml Streptomycin, Sigma) at 5% CO_2 37°C until 80-90% confluent. Approximately 5 x 10^4 cells were seeded into 96-well cell culture clusters and allowed to adhere for 2 h prior to lipopolysaccharide (LPS) activation and addition of conditioned medium. Naive RAW 264.7 cells were exposed to purified LPS from *E. coli* serotype O127:B8 (Sigma). Activation medium was made by adding 2 ng LPS to 20 μl conditioned medium per well. Macrophages were either pre-incubated or co-incubated with cell-free *Lactobacillus* conditioned medium. Recombinant mIL-10 (R&D Systems, Minneapolis, Min.) was used as a positive control. Cell viability was assessed by Trypan-blue (Invitrogen) exclusion. The presence of TNF-α in cell culture supernatant was measured with a sandwich enzyme immunoassay, Quantikine M® Mouse TNF-α Immunoassay (R & D Systems).

[0037] The effect of *Lactobacillus-conditioned* media on TNFα production by LPS-activated macrophages is shown in Figure 1, which shows that of the 45 strains tested, several different strains are capable of decreasing TNFα production by the activated macrophages. Figure 2 shows the fold change in TNFα expression with various *Lactobacillus* strains compared to LPS alone. The results of these studies are then used to select the most efficient strains. The strains mentioned in the figures but not specifically mentioned in the text are various strains of *Lactobacillus,* primarily *L. reuteri* that were tested.

[0038] In this example, *L. coryniformis* MM7, ATCC PTA-4660, was selected by using the method above, for addition to a standard yogurt. The *L. coryniformis* strain was grown and lyophilized, using standard methods for growing *Lactobacillus* in the dairy industry. This culture was then added to previously fermented milk, using traditional yogurt cultures, at a level of 10E+7 CFU/gram of yogurt, and the yogurt was used by humans as a prevention of gastritis caused by *H. pylori.*

Example 2 Use of the Conditioned medium

[0039] Using the method above, the conditioned medium from one effectively TNFα decreasing strain was selected, in this experiment the medium from *L. reuteri* ATCC PTA-4659. This medium was produced in larger scale by growing the strain in de Man, Rogosa, Sharpe (MRS) (Difco, Sparks, MD). Overnight cultures of lactobacilli were diluted to an OD_{600} of 1.0 (representing approximately 10^9 cells/ml) and further diluted 1:10 and grown for an additional 24 h. Bacterial cell-free conditioned medium was collected by centrifugation at 8500 rpm for 10 min at 4°C. Conditioned medium was separated from the cell pellet and then filtered through a 0.22 μm pore filter unit (Millipore, Bedford, Mass.).The conditioned medium was then lyophilized and formulated, using standard methods, to make a tablet. This tablet was used as a drug by humans to treat ulcer caused by *H. pylori.*

Example 3. DNA-Fingerprinting of *Lactobacillus reuteri* strains

[0040] The method of U.S. Patent Nos. 5,523,217 and 5,691,136 of Lupski et al. was used to do genomic fingerprinting of *L. reuteri* strains. This method utilizes amplification of the bacterial DNA by adding a pair of outwardly-directed primers to the bacterial sample. After amplification, the extension products of the resulting hybridization are separated by size, and the strain of bacteria is characterized by measuring the pattern of sized extension products. Duplicate gel images were obtained for 82 strains of *L. reuteri* by Bacterial BarCodes, Inc. (Houston, TX) using the Uprime E primer (one primer) The duplicate sets of data were comparable. There were a total of 11 clusters, which were different from each other, and eight outliers, which appeared to be unique.

[0041] The strains found to be effective in reducing the TNF-α (see Figures 1 and 2) do not group together using this method, showing that it is not sufficient to use DNA-

fingerprinting this way to find several strains with TNFα reducing capacity.

Example 4. Characterization of protein produced by effective *Lactobacillus* strains

[0042] Different effective *Lactobacillus* conditioned media, including the *L. reuteri* strain MM2-3 conditioned medium, were treated with various denaturing compounds to determine the nature of the putative immunomodulins derived from the bacteria. Thus, conditioned media were subjected to repetitive freeze-thawing, heat treatment, digestion with DNA digesting enzymes, proteases and inactivated proteases. The putative immunomodulin was in this way determined to be one or more proteins or peptides in nature. To determine the size of the putative protein immunomodulin, the conditioned medium was fractionated by filtration and the filtrates tested for effectiveness. In this way, the active component of the conditioned media of effective *Lactobacillus* strains was found to be approx 5 kDa in size or less.

Claims

1. A biologically pure culture of *Lactobacillus coryniformis* strain MM7, ATCC PTA-4660.

2. A biologically pure culture of *Lactobacillus reuteri* strain MM2-3, ATCC PTA-4659.

3. A cell-free culture supernatant isolated from a biologically pure culture of *Lactobacillus reuteri* strain MM2-3, ATCC PTA-4659.

4. A cell-free culture supernatant isolated from a biologically pure culture of *Lactobacillus coryniformis* strain MM7, ATCC PTA-4660.

5. The *Lactobacillus* strain of claim 1 or claim 2 or the cell-free culture supernatant of claim 3 or claim 4, for use in reducing gastrointestinal inflammation associated with *Helicobacter pylori* infection in the gastrointestinal tract in mammals.

6. A product comprising cells of the *Lactobacillus* strain of claim 1 or claim 2 or the cell-free supernatant of claim 3 or claim 4.

7. A food composition comprising an ingestible support and an *H. pylori* associated inflammation-reducing component derived from a strain of *Lactobacillus* selected from the group consisting of *Lactobacillus coryniformis* strain MM7, ATCC PTA-4660 and *Lactobacillus reuteri* strain MM2-3, ATCC PTA-4659, wherein said component comprises cells of a biologically pure culture of the strain of *Lactobacillus* of claim 1 or claim 2, or wherein said component comprises the cell-free culture supernatant of claim 3 or claim 4.

8. A pharmaceutical composition comprising a pharmaceutical carrier and an *H. pylori* associated inflammation-reducing component derived from a strain of *Lactobacillus* selected from the group consisting of *Lactobacillus coryniformis* strain MM7, ATCC PTA-4660 and *Lactobacillus reuteri* strain MM2-3, ATCC PTA-4659, wherein said component comprises cells of a biologically pure culture of the strain of *Lactobacillus* of claim 1 or claim 2, or wherein said component comprises the cell-free culture supernatant of claim 3 or claim 4.

9. A nutritional supplement comprising an ingestible support and an *H. pylori* associated inflammation-reducing component derived from a strain of *Lactobacillus* selected from the group consisting of *Lactobacillus coryniformis* strain MM7, ATCC PTA-4660 and *Lactobacillus reuteri* strain MM2-3, ATCC PTA-4659, wherein said component comprises cells of a biologically pure culture of the strain of *Lactobacillus* of claim 1 or claim 2, or wherein said component comprises the cell-free culture supernatant of claim 3 or claim 4.

Patentansprüche

1. Biologisch reine Kultur von *Lactobacillus coryniformis* Stamm MM7, ATCC PTA-4660.

2. Biologisch reine Kultur von *Lactobacillus reuteri* Stamm MM2-3, ATCC PTA-4659.

3. Zellfreier Kulturüberstand, der aus einer biologisch reinen Kultur von *Lactobacillus reuteri* Stamm MM2-3, ATCC PTA-4659 isoliert wurde.

4. Zellfreier Kulturüberstand, der aus einer biologisch reinen Kultur von *Lactobacillus coryniformis* Stamm MM7, ATCC PTA-4660 isoliert wurde.

5. Lactobacillus Stamm nach Anspruch 1 oder Anspruch 2 oder zellfreier Kulturüberstand nach Anspruch 3 oder Anspruch 4 zur Verwendung bei der Abschwächung einer Magen-Darm-Entzündung, die mit einer Infektion mit *Helicobacter pylori* im Magen-Darmtrakt von Säugetieren einhergeht.

6. Produkt, das Zellen des *Lactobacillus* Stamms nach Anspruch 1 oder Anspruch 2 oder des zellfreien Überstands nach Anspruch 3 oder Anspruch 4 umfasst.

7. Nahrungsmittelprodukt, das einen einnehmbaren Trägerstoff und eine Komponente umfasst, die eine

mit *H. pylori* einhergehende Entzündung abschwächt, die von einem *Lactobacillus* Stamm abgeleitet ist, der ausgewählt ist aus der Gruppe bestehend aus *Lactobacillus coryniformis* Stamm MM7, ATCC PTA-4660 und *Lactobacillus reuteri* Stamm MM2-3, ATCC PTA-4659, wobei die Komponente Zellen einer biologisch reinen Kultur des *Lactobacillus* Stamms nach Anspruch 1 oder Anspruch 2 umfasst oder wobei die Komponente einen zellfreien Kulturüberstand nach Anspruch 3 oder Anspruch 4 umfasst.

8. Pharmazeutische Zusammensetzung, die einen pharmazeutischen Trägerstoff und eine Komponente umfasst, die eine mit *H. pylori* einhergehende Entzündung abschwächt, die von einem *Lactobacillus* Stamm abgeleitet ist, der ausgewählt ist aus der Gruppe bestehend aus *Lactobacillus coryniformis* Stamm MM7, ATCC PTA-4660 und *Lactobacillus reuteri* Stamm MM2-3, ATCC PTA-4659, wobei die Komponente Zellen einer biologisch reinen Kultur des *Lactobacillus* Stamms nach Anspruch 1 oder Anspruch 2 umfasst oder wobei die Komponente einen zellfreien Kulturüberstand nach Anspruch 3 oder Anspruch 4 umfasst.

9. Nahrungsergänzungsmittel, das einen einnehmbaren Trägerstoff und eine Komponente umfasst, die eine mit *H. pylori* einhergehende Entzündung abschwächt, die von einem *Lactobacillus* Stamm abgeleitet ist, der ausgewählt ist aus der Gruppe bestehend aus *Lactobacillus coryniformis* Stamm MM7, ATCC PTA-4660 und *Lactobacillus reuteri* Stamm MM2-3, ATCC PTA-4659, wobei die Komponente Zellen einer biologisch reinen Kultur des *Lactobacillus* Stamms nach Anspruch 1 oder Anspruch 2 umfasst oder wobei die Komponente einen zellfreien Kulturüberstand nach Anspruch 3 oder Anspruch 4 umfasst.

Revendications

1. Culture biologiquement pure de la souche MM7 de *Lactobacillus coryniformis,* ATCC PTA-4660.

2. Culture biologiquement pure de la souche MM2-3 de *Lactobacillus reuteri,* ATCC PTA-4659.

3. Surnageant de culture exempt de cellules isolé d'une culture biologiquement pure d'une souche MM2-3 de *Lactobacillus reuteri,* ATCC PTA-4659.

4. Surnageant de culture exempt de cellules isolé d'une culture biologiquement pure d'une souche MM7 de *Lactobacillus coryniformis,* ATCC PTA-4660.

5. Souche de *Lactobacillus* selon la revendication 1 ou la revendication 2 ou surnageant de culture exempt de cellules selon la revendication 3 ou la revendication 4 pour utilisation dans la réduction d'une inflammation gastro-intestinale par infection à *l'Helicobacter pylori* dans le tractus gastro-intestinal chez les mammifères.

6. Produit comprenant des cellules de la souche de *Lactobacillus* selon la revendication 1 ou la revendication 2 ou le surnageant exempt de cellules selon la revendication 3 ou la revendication 4.

7. Composition alimentaire comprenant un support ingérable et un composant réducteur de l'inflammation associée au *H. pylori* provenant d'une souche de *Lactobacillus* choisie dans le groupe constitué de la souche MM7 de *Lactobacillus coryniformis,* ATCC PTA-4660, et de la souche *Lactobacillus reuteri,* ATCC PTA-4659, dans laquelle ledit composant comprend des cellules d'une culture biologiquement pure de la souche de *Lactobacillus* de la revendication 1 ou la revendication 2, ou dans laquelle ledit composant comprend le surnageant de culture exempt de cellules de la revendication 3 ou la revendication 4.

8. Composition pharmaceutique comprenant un véhicule pharmaceutique et un composant réducteur de l'inflammation associée au *H. pylori* obtenue à partir d'une souche de *Lactobacillus* choisie dans le groupe constitué de la souche *Lactobacillus coryniformis,* ATCC PTA-4660, et de la souche *Lactobacillus reuteri,* ATCC PTA-4659, dans laquelle ledit composant comprend des cellules d'une culture biologiquement pure de la souche de *Lactobacillus* selon la revendication 1 ou la revendication 2 ou dans laquelle ledit composant comprend le surnageant de culture exempt de cellules de la revendication 3 ou la revendication 4.

9. Supplément nutritif comprenant un support ingérable et un composant réducteur de l'inflammation associée au *H. pylori* provenant d'une souche de *Lactobacillus* choisie dans le groupe constitué de la souche MM7 de *Lactobacillus coryniformis,* ATCC PTA-4660, et de la souche *Lactobacillus reuteri,* ATCC PTA-4659, dans lequel ledit composant comprend des cellules d'une culture biologiquement pure de la souche de *Lactobacillus* de la revendication 1 ou la revendication 2, ou dans lequel ledit composant comprend le surnageant de culture exempt de cellules de la revendication 3 ou la revendication 4.

FIGURE 1

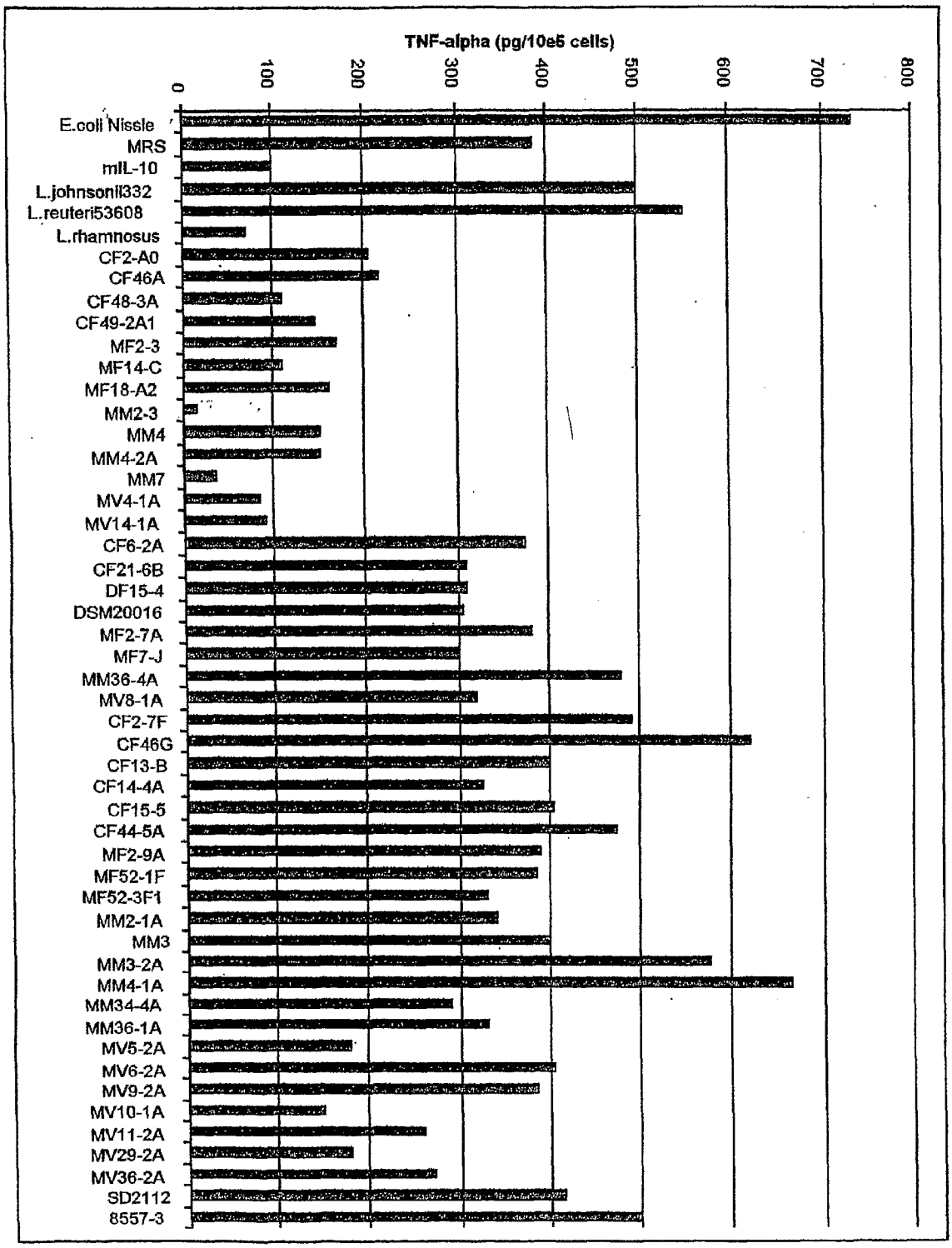
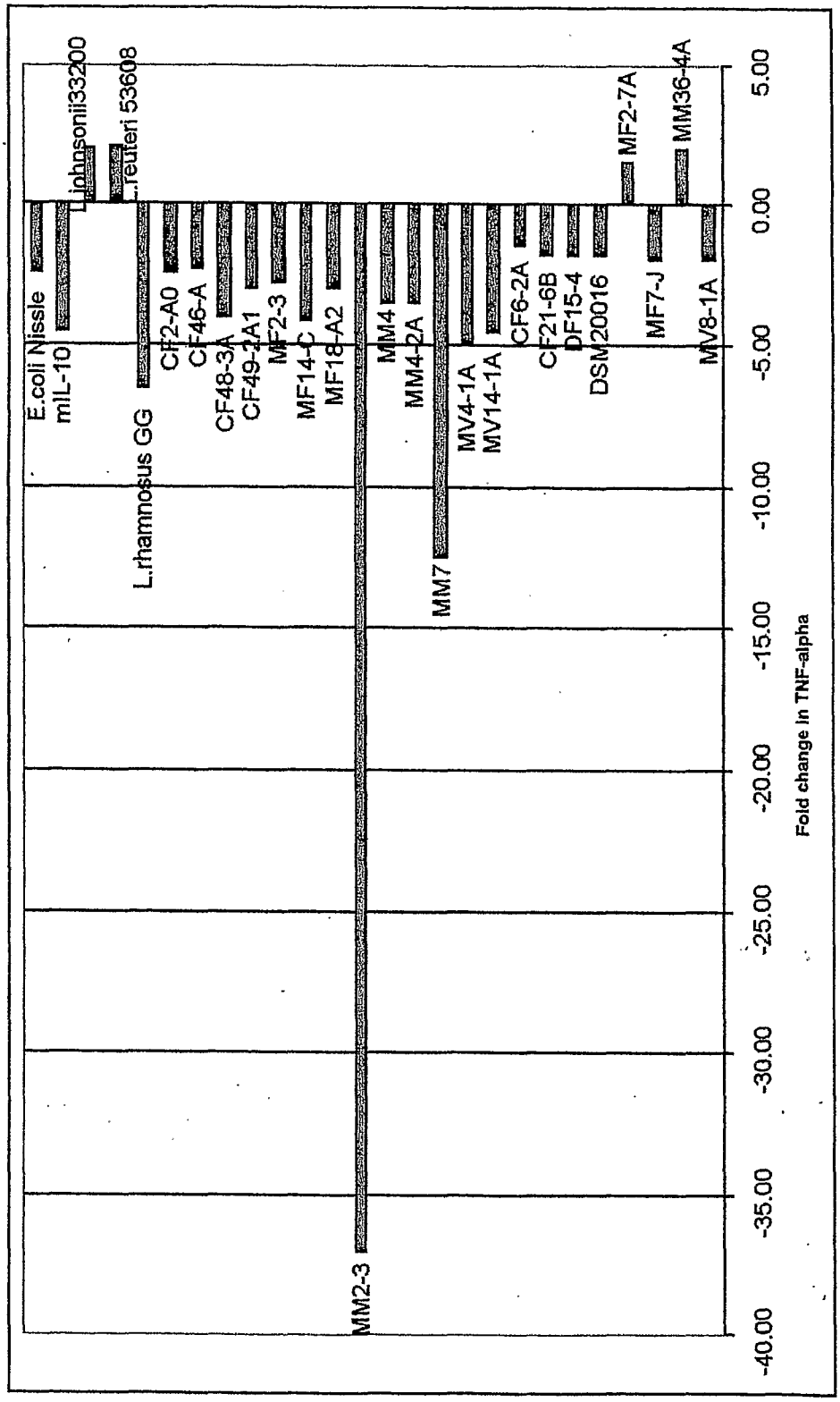

TNF-alpha (pg/10e5 cells)
0
100
200
300
400
500
600
700
800
E.coli Nissle
MRS
mIL-10
L.johnsonii332
L.reuteri53608
L.rhamnosus
CF2-A0
CF46A
CF48-3A
CF49-2A1
MF2-3
MF14-C
MF18-A2
MM2-3
MM4
MM4-2A
MM7
MV4-1A
MV14-1A
CF6-2A
CF21-6B
DF15-4
DSM20016
MF2-7A
MF7-J
MM36-4A
MV8-1A
CF2-7F
CF46G
CF13-B
CF14-4A
CF15-5
CF44-5A
MF2-9A
MF52-1F
MF52-3F1
MM2-1A
MM3
MM3-2A
MM4-1A
MM34-4A
MM36-1A
MV5-2A
MV6-2A
MV9-2A
MV10-1A
MV11-2A
MV29-2A
MV36-2A
SD2112
8557-3

FIGURE 2


E.coli Nissle
mIL-10
L.johnsonii33200
..reuteri 53608
L.rhamnosus GG
CF2-A0
CF46-A
CF48-3A
CF49-1A1
MF2-3
MF14-C
MF18-A2
MM2-3
MM4
MM4-2A
MM7
MV4-1A
MV14-1A
CF6-2A
CF21-6B
DF15-4
DSM20016
MF7-J
MV8-1A
MF2-7A
MM36-4A
5.00
0.00
-5.00
-10.00
-15.00
-20.00
-25.00
-30.00
-35.00
-40.00
Fold change in TNF-alpha

REFERENCES CITED IN THE DESCRIPTION

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TEJSAVTERMELŐ BAKTÉRIUMOK SZELEKCIÓJA ÉS ALKALMAZÁSA HELICOBACTER ÁLTAL OKOZOTT GYULLAÁSOK CSÖKKENTÉSÉRE

SZABADALMI IGÉNYPONTOK

1. A *Lactobacillus coryniformis* MM7, ATCC PTA-4660 törzs biológiailag tiszta tenyészete.

2. A *Lactobacillus reuteri* MM2-3, ATCC PTA-4659 törzs biológiailag tiszta tenyészete.

3. A *Lactobacillus reuteri* MM2-3, ATCC PTA-4659 törzs biológiailag tiszta tenyészetéből izolált sejtmentes tenyészet-felülúszó.

4. A *Lactobacillus coryniformis* MM7, ATCC PTA-4660 törzs biológiailag tiszta tenyészetéből izolált sejtmentes tenyészet-felülúszó.

5. Az 1. vagy 2. igénypont szerinti *Lactobacillus* törzs vagy a 3. vagy 4. igénypont szerinti sejtmentes tenyészet-felülúszó, *Helicobacter pylori* fertőzéssel összefüggő gastrointestinalis gyulladásnak emlősök gyomor- és bélrendszerében történő csökkentésében történő alkalmazásra.

6. Az 1. vagy 2. igénypont szerinti *Lactobacillus* törzs sejtjeit vagy a 3. vagy 4. igénypont szerinti sejtmentes tenyészet-felülúszót tartalmazó termék.

7. Élelmiszer, mely egy fogyasztható hordozót és egy *Lactobacillus coryniformis* MM7, ATCC PTA-4660 törzs és *Lactobacillus reuteri* MM2-3, ATCC PTA-4659 törzs közül választott *Lactobacillus* törzsből származó *H.pylori* fertőzéssel összefüggő gastrointestinalis gyulladást csökkentő komponenst tartalmaz, ahol a komponens az 1. vagy 2. igénypont szerinti *Lactobacillus* törzs biológiailag tiszta tenyészetének sejtjeit tartalmazza, vagy ahol a komponens a 3. vagy 4. igénypontok szerinti sejtmentes tenyészet-felülúszót tartalmazza.

8. Gyógyászati készítmény, mely egy gyógyászatilag elfogadható hordozót és egy *Lactobacillus coryniformis* MM7, ATCC PTA-4660 törzs és *Lactobacillus reuteri* MM2-3, ATCC PTA-4659 törzs közül választott *Lactobacillus* törzsből származó *H.pylori* fertőzéssel összefüggő gastrointestinalis gyulladást csökkentő komponenst tartalmaz, ahol a komponens az 1. vagy 2. igénypont szerinti *Lactobacillus* törzs biológiailag tiszta tenyészetének sejtjeit tartalmazza, vagy ahol a komponens a 3. vagy 4. igénypontok szerinti sejtmentes tenyészet-felülúszót tartalmazza.

9. Táplálék-kiegészítő, mely egy fogyasztható hordozót és egy *Lactobacillus coryniformis* MM7, ATCC PTA-4660 törzs és *Lactobacillus reuteri* MM2-3, ATCC PTA-4659 törzs közül választott *Lactobacillus* törzsből származó *H.pylori* fertőzéssel összefüggő gastrointestinalis gyulladást csökkentő komponenst tartalmaz, ahol a komponens az 1. vagy 2. igénypont szerinti *Lactobacillus* törzs biológiailag tiszta tenyészetének sejtjeit tartalmazza, vagy ahol a komponens a 3. vagy 4. igénypontok szerinti sejtmentes tenyészet-felülúszót tartalmazza.