The invention concerns new isolated Lactobacillus cells, which are capable of aggregating Helicobacter pylori under culture conditions of the human digestive tract, in particular of the stomach, and to the uses of such cells.
NOVEL LACTOBACILLUS STRAINS AND THEIR USE AGAINST HELICOBACTER PYLORI

FIELD OF THE INVENTION

[0001] The invention concerns new Lactobacillus strains and the uses thereof, in particular for pharmaceutical and/or dietary compositions.

PRIOR ART AND BACKGROUND OF THE INVENTION

[0002] Probiotic microorganisms comprise living or viable cells, which, in their living form, show advantageous effects in human or animal bodies. Probiotic compositions contain such microorganisms. Advantageous effects may in particular be the improvement of the microflora of the digestive tract. In particular, undesired other microorganisms can be inhibited in the microflora by immediate interactions between the probiotic microorganisms and the undesired microorganisms, by immediate interactions due to inhibitions of the metabolism of the undesired microorganism by expression products of the probiotic microorganism, or by intensification of the natural immune system. In general, it is assumed that a main mechanism is the competitive settlement of the gastrointestinal tract whereby undesired microorganisms cannot settle anymore on the mucosa to a disturbing extent or are displaced.

[0003] A group of probiotic microorganisms is for instance formed by Lactobacillus strains. These are typically gram-positive, microaerophilic or anaerobic bacteria fermenting sugar with the generation of acids, in particular of lactic acid.

[0004] From the document U.S. Pat. No. 5,716,615, a pharmaceutical composition is known in the art, which amongst others contains Lactobacilli. This pharmaceutical composition can be used, amongst others, for the treatment of diseases of the gastrointestinal tract.

[0005] From the document US 2005/0186190 A1, a dietetic or pharmaceutical composition is known in the art, which contains sphingomyelinas or Lactobacilli containing sphingomyelinas. This composition is suitable for treatments of infections with Helicobacter pylori.

[0006] From the document WO 2004/087891, Lactobacillus strains are known in the art, which are suitable for the production of pharmaceutical or dietetic compositions for the treatment of infections of the gastrointestinal tract with Helicobacter pylori.

[0007] From the document WO 2005/060937 A1, tablet-shaped formulations are known in the art, which contain viable Lactobacillus cells. These are suitable for the oral administration and treatment of infections of the gastrointestinal tract with pathogens.

[0008] From the document WO 2004/031368 A1, Lactobacillus strains are known in the art, which are suitable for the treatment of inflammations, which are associated with an infection with Helicobacter pylori.


[0010] Helicobacter pylori is a spiral-shaped bacterium colonizing the stomach, and by means of production of urease the pH value in the stomach is increased, and thus the bacteria are protected against the stomach acid. The bacteria penetrate the mucosa and deposit at epithelial cells of the stomach. Such an infection activates the body-own immune system, but the immune response is not sufficiently effective for eliminating the infection, with the consequence of an intensifying immune response. Finally, a chronic inflammation and illness with gastritis or stomach ulcers will occur. Up to now, it is not yet known, by means of which mechanisms Helicobacter pylori resists to the immune system.

[0011] About the effective mechanisms of the prior art Lactobacillus strains against Helicobacter pylori, various theories are presented in the above documents. Safe findings about the mechanisms do not exist, however.

[0012] Overall, it is desirable to develop Lactobacillus strains, which keep the Helicobacter pylori cell count in the stomach very low, and which are otherwise free from physiological side effects.

TECHNICAL OBJECT OF THE INVENTION

[0013] It is therefore the technical object of the invention to provide Lactobacillus strains, which inhibit the settlement of Helicobacter pylori on the stomach mucosa.

[0014] Further, it is the technical object of the invention to provide dietetic and/or pharmaceutical compositions, which are highly effective in particular in the prophylaxis of a Helicobacter pylori infection.

BASICS OF THE INVENTION AND PREFERRED EMBODIMENTS

[0015] For achieving this technical object, the invention teaches isolated, preferably viable Lactobacillus cells, which are capable to aggregate Helicobacter pylori under culture conditions of the human digestive tract, in particular of the stomach.

[0016] The invention is based on the surprising perception that certain selected Lactobacillus strains are capable of binding to free Helicobacter pylori to form aggregates. These relatively large aggregates are not capable anymore of penetrating the mucosa, and consequently Helicobacter pylori bacteria can no longer reach and infect die epithelial cells of the stomach. At last, the chronic inflammatory reaction of the immune system is not activated anymore, and an illness with gastritis or stomach ulcers is reliably prevented. The aggregates pass through the gastrointestinal tract and leave the body in a natural way. Even with an infection having occurred already, this mechanism of action of Lactobacillus strains according to the invention is helpful, since another infection with additional Helicobacter pylori bacteria is prevented and thus the existing infection can more easily be controlled by killing the present Helicobacter pylori bacteria. Usually, this is even possible for the natural immune system of the diseased person. In addition, Lactobacillus strains according to the invention are presumably also capable of inhibiting the urease activity of Helicobacter pylori, such that the Helicobacter pylori bacteria in the aggregates lose their protection against the attack of stomach acid. Insofar, a synergistic effect is also achieved.

[0017] The essential culture conditions of the human stomach tract comprise a pH value in the range from 1.8 to 4.5 and the presence of pepsin and NaCl. A reference medium, which
is characteristic for such culture conditions, comprises the following components: water, 5 g/l NaCl and 3 g/l pepsin, and the pH value is adjusted to 2.0 by means of HCl.

The term aggregation denotes the generation of cell aggregates having a size of at least 1 µm to 1.000 µm and more, comprising Lactobacillus cells and Helicobacter pylori cells, in suspensions, for instance according to the following examples, in particular in a reference medium, as described above.

For the purpose of the invention, various Lactobacillus strains were examined for their ability to aggregate Helicobacter pylori, and the following strains were identified and filed as strains according to the invention at the DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany: DSM 17646, DSM 17647, DSM 17648, DSM 17649, DSM 17650, DSM 17651, DSM 17652 and DSM 17653, DSM 17646, DSM 17649, DSM 17652 and DSM 17653 are Lactobacillus brevis strains. DSM 17647, DSM 17648 and DSM 17651 are Lactobacillus fermentum strains. DSM 17650 is a Lactobacillus pentosus strain.

The invention further concerns a pharmaceutical and/or dietetic composition comprising a physiologically effective dose of preferably viable Lactobacillus cells according to the invention and to a pharmaceutically tolerated carrier. Pharmaceutical compositions are compositions, which serve for therapeutic or prophylactic purposes only, and wherein besides the effective agent only auxiliary and/or carrier substances being usual in galenics are present. Dietetic compositions are compositions, which comprise, besides the effective agent, also food materials and nutritional supplements.

The invention further concerns the use of preferably viable Lactobacillus cells according to the invention for the production of a pharmaceutical or dietetic composition, in particular for the prophylaxis and/or treatment of diseases caused by infection with Helicobacter pylori, for instance gastrointestinal diseases. To these belong in particular gastritis, stomach ulcers and stomach cancers.

A pharmaceutical composition according to the invention may for instance be characterized by that it contains 10^2 to 10^15, preferably 10^6 or 10^8 to 10^12, in particular 10^8 to 10^10, Lactobacillus cells. Reference value is a unit of administration, for instance a tablet. Preferably, the composition is prepared for the oral administration. The Lactobacillus cells are suitably lyophilized.

The galenic preparation of a pharmaceutical composition according to the invention can be made in a way being usual in this technology. Suitable solid or liquid galenic preparation forms are for instance granulates, powders, dragees, tablets, (micro) capsules, suppositories, syrups, juices, suspensions or emulsions, for the production of which usual means are used, such as carrier substances, explosives, binding, coating, swelling, sliding or lubricating agents, tasting agents, sweeteners and solution mediators. As auxiliary substances are named here magnesium carbonate, titanium dioxide, lactose, mannite and other sugars, talcum, milk protein, gelatin, starch, cellulose and derivatives, animal and vegetable oils such as cod-liver oil, sunflower oil, peanut oil or sesame oil, polyethylene glycols and solvents, such as sterile water and mono or multi-valet alcohol, for instance glycerin. A pharmaceutical composition according to the invention can be produced by that cells of at least one Lactobacillus strain used according to the invention is mixed in a defined dose with a pharmaceutically suitable and physiologically well tolerated carrier and possibly further suitable active, additional or auxiliary substances, and is prepared in the desired form of administration. Carriers are in particular substances, which are selected from the group comprising “maltodextrin, microcrystalline cellulose, starch, in particular corn starch, leval, lactose, dextrose, and mixtures of such substances”. The composition may contain 0.1 to 95% by weight carrier and 5 to 99.9% by weight lyophilized Lactobacillus cells, relative to the total amount of cells and carriers, or consist thereof.

In the case of the dietetic composition, it may be provided that the composition contains 10^2 to 10^15, preferably 10^6 to 10^9, in particular 10^7 to 10^9, Lactobacillus cells. Reference value is a unit of administration, for instance a packing unit of a food material to be sold to an end user. The physiologically tolerated carrier will normally be a food material, which in particular is selected from the group comprising “milk products, fermented milk products, milk, yogurt, cheese, cereals, muesli bars, and children’s food preparations”.

The invention further concerns a method for the production of a pharmaceutical and/or dietetic composition according to the invention, wherein the lyophilized or not lyophilized, preferably viable Lactobacillus cells are mixed with the physiologically tolerated carrier and prepared for oral administration.

Finally, the invention concerns a method for the prophylaxis or treatment of a person that suffers from a disease caused by a Helicobacter pylori infection, in particular gastritis or stomach ulcer, or is suspected to fall ill with such a disease, wherein the person is administered a physiologically effective dose of a pharmaceutical and/or dietetic composition according to the invention one to five times per day. The administration may be performed over a limited time, for instance 1 to 30 weeks, or be unlimited in time. In particular, the latter is suitable for a permanent prophylaxis, also as a preventive against relapse diseases.

In the following, the invention will be explained in more detail based on examples representing embodiments only.

EXAMPLE 1

Storage of Used Strains

The storage of the Lactobacillus strains took place in a frozen state. 1 ml of a culture cultivated to a stationary phase (OD_600nm=4.8) in MRS medium (55 g/l, pH 6.5; Difco, USA) was mixed with 500 µl of a 50% by volume sterile glycerin solution, and the mixture was deep-frozen to −80° C.

The storage of Helicobacter pylori took place in a frozen state. 1 ml of a culture cultivated to a stationary phase in Brucella broth (28 g/l, pH 7.0; BD, USA), supplemented with 5% by volume defibrillated horse blood (Oxoid) was mixed with 500 µl of a 50% by volume sterile glycerin solution, and the mixture was deep-frozen to −80° C.

The horse blood was frozen before use and decomposed at 20° C., in order to destroy blood cells.

EXAMPLE 2

Aggregation of Helicobacter pylori by Lactobacillus Strains According to the Invention

The cultivation of the Lactobacilli took place in closed Falcon tubes in MRS medium at 37° C. for 24-48 h.
[0032] *Helicobacter pylori* was cultivated for 5 to 6 days in an Erlenmeyer flask under microaerophilic conditions and otherwise as described in Example 1.

[0033] After the cultivation, the cell morphology was investigated by microscope. Assays were made with cultures consisting of cells with a sigmoidal morphology as well as of cells with coccolid morphology. Cultures with mixed morphology were also investigated.

[0034] The respective cells were harvested by centrifugation at 3,200 g for 10 min. and the supernatant was removed. The cells were washed once in 5 ml buffer and resuspended in 5 ml buffer (PBS buffer containing 1.5 g/l Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O, 0.2 g/l KH<sub>2</sub>PO<sub>4</sub> and 8.8 g/l NaCl). The pH value was adjusted with HCl to 7.0. The OD<sub>600</sub> value was measured and adjusted to 2 by addition of buffer.

[0035] 2.5 ml of every cell suspension thus obtained (*Helico- bacter pylori/Lactobacillus*) were mixed, and the mixture was vortexed for 10 min. The result was investigated by microscope. Control experiments for self-aggregation were performed by separate investigation of cultures with *Lactobacillus* and *Helicobacter pylori* alone, respectively.

[0036] In Fig. 1, a suspension with a mixture of *Lactobacillus* and *Helicobacter pylori* can be seen where large aggregates have been formed, whereas such aggregates are absent in the control experiments. This result is obtained for all strains according to the invention. In the Fig. 1, the *Lactobacillus* strain DSM 17648 only is shown in an exemplary manner.

[0037] Fig. 1A shows a typical aggregate of *Helicobacter pylori* by the strain DSM 17647. Fig. 1B shows the strain DSM 17648 alone. Fig. 1C shows *Helicobacter pylori* alone. The magnification is 1,000. It can be seen that the size of the aggregates obtained in the mixture is in the range from 2 µm to 1,000 µm and more.

[0038] This variant, too, of an aggregation test is suitable for identifying *Lactobacillus* strains according to the invention.

**EXAMPLE 4**

Effect of the Lyophilization of *Lactobacillus*

[0043] The bacteria were drawn according to Example 1. Aliquots of 1 ml of the *Lactobacillus* cultures were harvested by centrifugation at 3,200 g for 10 min. The supernatant was removed, and the pellets were lyophilized for 2 h under vacuum. Pellets thus obtained of each of the *Lactobacillus* strains according to the invention were resuspended in 1 ml PBS buffer, pH 7.0. The resuspended *Lactobacillus* cells were mixed in a volume ratio of 1:1 with freshly drawn *Helicobacter pylori* cultures, and the aggregation was determined as in Examples 2 and 3. The capability of the *Lactobacillus* cells to induce an aggregation of *Helicobacter pylori* was not affected by the lyophilization, as shown by investigations according to the above examples (a photographic documentation was however not made).

**EXAMPLE 5**

Determination of the Species

[0044] The taxonomic determination of the *Lactobacillus* strains according to the invention was performed by using the hydrocarbon fermentation patterns thereof. This was determined by using the API 50 CH system (bioMérieux, France), and the analysis was made with the APILAB PLUS software (Release 3.3.3 of the same supplier). The determination was performed according to supplier’s instructions.

**EXAMPLE 6**

Production of a Pharmaceutical Composition by Using *Lactobacillus* Strains According to the invention

[0045] Cells of a *Lactobacillus* strain or of several *Lactobacillus* strains according to the invention are drawn as in Example 4, and are lyophilized. Then, the pellet is ground to a particle size of maximum approx. 1 mm diameter. The obtained granulate is mixed in the following ratios (% by weight) with carrier or auxiliary substances:

- 20% granulate
- 7% silicon dioxide (Syloid AL-1FP, GRACE Davidson)
- 1% magnesium stearate (MF-2-V, Ackros)
- 77% microcrystalline cellulose (Avicel PH 112, FMC)

[0050] Mixing is performed in a Quintech Mixermixer at position 70 level II. All components are added at the same time. Mixing is made for approx. 120 s. Then, the obtained mixture is pressed in a commercial tablet press under standard conditions, however with a pressure force as low as
The pharmaceutical composition or the use according to one of claims 3 to 6, wherein the composition is prepared for oral administration.

8. The pharmaceutical composition or the use according to one of claims 3 to 7, wherein the Lactobacillus cells are lyophilized.

9. The pharmaceutical composition or the use according to one of claims 3 to 8, wherein the carrier is selected from the group comprising “maltodextrin, microcrystalline cellulose, starch, in particular corn starch, levulose, lactose, dextrose, and mixtures of such substances”.

10. The pharmaceutical composition or the use according to one of claims 3 to 9, wherein the composition contains 0.1 to 95% by weight carrier and 5 to 99.9% by weight lyophilized Lactobacillus cells.

11. The dietetic composition or the use according to one of claims 3 to 5, wherein the composition contains 10^2 to 10^15, preferably 10^6 to 10^9, in particular 10^7 to 10^9, Lactobacillus cells.

12. The dietetic composition or the use according to one of claim 3 to 5 or 11, wherein the physiologically tolerated carrier is a food material, in particular selected from the group comprising “milk products, fermented milk products, milk, yogurt, cheese, cereals, muesli bars, and children’s food preparations”.

13. A method for producing a pharmaceutical and/or dietetic composition according to claims 3 to 12, wherein a physiologically effective quantity of the lyophilized or non-lyophilized Lactobacillus cells is mixed with the physiologically tolerated carrier and prepared for oral administration.

14. A method for the prophylaxis and/or treatment of a person who suffers from a disease caused by a Helicobacter pylori infection or is suspected to fall ill with such a disease, wherein the person is administered a physiologically effective dose of a pharmaceutical and/or dietetic composition according to claims 3 to 12 one to five times per day.

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