Moreover, it has been described that the partial or complete loss of the NHE3 protein (*Sodium Hydrogen Exchanger 3*) in mice with mucoviscidosis reduced the incidence of intestinal obstructions, notably by increasing intestinal fluidity, and it was concluded that NHE3 is a potential target for regulating the fluidity of the intestinal tract in patients with mucoviscidosis and with altered expression of CFTR.

Therefore, there is still a need for new strategies for treating and/or preventing diarrhea.

Summary

In general, the present disclosure is based on the finding that, in a quite original manner, certain probiotic strains are capable of acting directly on diarrhea, by inducing absorption of water present in excess in the colon. Surprisingly and unexpectedly, the inventors showed that certain probiotic strains are capable of inhibiting the expression of transporters involved in the secretion of water in the colon and/or of stimulating the expression of transporters involved in the absorption of water in the colon, and can be used for treating and/or preventing diarrhea.

Thus, in a quite original manner, these probiotic strains do not act as inhibitors of the transporters, *i.e.* do not act by binding to the transporters, thus hampering their functioning, but act on their expression and therefore on the number of transporters present.

Moreover, as these probiotic strains act directly on the absorption of water present in excess in the colon, they have the advantage that they can be used for preventing and/or treating any type of diarrhea, in particular regardless of whether the diarrhea is of infectious or noninfectious origin and in the presence or absence of a dysbiosis.

In one aspect, the present invention relates to a method of selecting a probiotic strain for treating and/or preventing diarrhea, said method comprising the steps of:

- measuring the effect of at least one test strain on the expression of the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) protein, and
- selecting at least one strain that induces a decrease in the expression of the CFTR protein.

In ~~one aspect~~an embodiment, the present ~~invention~~ disclosure relates to a method of selecting a probiotic strain for treating and/or preventing diarrhea, said method comprising the steps of:

- measuring the effect of at least one test strain on the expression of at least one transporter involved in the absorption or secretion of water in the colon, and
- selecting at least one strain that induces a decrease in the expression of at least one transporter involved in the secretion of water in the colon and/or an increase in at least one transporter involved in the absorption of water in the colon.

More specifically, the present disclosure relates to a method of selecting a probiotic strain for treating and/or preventing diarrhea, said method comprising the steps of:

- measuring the effect of at least one test strain on expression of the CFTR protein (*Cystic Fibrosis Transmembrane Conductance Regulator*) and/or on expression of the NHE3 protein (*Sodium Hydrogen Exchanger 3*), and
- selecting at least one strain that induces a decrease in expression of the CFTR protein and/or an increase in expression of the NHE3 protein.

In certain embodiments, the test strain is a yeast strain or a bacterial strain. In particular, a bacterial strain to be tested can be a strain of *Bacillus*, preferably a strain of *Bacillus subtilis*.

In certain embodiments, expression of the CFTR protein and/or of the NHE3 protein is measured by electrophoresis, Western blot, immunoassay, immunohistochemistry, immunocytochemistry, mass spectrometry, RT-PCR or Northern blot.

In another embodiment, the present disclosure relates to a probiotic strain selected using a method disclosed herein, for use in the treatment and/or prevention of diarrhea.

In one aspect, the invention relates to the use of a probiotic strain, or of cells obtained by culture of said probiotic strain or of a composition comprising said probiotic strain or said cells, for the manufacture of a medicament for the treatment and/or prevention of diarrhea, wherein said probiotic strain, or said cells or said composition induces a decrease in expression of the CFTR protein and an increase in the expression of the NHE3 protein.

In ~~one aspect~~ an embodiment, the ~~invention~~ disclosure relates to a probiotic strain that induces a decrease in expression of at least one transporter involved in the secretion of water in the colon and/or an increase in the expression of at least one transporter involved in the absorption of water in the colon, cells obtained by culture of said probiotic strain or composition comprising said probiotic strain or said cells, for use in the treatment and/or prevention of diarrhea.

In certain embodiments, the probiotic strain is a strain of *Bacillus subtilis*, preferably the strain of *Bacillus subtilis* CU1 deposited at the CNCM (Collection Nationale de Cultures de Microorganismes / National Collection of Cultures of Microorganisms, 25 rue du Docteur Roux, 75724 Paris Cedex 15) on October 25, 2001 under number I-2745.

In embodiments, the present disclosure relates to cells obtained by culture of a probiotic strain selected using a method disclosed herein, to be used in the treatment and/or prevention of diarrhea.

In particular, the disclosure relates to cells of *Bacillus subtilis* obtained by culture of the strain of *Bacillus subtilis* CU1 deposited at the CNCM on October 25, 2001 under number I-2745, for use in the treatment and/or prevention of diarrhea.

The present disclosure also relates to a method of treating and/or preventing diarrhea in a patient comprising administration, to the patient, of an effective amount of cells obtained by culture of a probiotic strain selected using a method disclosed herein.

In certain embodiments, the method of treating and/or preventing diarrhea comprises administration of cells of *Bacillus subtilis* obtained by culture of a probiotic strain of *Bacillus subtilis*, preferably the strain of *Bacillus subtilis* CU1 deposited at the CNCM on October 25, 2001, under number I-2745.

In another embodiment, the present disclosure relates to a composition for use in the treatment and/or prevention of diarrhea, said composition comprising at least one probiotic strain according to the invention, or cells obtained by culture of said strain. In particular, the composition comprises cells of *Bacillus subtilis* obtained by culture of the strain of *Bacillus subtilis* CU1 deposited at the CNCM on October 25, 2001, under number I-2745.

Yet another ~~aspect~~ embodiment relates to the use of a probiotic strain, cells or composition as disclosed herein in the manufacture of a medicament for the treatment and/or prevention of diarrhea.

The forms of diarrhea that can be treated prophylactically and/or therapeutically according to the invention include acute diarrhea and chronic diarrhea.

In certain embodiments, the diarrhea treated according to the invention is diarrhea of infectious origin that is induced by a viral, bacterial or parasitic pathogen.

In other embodiments, the diarrhea treated according to the invention is diarrhea of noninfectious origin.

Diarrhea of noninfectious origin is for example diarrhea induced by a food intolerance, diarrhea induced by a fatty diet, diarrhea induced by alcohol, diarrhea induced by a psychological factor, diarrhea induced by the administration of a medicinal product, diarrhea induced by the administration of a therapeutic procedure, diarrhea associated with a disease or with a clinical condition, or diarrhea associated with drug withdrawal.

A more detailed description of certain preferred embodiments of the invention is given below.

Detailed Description of the Invention

As mentioned above, the present invention relates to the identification of probiotic strains capable of acting on the absorption of water in the colon by direct action at the level of the transporters involved in the regulation of the intestinal fluid, and their use as medicinal products in the treatment and/or prevention of diarrhea.

Claims

1. A method of selecting a probiotic strain for treating and/or preventing diarrhea, said method comprising the steps of:
   - measuring the effect of at least one test strain on the expression of at least one transporter involved in the secretion of water in the colon or of at least one transporter involved in the absorption of water in the colon, and
   - selecting at least one strain that induces a decrease in the expression of at least one transporter involved in the secretion of water in the colon and/or an increase in the expression of at least one transporter involved in the absorption of water in the colon.

2. The method of selection as claimed in claim 1, said method comprising the steps of:
   - measuring the effect of at least one test strain on expression of the CFTR protein and/or of the NHE3 protein, and
   - selecting at least one strain that induces a decrease in expression of the CFTR protein and/or an increase in expression of the NHE3 protein.

3. The method of selection as claimed in claim 1 or claim 2, characterized in that the test strain is a yeast strain or a bacterial strain.

4. The method of selection as claimed in claim 3, characterized in that the bacterial strain is a strain of *Bacillus*, preferably a strain of *Bacillus subtilis*.

5. The method of selection as claimed in any one of claims 1 to 4, characterized in that the expression of the CFTR protein and/or of the NHE3 protein is measured by electrophoresis, Western blot, immunoassay, immunohistochemistry, immunocytochemistry, mass spectrometry, RT-PCR or Northern blot.

6. A probiotic strain that induces a decrease in expression of at least one transporter involved in the secretion of water in the colon and/or an increase in the expression of at least one transporter involved in the absorption of water in the colon, cells obtained by culture of said probiotic strain or composition comprising said probiotic strain or said cells, for use in the treatment and/or prevention of diarrhea.

7. The probiotic strain, cells or composition for use in the treatment and/or prevention of diarrhea as claimed in claim 6, characterized in that the probiotic strain is a strain of *Bacillus subtilis*.

8. The probiotic strain, cells or composition for use in the treatment and/or prevention of diarrhea as claimed in claim 7, characterized in that the probiotic strain is the strain of *Bacillus subtilis* CU1 deposited at the CNCM on October 25, 2001 under number I-2745.

9. The probiotic strain, cells or composition for use in the treatment and/or prevention of diarrhea as claimed in any one of claims 6 to 8, characterized in that the diarrhea is acute diarrhea or chronic diarrhea.

10. The probiotic strain, cells, or composition for use in the treatment and/or prevention of diarrhea as claimed in any one of claims 6 to 9, characterized in that the diarrhea is diarrhea of infectious origin or diarrhea of noninfectious origin.

11. Use of a probiotic strain, cells or composition as defined in any one of claims 6 to 8 in the manufacture of a medicament for the treatment and/or prevention of diarrhea.

12. The use of claim 11, wherein the diarrhea is acute diarrhea or chronic diarrhea, or wherein said diarrhea is diarrhea of infectious origin or diarrhea of noninfectious origin.

Claims

1. A method of selecting a probiotic strain for treating and/or preventing diarrhea, said method comprising the steps of:
   - measuring the effect of at least one test strain on the expression of the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) protein, and
   - selecting at least one strain that induces a decrease in the expression of the CFTR protein.

2. The method of selection as claimed in claim 1, said method further comprising the steps of:
   - measuring the effect of at least one test strain on expression of the NHE3 (Sodium Hydrogen Exchanger 3) protein, and
   - selecting at least one strain that induces an increase in expression of the NHE3 protein.

3. The method of selection as claimed in claim 1 or claim 2, characterized in that the test strain is a yeast strain or a bacterial strain.

4. The method of selection as claimed in claim 3, characterized in that the bacterial strain is a strain of *Bacillus*.

5. The method of selection as claimed in claim 4, wherein the strain is a strain of *Bacillus subtilis*.

6. The method of selection as claimed in any one of claims 1 to 5, characterized in that the expression of the CFTR protein and/or of the NHE3 protein is measured by electrophoresis, Western blot, immunoassay, immunohistochemistry, immunocytochemistry, mass spectrometry, RT-PCR or Northern blot.

7. Use of a probiotic strain, or of cells obtained by culture of said probiotic strain or of a composition comprising said probiotic strain or said cells, for the manufacture of a medicament for the treatment and/or prevention of diarrhea, wherein said probiotic strain, or said cells or said composition induces a decrease

in expression of the CFTR protein and an increase in the expression of the NHE3 protein.

8. The use as claimed in claim 7, characterized in that the probiotic strain is a strain of *Bacillus subtilis*.

9. The use as claimed in claim 8, characterized in that the probiotic strain is the strain of *Bacillus subtilis* CU1 deposited at the CNCM (National Collection of Cultures of Microorganisms) on October 25, 2001 under number I-2745.

10. The use as claimed in any one of claims 7 to 9, characterized in that the diarrhea is acute diarrhea or chronic diarrhea.

11. The use as claimed in any one of claims 7 to 10, characterized in that the diarrhea is diarrhea of infectious origin or diarrhea of noninfectious origin.