BACTERIOPHAGE THERAPY

FIELD OF THE INVENTION

The present invention lies in the field of bacteriophage therapy for use in the treatment of inflammatory bowel diseases.

BACKGROUND

Bacteriophages are viruses that infect bacteria by specific interaction.

Crohn's disease (CD), also known as regional enteritis, is an inflammatory disease of the intestines that may affect any part of the gastrointestinal tract from mouth to anus, causing a wide variety of symptoms. It primarily causes abdominal pain, diarrhea, vomiting, or weight loss, but may also cause complications outside the gastrointestinal tract such as skin rashes, arthritis, inflammation of the eye, tiredness, and lack of concentration.

Although the exact cause of CD is still unknown, a combination of environmental factors and genetic predisposition seems to cause the disease. CD is thought to be an autoimmune disease, in which the body's immune system attacks the gastrointestinal tract, causing inflammation; it is classified as a type of inflammatory bowel disease (IBD).

In patients with CD, abnormal expression of carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) is observed at the apical surface of the ileal epithelium and CD ileal lesions are colonized by pathogenic adherent-invasive *Escherichia coli* (AIEC).

There is no known pharmaceutical or surgical cure for Crohn's disease. In particular, neither IBD in general nor CD in particular can be treated with antibiotics (aiming at combatting pathogenic *E. coli*). Treatment options are restricted to controlling symptoms, maintaining remission, and preventing relapse.

SUMMARY

The subject disclosure provides a pharmaceutical composition comprising: (i) at least one bacteriophage strain capable of producing a lytic infection in an adherent-invasive *Escherichia coli* strain; and (ii) a pharmaceutically acceptable carrier; for the treatment of inflammatory bowel disease (IBD).

The subject disclosure further provides a method of treating inflammatory bowel disease comprising administering to a subject in need thereof at least one bacteriophage strain capable of producing a lytic infection in an adherent-invasive *Escherichia coli* strain thereby treating the subject.

In one aspect, there is provided use of at least one bacteriophage strain capable of producing a lytic infection in an adherent-invasive *Escherichia coli* strain in the manufacture of a medicament for treating inflammatory bowel disease in a subject in need thereof, wherein the medicament is to be administered to the subject.

In one aspect, there is provided a bacteriophage strain P1 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4694 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one aspect, there is provided a bacteriophage strain P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4695 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one aspect, there is provided a bacteriophage strain P3 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4696 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one aspect, there is provided a bacteriophage strain P4 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4697 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one aspect, there is provided a bacteriophage strain P5 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4698 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one aspect, there is provided a bacteriophage strain P6 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4699 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one aspect, there is provided a bacteriophage strain P8 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4700 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

The subject disclosure also makes use of bacteriophage strain CLB_P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4675 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one aspect, there is provided a pharmaceutical composition comprising a combination of two or more of

– the bacteriophage strain P1 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4694 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P1;

– the bacteriophage strain P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4695 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P2;

– the bacteriophage strain P3 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4696 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P3;

– the bacteriophage strain P4 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4697 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P4;

– the bacteriophage strain P5 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4698 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P5;

– the bacteriophage strain P6 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4699 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P6;

– the bacteriophage strain P8 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4700 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P8;

– the bacteriophage strain CLB_P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4675 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain CLB_P2.

For the purpose of the present disclosure, a variant of a bacteriophage strain is regarded as having the same lytic activity as said bacteriophage strain if it performs at least "+" against at least one of the AIEC strains LF82, 07081, 07082, 07076 and 06075 in the "*In vitro* assay of the infectivity of bacteriophages in AIEC strains" described in Example 3 below. In a preferred embodiment, a variant is regarded as having the same lytic activity if it performs at least "+" against all five AIEC strains LF 82, LF 06075, LF 07076, LF 07081 and LF 07082 (AIEC strains LF 06075, LF 07076, LF 07081 and LF 07082 are also abbreviated herein as 06075, 07076, 07081 and 07082, respectively). These AIEC strains have been deposited by Université Lille 2 – Droit et

Santé, 42 Rue Paul Duez, 59000 Lille (France) with the French National Collection at Institut Pasteur under Accession Numbers CNCM I-4712 (LF 82), CNCM I-4713 (LF 06075), CNCM I-4714 (LF 07076), CNCM I-4715 (LF 07081) and CNCM I-4716 (LF 07082).

For the purpose of the present disclosure, a variant of one of the bacteriophage strains P1 to P6, P8 and CLB_P2 is regarded as having the same phenotypic characteristics as said bacteriophage strain if it has at least 80% sequence identity on at least 70% of length, preferably at least 90% sequence identity on at least 80% of length and more preferably complete sequence identity on at least 90% of length (as determined by the BLAST algorithm) with the major capsid protein of bacteriophage wV8 (for variants of P1 to P6) or bacteriophage RB69 (for variants of P8) or bacteriophage JS98 (for variants of CLB_P2), as described below in the section "Identification of Major Capsid Proteins".

In a preferred embodiment, a variant of a bacteriophage has the same lytic activity as the bacteriophage. In another embodiment, a variant of a bacteriophage has the same lytic activity and the same phenotypic characteristics as the bacteriophage.

DETAILED DESCRIPTION OF THE INVENTION

The subject invention provides a pharmaceutical composition comprising: (i) at least one bacteriophage strain capable of producing a lytic infection in an adherent-invasive Escherichia coli strain; and (ii) a pharmaceutically acceptable carrier; for the treatment of inflammatory bowel disease.

The subject invention further provides for a method of treating inflammatory bowel disease comprising administering to a subject in need thereof at least one bacteriophage strain capable of producing a lytic infection in an adherent-invasive *Escherichia coli* strain thereby treating the subject.

An "*adherent-invasive Escherichia coli (AIEC) strain*" as used herein should be understood as referring to an *E. coli* strain having a mean invasion potential of equal to or higher than 0.1% in a cell culture of the intestinal cell line I-407. In other words, an AIEC strain has the ability to invade an intestinal cell culture of I-407 with an invasion

index equal or superior to 0.1% of the original inoculum (taken as 100%), when tested in accordance with the invasion assay described below in the section "Invasion Assay" (see also *Darfeuille-Michaud et al. (2004), Gastroenterology 127:412-421*).

Non-limiting examples of AIEC strains are LF82, LF82SK (deposited by Université d'Auvergne, 49 Boulevard François Mitterand, 63001 Clermont-Ferrand (France) with the French National Collection at Institut Pasteur under Accession Number CNCM I-4723), those listed in Table 1 herein below and those listed in the following itemization (cf. *Darfeuille-Michaud et al. (2004), Gastroenterology 127:412-421, especially page 417, Table 2*): LF31, LF71, LF123, LF138, LF9, LF15, LF28, LF50, LF65, LF119, LF128, LF130, LF73, LF100, LF110, LF134, LF105, LF49-2, LB11, and LF45-2. In one embodiment, the adherent-invasive *Escherichia coli* strain is LF82, 07081, 07082, 07076 or 06075, in particular LF82.

In one embodiment, the adherent-invasive *Escherichia coli* strain is present in the colon of the subject. In another embodiment, the adherent-invasive *Escherichia coli* strain is present in the ileum of the subject. In yet another embodiment, the adherent-invasive *Escherichia coli* strain is present in one or more intestinal parts (small and/or large) of the subject.

In one embodiment, the at least one bacteriophage strain is P1 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4694 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one embodiment, the at least one bacteriophage strain is P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4695 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one embodiment, the at least one bacteriophage strain is P3 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession

Number CNCM I-4696 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one embodiment, the at least one bacteriophage strain is P4 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4697 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one embodiment, the at least one bacteriophage strain is P5 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4698 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one embodiment, the at least one bacteriophage strain is P6 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4699 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one embodiment, the at least one bacteriophage strain is P8 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4700 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one embodiment, the at least one bacteriophage strain is CLB_P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4675 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

In one aspect, it is envisaged that the pharmaceutical composition comprises more than one bacteriophage strain, also named "*a bacteriophage cocktail*". The bacteriophage cocktail of the present invention comprises any combination of two or more of P1, P2, P3, P4, P5, P6, P8 and CLB_P2 and variants thereof having the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics. Preferably, the bacteriophages in a bacteriophage cocktail intended for treatment of a specific subject or group of subjects will be selected on the basis of the AIEC strain or AIEC strains identified and selected for combatting.

Non-limiting examples of inflammatory bowel diseases are Crohn's disease (CD), ulcerative colitis (UC), chronic inflammatory bowel disease (chronic IBD) such as but not limited to microscopic colitis, celiac disease and vasculitis. In one embodiment, the IBD is CD or UC. In another embodiment, the inflammatory bowel disease is recurrence of ileal lesions after surgery (such as surgery for the removal of at least a part of the small intestine in CD patients). The recurrence can be measured by the Rutgeerts score.

In one embodiment, the IBD is not caused by a bacterial infection. This embodiment is based on the observation that IBD is an autoimmune disease which is not generally considered a bacterial disease. Instead, a bacterial infection may be concomitant to IBD, but is not necessarily the causative agent. This observation adds to the surprising finding of the present invention, namely applying bacteriophage therapy for the treatment of a disease which is not caused by bacteria.
For that reason, there can be – as an example – AIEC strains in family members of subjects suffering from an IBD, although these family members do not suffer from this disease. Likewise, AIEC strains can also be found in subjects neither suffering from IBD nor being related to subjects suffering from IBD, as can also be seen from Table 1 below.

"*Treating*" as used herein should be understood to encompass a decrease in one or more symptoms characteristic of the disease; a decrease in the rate of progression of the disease; recovery from the disease, cure from the disease, maintenance of remission and prophylaxis such as prevention of relapse.

A "*subject*" as used herein can be a male or a female subject. A subject can be a human being or any other mammal.

The dose and regimen of administration of a pharmaceutical composition of the invention will necessarily be dependent upon the therapeutic effect to be achieved (e.g. treatment of IBD) and may vary with the particular bacteriophage strains in the composition, the route of administration, and the age and condition of the individual subject to whom the medicament is to be administered.

A dosage for humans is likely to contain a dose of bacteriophage between 10^4 and 10^{11} plaque forming units (pfu). The desired dose may be presented as one dose per day or as multiple sub-doses administered at appropriate intervals.

In the context of the present invention the term "*pharmaceutically acceptable carrier*" relates to pharmaceutically-acceptable, non-toxic carriers, fillers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration.

The pharmaceutical compositions of the present invention may further comprise pharmaceutically acceptable auxiliary agents, and optionally other therapeutic agents. Auxiliary agents, also named accessory ingredients, encompass those conventional in the art such as, but not limited to matrix-forming agents, thickeners, binders, lubricants, pH adjusting agents, protecting agents, viscosity enhancers, wicking agents, disintegrants, including non-effervescent and effervescent disintegrants, surfactants, anti-oxidants, wetting agents, colorants, flavoring agents, taste-masking agents, sweeteners, preservatives and so forth. In addition to being pharmaceutically acceptable, the auxiliary agents must be "*acceptable*" in the sense that they are compatible with the other ingredients of the composition, including the bacteriophage.

Pharmaceutical compositions and routes of administration include those suitable for or via oral (including buccal, sublingual and intraorbital), rectal, nasal, topical (including transdermal), ocular, otic, vaginal, bronchial, pulmonary or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intraperitoneal, intrapleural, intravesicular and intrathecal) administration or administration via an implant. The

pharmaceutical composition or route of administration may be adapted to provide a targeted effect of bacteriophage strain of the invention. In a specific embodiment, a pharmaceutical composition of the invention is administered orally. The compositions may be prepared by any method well known in the art of pharmacy. Such methods include the step of bringing in association a bacteriophage strain of the invention with a pharmaceutically acceptable carrier and optionally one or more auxiliary agents.

Pharmaceutical compositions suitable for oral administration may be presented as discrete dosage units (dosage forms) such as pills, tablets, dragees or capsules, or as a powder or granules, or as a solution or suspension. The pharmaceutical composition may also be presented as a bolus or paste. The compositions can further be processed into a suppository or enema for rectal administration.

For parenteral administration, suitable compositions include aqueous and non-aqueous sterile injections. The compositions may be presented in unit-dose or multi-dose containers, for example sealed vials and ampoules, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of sterile liquid carrier, for example water, prior to use.

For transdermal administration, e.g., gels, patches or sprays can be contemplated.

Compositions or formulations suitable for pulmonary administration, e.g., by nasal inhalation, include fine dusts or mists which may be generated by means of metered dose pressurized aerosols, nebulizers or insufflators.

The invention further includes a kit comprising a pharmaceutical composition of the invention and instructions for the use of the composition for a use as hereinbefore described, optionally together with packaging material.

The subject invention further provides a bacteriophage strain P1 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4694 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

The subject invention further provides a bacteriophage strain P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4695 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

The subject invention further provides a bacteriophage strain P3 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4696 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

The subject invention further provides a bacteriophage strain P4 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4697 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

The subject invention further provides a bacteriophage strain P5 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4698 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

The subject invention further provides a bacteriophage strain P6 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4699 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

The subject invention further provides a bacteriophage strain P8 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4700 or a variant thereof, wherein the variant has the same lytic

activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

EXAMPLES

The invention is further described in the following examples, which are not in any way intended to limit the scope of the invention as claimed.

METHODS

INVASION ASSAY

The Intestine-407 (I-407) cell line derived from human embryonic jejunum and ileum was used as a model of undifferentiated intestinal epithelial cells. It was purchased from Flow Laboratories (Flow Laboratories Inc., Mc Lean, VA).

Intestine-407 cells were seeded in 24-well tissue culture plates (Polylabo, Strasbourg, France) at a density of 4,105 cells/well and incubated for 20 hours. The cell monolayers were washed twice with PBS (pH 7.2). Bacterial invasion of epithelial cells was measured using the gentamicin protection assay (Falkow et al. (1987), Rev. Infect. Dis. 9 (Suppl. 5):S450-455). Each monolayer was inoculated in 1 mL of the cell culture medium lacking antibiotics with a multiplicity of infection of 10 bacteria per epithelial cell. After a 3-hour incubation period at 37°C with 5% CO_2, the monolayers were washed 3 times with PBS. Fresh cell culture medium containing 100 µg/mL of gentamicin (Sigma, St. Louis, MO) was added for 1 hour to kill extracellular bacteria before lysis of the monolayers with 1% Triton X-100 (Sigma) in deionized water. This concentration of Triton X-100 had no effect on bacterial viability for at least 30 minutes. The samples were diluted and plated onto Mueller–Hinton agar plates to determine the number of colony-forming units. All results of *E. coli* invasive ability with Intestine-407 cell line were expressed as the percentage of intracellular bacteria compared with the initial inoculum, taken as 100%. All of the assays were performed at least 3 times in separate experiments.

IDENTIFICATION OF MAJOR CAPSID PROTEINS

Virion proteins were obtained by boiling 60 µl of a suspension of 10^{11} pfu/ml of each bacteriophage for 10 min. 20 µl of the suspension were run on a precast 4–12% polyacrylamide gel. The gel was stained with Coomassie blue and the major bands

were excised, subjected to trypsin digestion and analyzed by mass spectrometry at the Institut Pasteur microsequencing facility.

The peptide masses obtained were compared with the information in protein databases, allowing the identification of the closest known protein, i.e. wV8 for P1 to P6 and RB69 for P8 and JS98 for CLB_P2 (see A. Villegas et al, Virology Journal 2009, 6:41 for characterization of wV8 and S. Zuber et al., Journal of Bacteriology 2007, 189:22, 8206 for characterization of RB69 and JS 98).

Alignment of the major capsid protein of bacteriophage wV8 with peptides obtained from mass spectrometry of the major capsid proteins of bacteriophages P1 to P6:

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wV8 :  MLTNSEKSRFFLADLTGEVQSIPNTYGYISNLGLFRSAPITQTTFLMDLTDWDVSLLDAVDRDSRKAE
P1 :             FFLADLTGEVQSIPNTYGYISNLGLFR
P2 :           SRFFLADLTGEVQSIPNTYGYISNLGLFRSAPITQTTFLMDLTDWDVSLLDAVDR
P3 :
P4 :
P5 :
P6 :

wV8 :  TSAPERVRQISFPMMYFKEVESITPDEIQGVRQPGTANELTTEAVVRAKKLMKIRTKFDITREFLFMQ
P1 :           QISFPMMYFKEVESITPDEIQGVRQPGTANELTTEAVVR         TKFDITREFLFMQ
P2 :           QISFPMMYFKEVESITPDEIQGVRQPGTANELTTEAVVR         TKFDITREFLFMQ
P3 :           QISFPMMYFKEVESITPDEIQGVRQPGTANELTTEAVVR         TKFDITREFLFMQ
P4 :           QISFPMMYFKEVESITPDEIQGVRQPGTANELTTEAVVR         TKFDITREFLFMQ
P5 :           QISFPMMYFKEVESITPDEIQGVRQPGTANELTTEAVVR         TKFDITREFLFMQ
P6 :           QISFPMMYFKEVESITPDEIQGVRQPGTANELTTEAVVR         TKFDITREFLFMQ

wV8 :  ALKGKVVDARGTLYADLYKQFDVEKKTVYFDLDNPNADIDAAIEELRMHMEDEAKTGTVINGEEIHVV
P1 :   ALK       GTLYADLYK      KTVYFDLDNPNADIDASIEELR         TGTVINGEEIHVV
P2 :   ALK       GTLYADLYK                                     TGTVINGEEIHVV
P3 :   ALK       GTLYADLYK       TVYFDLDNPNADIDASIEELR         TGTVINGEEIHVV
P4 :   ALK       GTLYADLYK                                     TGTVINGEEIHVV
P5 :   ALK       GTLYADLYK       TIYFDLDNPNADIDASIEELR         TGTVINGEEIHVV
P6 :   ALK       GTLYADLYKQFDVEK TIYFDLDNPNADIDASIEELR         TGTVINGEEIHVV

wV8 :  VDRLFFSKLVKHPKIRDAYLAQQTPLAWQQITGSLRTGGTDGVQAHMNTFYYGGVKFVQYNGKFKDKR
P1 :   VDR           IRDAYLAQQTPLAWQQITGSLR                    FVQYNGK
P2 :   VDRLFFSK      IRDAYLAQQTPLAWQQITGSLRTGGTDGVQAHMNTFYYGGVKFVQYNGK
P3 :   VDR             DAYLAQQTPLAWQQITGSLR                    FVQYNGK
P4 :   VDR             DAYLAQQTPLAWQQITGSLR                    FVQYNGK
P5 :   VDR             DAYLAQQTPLAWQQITGSLRTGGADGVQAHMNTFYYGGVKFVQYNGK
P6 :                   DAYLAQQTPLAWQQITGSLRTGGADGVQAHMNTFYYGGVK

wV8 :  GKVHTLVSIDSVAATVGVGHAFPNVSMLGEANNIFEVAYGPCPKMGYANTLGQELYVFEYEKDRDEGI
P1 :                                                MGYANTLGQELYVFEYEKDR
P2 :                                                MGYANTLGQELYVFEYEKDR
P3 :
P4 :
P5 :
P6 :
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wV8 :  DFEAHSYMLPYCTRPQLLVDVRSDAKPD
P1 :                  PQLLVDVR
P2 :                  PQLLVDVR
P3 :                  PQLLVDVR
P4 :                  PQLLVDVR
P5 :                  PQLLVDVR
P6 :                  PQLLVDVR
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Alignment of the major capsid protein of bacteriophage RB69 with peptides obtained from mass spectrometry of the major capsid protein of bacteriophage P8:

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RB69 : MTTIKTKAQLVDKWKELLEGEGLPEIANSKQAIIAKIFENQEKDFEVSPEYKDEKIAQAFGSFLTEAE
P8 :

RB69 : IGGDHGYNAQNIAAGQTSGAVTQIGPAVMGMVRRAIPNLIAFDICGVQPMNSPTGQVFALRAVYGKDP
P8 :

RB69 : IAAGAKEAFHPMYAPDAMFSGQGAAKKFPALAASTQTKVGDIYTHFFQETGTVYLQASAQVTISSSAD
P8 :         EAFHPMYAPDAMFSGQGAAK

RB69 : DAAKLDAEIIKQMEAGALVEIAEGMATSIAELQEGFNGSTDNPWNEMGFRIDKQVIEAKSRQLKAAYS
P8 :                                                                AAYS

RB69 : IELAQDLRAVHGMDADAELSGILATEIMLEINREVVDWINYSAQVGKSGMTNIVGSKAGVFDFQDPID
P8 :   IELAQDLR                          EVVDWINYSAQVGK          AGVFDFQDPID

RB69 : IRGARWAGESFKALLFQIDKEAVEIARQTGRGEGNFIIASRNVVNVLASVDTGISYAAQGLASGFNTD
P8 :   IR   WAGESFK                QTGRGEGNFIIASR

RB69 : TTKSVFAGVLGGKYRVYIDQYAKQDYFTVGYKGANEMDAGIYYAPYVALTPLRGSDPKNFQPVMGFKT
P8 :      SVFAGVLGGKYRVYIDQYAKQDYFTVGYKGANEMDAGIYYAPYVALTPLR     NFQPVMGFKT

RB69 : RYGIGVNPFAESSLQAPGARIQSGMPSILNSLGKNAYFRRVYVKGI
P8 :   RYGIGVNPFAESSLQAPGARIQSGMPSILNSLGK
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Alignment of the major capsid protein of bacteriophage JS98 with peptides obtained from mass spectrometry of the major capsid protein of bacteriophage CLB_P2.

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JS98:   MKKNALVQKWSALLENEALPEIVGASKQAIIAKIFENQEQDILTAPEYRDEKISEAFGSFLTEAEI
CLB_P2:

JS98:   GGDHGYDATNIAAGQTSGAVTQIGPAVMGMVRRAIPHLIAFDICGVQPLNNPTGQVFALRAVYGKD
CLB_P2:                                                              AVYGKD

JS98:   PIAAGAKEAFHPMYAPNAMFSGQGAAETFEALAASKVLEVGKIYSHFFEATGSAHFQAVEAVTVDA
CLB_P2: PIAAGAK

JS98:   GATDAAKLDAAVTALVEAGQLAEIAEGMATSIAELQEGFNGSTDNPWNEMGFRIDKQVIEAKSRQL
CLB_P2:

JS98:   KASYSIELAQDLRAVHGMDADAELSGILATEIMLEINREVIDWINYSAQVGKSGMTNTVGAKAGVF
CLB_P2:  ASYSIELAQDLR                          EVIDWINYSAQVGK          AGVF

JS98:   DFQDPIDIRGARWAGESFKALLFQIDKEAAEIARQTGRGAGNFIIASRNVVNVLAAVDTSVSYAAQ
CLB_P2: DFQDPIDIR   WAGESFKALLFQIDKEAAEIAR    GAGNFIIASR

JS98:   GLGQGFNVDTTKAVFAGVLGGKYRVYIDQYARSDYFTIGYKGSNEMDAGIYYAPYVALTPLRGSDP
CLB_P2:             AVFAGVLGGKYRVYIDQYAR         GSNEMDAGIYYAPYVALTPLR

JS98:   KNFQPVMGFKTRYGIGINPFADPAAQAPTKRIQNGMPDIVNSLGLNGYFRRVYVKGI
CLB_P2:  NFQPVMGFKTRYGIGINPFADPAAQAPTKRIQNGMPDIVNSLGLNGYFR
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EXAMPLE 1

Isolation of AIEC strains

One hundred and sixty-six (166) adherent-invasive *Escherichia coli* (*E. coli*) strains, including *E. coli* strain LF82 (Table 1), were isolated as follows: The AIEC strains were isolated from fresh feces of CD patients, their family members and control subjects. The feces were diluted in tenfold dilutions up to -9. Each dilution was plated on different media. After incubation, colonies were sub-cultured, identified and the strains were tested for invasion capacity.

In detail, immediately after emission, fresh feces were introduced in a sterile container. The atmosphere was rendered anaerobic by addition of a moistened Anaerocult®. Samples were treated the day of sampling. About 1 g of feces were introduced in 9 mL of cysteinated ¼ strength Ringer solution in pre-weighed tubes; they were reweighed after introduction of the sample to determine its exact weight (first tenfold dilution). Eight further tenfold dilutions were made and 0.1 mL of each dilution was plated on different non-selective and selective media incubated in appropriated conditions: Columbia blood agar (CS) and CSH agar incubated for one week under anaerobic conditions, MRS medium incubated for 48h in an atmosphere enriched in CO_2, McConkey and Cetrimide agar incubated for 48h in air. All incubations were done at 37°C. After incubation, colonies were counted, subcultured and identified by established phenotypic criteria.

A control subject was selected *vis-à-vis* a CD patient so that the control subject was of the same sex and age as the CD patient and had a similar family size as the CD patient (to take microflora variation within a family into consideration).

The protocol was approved by the local ethical committee in 2000. The patients were followed by the EPIMAD register, which is organized under an agreement between the Institut National de la Santé et de la Recherche Médicale (INSERM) and the Institut National de Veille Sanitaire (InVS) and is also supported by the François Aupetit Association, Lion's Club of Northwestern France, Ferring Laboratories, the Société Nationale Française de Gastroentérologie and Lille University Hospital.

Table 1 – AIEC strains

Number	Reference	Invasion I-407 Mean (%)	Invasion I-407 SEM (%)	Culture Medium [1]	Dilution	Level of *E. coli* (log UFC/g) [2]	Total Count (logUFC/g) [3]
	LF82	1.29	0.8	McC	-4	5.7	5.9
AIEC isolated from CD patient							
06259	C4-1	2.050	0.500	McC	-5	5.87	10.52
06254	C34-12	2.163	0.738	Cet	-2	2.91	10.14
06256	C34-2	0.550	0.170	McC	-7	7.91	10.14
06072	C39-1	0.2075	0.147	McC	-6	7.02	9.93
06073	C39-2	0.1374	0.097	McC	-6	7.02	9.93
06075	C39-4	0.2334	0.165	McC	-5	6.02	9.93
06076	C39-7	0.5900	0.417	Cet	-2	3.02	9.93
06087	C42-1	0.1095	0.055	McC	-5	5.82	9.58
06088	C42-2	0.1954	0.098	McC	-5	5.82	9.58
06089	C42-3	0.1930	0.097	Cet	-3	3.82	9.58
06398	C76-10	0.131	0.036	CS ana	-5	6.09	9.99
06011	C84-2	0.2580	0.129	McC	-6	6.96	9.23
06023	C97-1	0.1173	0.068	McC	-7	8.14	10.03
06024	C97-2	0.1303	0.075	McC	-6	7.14	10.03
06026	C98-1	0.1439	0.072	McC	-6	7.2	9.34
06027	C98-2	0.1122	0.065	McC	-6	7.2	9.34
06028	C98-4	0.2310	0.133	Cet	-2	3.20	9.34
06029	C99-1	1.0657	0.615	McC	-6	6.99	10.17
06030	C99-2	0.1613	0.081	McC	-6	6.99	10.17
06031	C99-3	0.2330	0.135	McC	-5	5.99	10.17
06033	C99-9	0.4667	0.269	Cet	-2	2.99	10.17
06150	C187-13	0.6675	0.472	CS ana	-7	7.93	9.97
06151	C187-14	1.0350	0.732	CS ana	-7	7.93	9.97
06152	C187-15	0.4375	0.253	CS ana	-7	7.93	9.97
06166	C190-1	0.2251	0.130	McC	-8	9.28	10.98
06167	C190-2	0.1247	0.072	McC	-8	9.28	10.98
06168	C190-3	0.1688	0.097	McC	-7	8.28	10.98
06169	C190-4	0.1373	0.079	McC	-6	7.28	10.98
06170	C190-6	0.7065	0.408	Cet	-3	4.28	10.98
06171	C190-8	0.5827	0.336	Cet	-2	3.28	10.98
06172	C190-7	0.5385	0.311	Cet	-2	3.28	10.98
06173	C190-12	0.5182	0.299	CS ana	-9	10.28	10.98

Number	Reference	Invasion I-407 Mean (%)	Invasion I-407 SEM (%)	Culture Medium [1]	Dilution	Level of *E. coli* (log UFC/g) [2]	Total Count (logUFC/g) [3]
06280	C203-7	0.185	0.087	Cet	-3	3.96	9.94
06281	C203-9	0.393	0.023	Cet	-2	2.96	9.94
06283	C204-4	0.253	0.092	McC	-6	7.00	9.78
06271	C205-2	0.153	0.052	McC	-6	6.93	9.97
06278	C205-9	0.160	0.005	Cet	-2	2.93	9.97
06351	C215-8	0.548	0.397	Cet	-5	5.93	9.91
06352	C215-9	0.262	0.143	Cet	-5	5.93	9.91
06353	C215-12	1.960	1.340	Cet	-3	3.93	9.91
06354	C215-13	1.339	1.281	Cet	-3	3.93	9.91
06356	C215-10	2.260	1.540	Cet	-3	3.93	9.91
06357	C215-11	2.195	1.355	Cet	-3	3.93	9.91
06358	C215-1	1.110	0.590	McC	-6	7.93	9.91
06359	C215-2	1.523	0.928	McC	-6	7.93	9.91
06360	C215-3	0.165	0.064	McC	-4	4.93	9.91
06361	C215-4	0.315	0.135	McC	-3	3.93	9.91
06362	C215-5	0.980	0.720	McC	-3	3.93	9.91
07074	C43-1	1.5825	1.3675	McC	-6	7.26	9.66
07075	C44-1	0.1822	0.0755	McC	-5	6.01	9.91
07076	C44-2	0.5950	0.3350	McC	-5	6.01	9.91
07077	C44-3	0.1432	0.0486	McC	-4	5.01	9.91
07078	C44-4	0.3086	0.1764	McC	-4	5.01	9.91
07081	C44-9	0.5525	0.2675	Cet	-2	3.01	9.91
07082	C45-1	0.4675	0.0925	McC	-5	5.94	9.46
07086	C45-9	0.2110	0.0842	Cet	-2	2.94	9.46
07093	C50-2	1.3125	0.9375	McC	-5	5.99	7.69
07035	C66-2	0.6475	0.3125	McC	-7	8.01	9.53
07045	C71-1	0.2079	0.1226	McC	-5	5.95	10.58
07046	C71-2	0.2030	0.0719	McC	-4	4.95	10.58
07048	C71-5	0.2388	0.1382	Cet	-2	2.95	10.58
07051	C100-11A	0.6325	0.3425	MRS	-4	5.07	10.47
07003	C112-4	0.2513	0.0861	McC	-5	6.14	10.88
07006	C112-10	0.8913	0.1863	Cet	-2	3.14	10.88
07022	C121-8	0.1903	0.0448	Cet	-5	6.14	10.88
07101	C55-1	0.678	0.022	McC	-6	6.95	10.38
07103	C55-3	9.175	2.775	McC	-5	5.95	10.38
07107	C55-8A	4.425	0.075	Cet	-2	2.95	10.38

Number	Reference	Invasion I-407 Mean (%)	Invasion I-407 SEM (%)	Culture Medium [1]	Dilution	Level of *E. coli* (log UFC/g) [2]	Total Count (logUFC/g) [3]
07111	C60-1	0.232	0.028	McC	-6	6.84	8.20
07113	C60-3	0.340	0.105	McC	-4	4.84	8.20
07126	C231-1	0.323	0.097	McC	-7	7.94	10.62
07127	C231-2	0.141	0.030	McC	-6	6.94	10.62
07128	C231-5	0.365	0.095	Cet	-3	3.94	10.62
07134	C233-1	0.645	0.090	McC	-3	3.98	6.18
07135	C233-3	1.510	0.390	McC	-2	2.98	6.18
07136	C233-2	2.090	0.260	McC	-3	3.98	6.18
07137	C233-11	1.108	0.168	CSH	-3	3.98	6.18
AIEC isolated from family members of CD patients							
06066	C22-9	0.2710	0.192	CS ana	-7	7.94	10.12
06381	C33-5	0.465	0.185	Cet	-2	2.90	9.46
06258	C35-5	0.873	0.428	McC	-6	7.64	10.14
06086	C41-7	0.1242	0.072	Cet	-2	2.91	10.14
06384	C47-2	0.180	0.010	McC	-6	7.07	9.62
06386	C47-4	0.121	0.047	McC	-5	6.07	9.62
06097	C64-2	1.4550	1.029	McC	-5	6.03	9.80
06099	C64-5	0.1175	0.068	Cet	-2	3.03	9.80
06100	C64-6	1.1225	0.794	Cet	-2	3.03	9.8
06006	C81-1	0.2850	0.202	McC	-5	5.96	9.64
06007	C81-2	0.3200	0.226	McC	-5	5.96	9.64
06016	C85-1	0.7540	0.435	McC	-7	8.01	10.16
06019	C85-5	1.4775	1.045	Cet	-2	3.01	10.16
06394	C87-7	0.130	0.030	MRS	-2	3.03	9.42
06020	C92-1	0.1253	0.072	McC	-5	6.09	9.64
06021	C92-2	0.1678	0.097	McC	-4	5.09	9.64
06022	C92-4	0.1229	0.071	McC	-2	3.09	9.64
06396	C95-1	4.975	2.575	McC	-6	7.1	9.75
06037	C102-1	0.6767	0.391	McC	-6	6.90	8.55
06040	C102-7	0.2342	0.135	CS ana	-6	6.9	8.55
06080	C107-2	0.5050	0.357	McC	-6	7.1	9.55
06042	C107-3	0.1900	0.110	McC	-6	7.1	9.55
06043	C107-5	1.7600	1.245	McC	-6	7.1	9.55
06045	C107-10	0.1975	0.140	Cet	-2	3.1	9.55
06046	C108-2	0.3925	0.278	McC	-6	7.12	10.54
06049	C108-10	0.2425	0.171	CS ana	-7	8.12	10.54

Number	Reference	Invasion I-407 Mean (%)	Invasion I-407 SEM (%)	Culture Medium [1]	Dilution	Level of *E. coli* (log UFC/g) [2]	Total Count (logUFC/g) [3]
06057	C133-1	0.2475	0.175	McC	-6	6.98	10.49
06101	C133-4	0.1809	0.090	Cet	-2	2.98	10.49
06160	C189-2	1.3483	0.778	McC	-6	7.07	10.19
06164	C189-16B	0.3295	0.190	CSH	-8	8.07	10.19
06176	C191-4	0.3975	0.281	McC	-5	5.96	10.34
06177	C191-5	0.3185	0.225	McC	-5	5.96	10.34
06293	C207-6	0.175	0.111	Cet	-2	2.93	10.13
06295	C208-6	0.116	0.047	Cet	-2	2.91	10.57
06301	C211-1	0.285	0.242	McC	-2	2.87	10.07
06329	C218-2	0.649	0.439	McC	-6	6.88	10.06
06338	C218-13	0.208	0.047	Cet	-5	5.88	10.06
06341	C218-16	0.304	0.218	Cet	-4	4.88	10.06
07064	C225-1	0.1280	0.0058	McC	-4	4.90	10.10
07065	C225-2	0.8354	0.7146	McC	-4	4.90	10.10
07066	C225-5	0.9200	0.4150	McC	-6	6.87	9.49
07067	C225-6	1.0792	0.5977	McC	-5	5.87	9.49
07068	C226-1	0.1193	0.0334	McC	-5	6.06	10.58
07073	C227-4	0.2164	0.1568	Cet	-2	2.88	10.06
07120	C228-2	0.228	0.013	McC	-2	3.17	9.72
07121	C229-1	0.126	0.012	McC	-5	6.06	9.50
07122	C229-2	0.117	0.034	McC	-4	5.06	9.50
07123	C229-7	0.190	0.045	Cet	-5	6.06	9.50
07131	C232-5	0.190	0.122	Cet	-2	2.98	9.60
07138	C235-1	0.658	0.193	McC	-5	6.02	9.42
AIEC isolated from control subjects							
06235	C174-6	2.833	2.468	Cet	-2	3.05	10.59
06242	C177-1	0.251	0.175	McC	-6	6.99	9.95
06103	C177-13	0.1461	0.073	CS ana	-7	7.99	9.95
06105	C177-2	0.1571	0.079	McC	-5	5.99	9.95
06106	C178-23	0.4527	0.261	CSH	-5	6.03	10.24
06108	C179-7	0.1103	0.064	Cet	-2	3.07	10.53
06142	C181-5	1.1525	0.815	Cet	-3	4.01	9.96
06143	C183-12	0.2117	0.122	CSH	-7	8.2	9.95
06121	C183-2	0.1867	0.108	McC	-4	5.2	9.95
06122	C183-5	1.1025	0.780	Cet	-2	3.20	9.95
06145	C184-17	0.1095	0.077	CSH	-5	6.09	9.64

Number	Reference	Invasion I-407 Mean (%)	Invasion I-407 SEM (%)	Culture Medium [1]	Dilution	Level of *E. coli* (log UFC/g) [2]	Total Count (logUFC/g) [3]
06146	C185-22	0.3933	0.227	CSH	-5	5.88	9.88
06126	C185-1	0.6050	0.428	McC	-4	4.88	9.88
06135	C185-2	0.4500	0.318	McC	-5	5.88	9.88
06136	C185-3	0.4875	0.345	McC	-2	2.88	9.88
06137	C185-6	0.3125	0.221	Cet	-2	2.88	9.88
06127	C186-1	0.1063	0.053	McC	-6	6.86	10.04
06129	C186-4	0.4700	0.332	Cet	-3	3.86	10.04
06158	C188-15	0.1052	0.061	CS ana	-6	6.94	9.60
06196	C192-11	0.508	0.279	Cet	-2	2.92	9.44
06197	C195-1	0.206	0.157	McC	-5	5.95	9.72
06198	C195-2	1.806	1.363	McC	-4	4.95	9.72
06200	C195-6	2.498	1.482	Cet	-2	2.95	9.72
06201	C196-1	0.218	0.105	McC	-7	7.87	9.61
06204	C196-4	0.307	0.163	McC	-5	5.87	9.61
06212	C197-1	3.133	1.438	McC	-6	6.86	10.32
06213	C197-2	0.445	0.042	McC	-6	6.86	10.32
06216	C197-6	0.886	0.782	Cet	-2	2.86	10.32
06217	C198-1	0.143	0.112	McC	-6	7.07	10.03
06218	C198-2	0.113	0.096	McC	-6	7.07	10.03
06221	C199-3	5.367	3.132	McC	-4	5.06	9.79
06222	C199-4	1.353	0.942	McC	-3	4.06	9.79
06223	C199-5	2.980	2.122	McC	-3	4.06	9.79
06224	C199-6	5.398	2.837	McC	-3	4.06	9.79
06225	C200-1	0.538	0.277	McC	-3	4.06	9.79
07032	C222-1	0.1038	0.0783	McC	-6	7.14	10.88
07033	C222-2	1.2425	0.6575	McC	-6	7.14	10.88
07125	C230-1	0.300	0.055	McC	-5	6.0	10.2

1 McC = McConkey Agar (bioMérieux)
Cet = Cetrimide Agar (bioMérieux)
CS ana = anaerobic Columbia blood agar
MRS = Man Rogosa Sharp Agar (Oxoid)
CSH = Columbia SH Agar

2 The "level of *E. coli*" refers to the amount of the AIEC strain in the feces.

3 "Total Count" refers to all bacterial species in feces.

CS ana culture medium has the following composition (per liter medium):

- 39 g of Columbia blood agar base (Oxoid)
- 5 g of glucose

- 0.3 g of cysteine chlorohydrate
- 5 g of agar
- pH 7.0 ± 0.2

The mixture is sterilized for 15 minutes at 121°C. Just before plating, 5% of horse blood is added.

CSH culture medium has the following composition (per liter medium):

- 39 g of Columbia blood agar base (Oxoid)
- 3 g of cysteine chlorohydrate
- pH 6.8 ± 0.2

The mixture is sterilized for 15 minutes at 121°C. Just before plating, 2 ml of sterile ammonium citrate solution (0.25 g/10 ml water) are added. After incubation, bacteria using cysteine (and releasing sulfide) result in black colonies on this medium.

EXAMPLE 2

Phage isolation

Phages were isolated from sewage water as follows: sewage water was filtered at 0.2 µm and mixed with an equal volume of 2X Luria-Bertani (LB) medium. This mixture was inoculated with a fresh culture of LF82 strain and incubated on a shaker at 37°C overnight. Chloroform (1/10 volume) was added to the flask and placed on a shaker for one hour. The medium was centrifuged at 10,000 g for 10 min. 1 ml of the supernatant was collected and 1/10 vol. of chloroform was added. After a brief mix by vortex, the Eppendorf tube was centrifuged at 7,500 g for 5 min. To determine if phages were present in this extract, a drop (10 µl) of the supernatant was applied on an LB agar plate and allowed to dry. Using a platinum wire, the plate was streaked from the drop through the rest of the plate to isolate individual phages. 1 ml of a growing culture of LF82 strain was applied to cover the entire plate; the excess was removed and the plate was incubated at 37°C overnight. One or two plaques were picked up and resuspended in 200 µl of SM buffer (10 mM TrisHCl pH7, NaCl 200 mM, gelatin 0.03%). 20 µl of chloroform was added in each tube and tubes were briefly mixed by vortex and centrifuged at 7,500 g for 5 min. 10 µl of the supernatant was applied on a LB plate and allowed to dry and the previous procedure was repeated at least three times. Once the majority of isolated plaques were homogenous, 10 µl of the last resuspended plaque were added to 1 ml of growing culture of LF82 strain at OD 0.1 at 600 nm. This culture tube was incubated at 37°C for 2 to 4 hours until lysis occurred. After addition

of 1/10 vol. of chloroform, the culture was transferred to an Eppendorf tube, centrifuged at 7,500 g for 5 min and cooled to 4°C, thereby obtaining the primary stock. Several dilutions of this stock were kept at 4°C and used to infect a larger volume of culture in order to prepare larger amounts of phages. Seven (7) phages were obtained as follows:

- vB_EcoM_LF82_P1 (herein before and after P1) deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4694;
- vB_EcoM_LF82_P2 (herein before and after P2) deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4695;
- vB_EcoM_LF82_P3 (herein before and after P3) deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4696;
- vB_EcoM_LF82_P4 (herein before and after P4) deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4697;
- vB_EcoM_LF82_P5 (herein before and after P5) deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4698;
- vB_EcoM_LF82_P6 (herein before and after P6) deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4699; and
- vB_EcoM_LF82_P8 (herein before and after P8) deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4700.

CLB_P2, deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4675, and its isolation is described in detail in Maura et al. Environmental Microbiology (2012) 14(8), 1844–1854.

P1 to P6 phages belong to the wV8 bacteriophage family.

P8 belongs to the RB69 bacteriophage family.

CLB_P2 belongs to the JS98 bacteriophage family.

The classification into the wV8, RB69 and JS98 bacteriophage families was done based on the sequence of the major capsid protein.

EXAMPLE 3

***In vitro* assays of the infectivity of bacteriophages in AIEC strains**

Plaque assay was carried out by contacting serial dilutions of bacteriophage solutions (from not diluted to 10^{-8} dilution) with a Petri dish which surface was covered by one bacterium. After overnight incubation at 37°C plaques were counted. When the bacterium tested was the bacterial host (reference host) used to isolate bacteriophages it was considered that the plaque assay gave an efficiency of 100%. When the bacterium tested was not the original host, then the results were expressed by comparison to the reference host. A result greater than 80% (+++) means that the bacterium is a highly efficient host compared to the reference host, while a result between 0.1 and 80% (++) means that the bacterium is an efficient host, and a result below 0.1% (+) but above 0 means that the bacterium is a moderately efficient host, and finally 0 (-) means that the bacterium is totally resistant.

Results

Table 2 shows the result of the host spectrum of the 8 phages (as isolated/identified in Example 2) on 38 strains (out of the 166 strains isolated in Example 1, Table 1)

Table 2 Strains tested and effective efficiency of plating (EOP) obtained for each bacteriophage

Bacterial Strain	Bacteriophage							
	P1	P2	P3	P4	P5	P6	P8	CLB_P2
LF82	+++	+++	+++	+++	+++	+++	+++	+++
06023	-	-	-	-	-	-	-	++
06030	-	-	-	-	-	-	+	+++
06033	++	++	++	++	++	++	+	+++
06066	-	-	-	-	-	-	+++	-

06072	-	-	-	-	-	-	+	++
06073	-	-	-	-	-	-	+	+++
06075	+++	+++	+++	+++	+++	+++	+	++
06088	++	++	++	++	++	++	-	-
06089	++	++	++	++	++	++	-	-
06122	++	++	++	++	++	++	-	-
06150	-	-	-	-	-	-	+	-
06351	++	++	++	++	++	++	-	++
06353	+	+	+	+	+	+	-	-
06354	-	-	-	-	-	-	-	-
06356	++	+	+	+	+	+	-	-
06357	+	+	+	+	+	+	-	-
06358	++	+	+	+	+	+	-	-
06359	++	+	+	+	+	+	-	-
06361	+	++	+	+	+	+	+	-
06362	-	-	-	-	-	-	-	-
07045	-	-	-	-	-	-	-	-
07046	-	-	-	-	-	-	-	++
07048	-	-	-	-	-	-	-	-
07051	-	-	-	-	-	-	-	-
07075	-	-	-	-	-	-	-	-
07076	++	+++	++	+	+	+	+++	++
07077	-	-	-	-	-	-	-	-
07078	++	+++	+	+	+	+	+	++
07081	++	+++	++	+	+	++	+++	++
07082	+++	+++	+++	+++	+++	+++	+++	++
07107	+++	++	+++	++	+++	+++	-	+++
07126	+++	+++	+++	+++	+++	+++	-	++
07127	+++	++	+++	+++	++	++	-	++
07128	-	-	-	-	-	-	-	++
07134	-	-	-	-	-	-	-	-
07135	-	-	-	-	-	-	-	++
07136	-	-	-	-	-	-	-	-

07137	-	-	-	-	-	-	++	-

Table 3 number of strains infected by phages:

Efficacy	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P8**	**CLB_P2**
+	3	5	6	9	9	8	8	0
++	10	8	8	6	6	7	1	13
+++	5	6	5	4	4	4	4	5
Total/38	18	19	19	19	19	19	13	18

Numbers indicate the number of strains infected by one bacteriophage

EXAMPLE 4

***In vivo* replication of bacteriophages in the gut of mice**

In vivo replication of bacteriophages in the gut of mice was evaluated as follows:

First, the strain LF82 was engineered to carry two antibiotic resistance genes conferring respectively resistance to Streptomycin and Kanamycin. This new bacterial strain was named LF82SK and its invasive properties were verified as to be similar to the original LF82 strain.

Three (3) groups of two (2) mice each:

Group 1: non-colonized mice + phages

Group 2: LF82SK colonized mice

Group 3: LF82SK colonized mice + phages

Streptomycin (5 g/L) was added to drinking water of all animals 3 days before day 0 and kept along the experiment.

At day 0, LF82SK was administered to Group 2 and 3 in order to allow the strain to colonize mice's gut.

At day 4, 200 µl of a cocktail of P2 + P6 bacteriophages was administered to Group 1 and 3 (gavage solution 10^8 pfu/ml) once in the morning and once in the afternoon. P2+P6 bacteriophages were also added to the drinking water (10^8 pfu/ml). At day 5 in the morning, mice were sacrificed to evaluate the number of bacteria and bacteriophages in the ileum and in the feces.

Results:

Bacteria (E. coli):

Group 1: no bacteria;

Group 2: in ileum – 10^6 cfu/g organ; in feces – 10^8 cfu/organ;

Group 3: in ileum and feces: bacteria all lysed by phages.

Phages:

Group 1: in ileum – 10^6 pfu/g organ; in feces – 10^7 pfu/organ;

Group 2: no phages

Group 3: in ileum – 10^6 pfu/g organ; in feces – 10^{10} pfu/organ;

In the feces, there were 100 times more phages in Group 3 than in Group 1 showing the multiplication of the phages *in vivo*.

EXAMPLE 5

***In vivo* replication of bacteriophages in the gut of mice**

In vivo replication of bacteriophages in the gut of mice was evaluated as follows:

12 mice were dispatched into three (3) groups of four (4) mice each:

Group 1: non-colonized mice + phages

Group 2: LF82SK-colonized mice

Group 3: LF82SK-colonized mice + phages

Streptomycin (5 g/L) was added to drinking water of all animals 3 days before day 0 and kept along the experiment.

At day 0, LF82SK was given to mice of Group 2 and 3 in order to allow the strain to colonize mice's gut.

At day 4, bacteriophages (cocktail of P2+P6+P8 at 10^8 pfu/mL each) were added in the drinking water of Group 1 and 3.

At day 5, mice were sacrificed to evaluate the number of bacteria and bacteriophages in the ileum and in the feces. 100 µl of ileal homogenates from the three groups were taken to extract whole DNA using Maxwell® 16 Tissue DNA purification kit from Promega.

Results:

Bacteria (E. coli):

Group 1: no bacteria;

Group 2: in ileum – $3.2 \cdot 10^6$ cfu/g of organ; in feces – $1.2 \cdot 10^9$ cfu/g of feces;

Group 3: in ileum and feces: bacteria all lysed by phages.

Phages:

Group 1: in ileum – $1.4 \cdot 10^6$ pfu/g of organ ; in feces – $5.2 \cdot 10^6$ pfu/g of feces;

Group 2: no phages

Group 3: in ileum – $2.6 \cdot 10^6$ pfu/g of organ ; in feces – $1.0 \cdot 10^9$ pfu/g of feces;

In the feces, there were 200 times more phages in Group 3 than in Group 1 showing the multiplication of the phages *in vivo*.

DNA extracted from ileal sections was used to run quantitative PCR using two sets of primers. One set of primers (SEQ ID NO: 30-31) served to amplify DNA from "all bacteria" present in the sample while the second set (SEQ ID NO: 32-33) was used to amplify specifically DNA from "*E. coli*" bacteria. After normalization, results were expressed as the ratio of *E. coli* versus all bacteria.

Group 1: qPCR amplifications were successful with all bacteria primers but not with *E. coli* primers. The ratio could not be calculated.

Group 2: qPCR amplifications were successful with both set of primers. The average ratio was 0.6 (60% of total bacteria were *E. coli* bacteria)

Group 3: qPCR amplifications were successful with both set of primers. The average ratio was 0.1 (10% of total bacteria were *E. coli* bacteria). Note that one mouse displayed a ratio of 0.4 while the three others displayed much lower values (0.06; 0.0002; 0.002).

In consequence bacteriophages were able to reduce the level of ileal colonization of LF82 bacteria by at least one order of magnitude in three mice out of four.

EXAMPLE 6

***In vivo* assay of the infectivity of bacteriophages**

Two cocktails of phages are selected for testing in wild-type (WT) mice and in CEACAM6 mice infected with the LF82 *E. coli* strain isolated from the CD patients.

In both WT mice and in CEACAM6 mice infected with the LF82 *E. coli* strain isolated from the CD patients, bacteriophages are administered to the mice by oral gavage in CMC. This kind of administration has many advantages: known quantity of bacteriophage administration and immediate gastric acidity neutralization. Phages are daily administered to the mice during the entire study.

- Mice are sacrificed at 5 days after LF82 administration.
- Main criteria: quantification of LF82 in ileal and colonic adherent flora of the mice.
- Minor criteria:
    - Evaluation of weight
    - Stool consistency.
    - Presence of fecal blood (macro and bio)
- Luminal flora (conventional flora + LF82 + phages)
    - At sacrifice: Macroscopic and histologic examinations, adherent ileal and colonic flora + LF82 + phages,
    - At sacrifice: Macroscopic and histologic examinations, adherent ileal and colonic flora + LF82 + phages,

Biological parameters of inflammation are monitored, and bacteriophage translocation in the mesenteric lymph nodes (MLN), liver and spleen is searched for.

Inflammation markers (MPO, pro-inflammatory cytokines IL-6, IL-12 and anti-inflammatory cytokines IL-10) are monitored. Bacteriophage and AIEC translocation in MLN, liver and spleen is searched for.

Follow-up of bacteriophage elimination takes place in stools of mice receiving the bacteriophage cocktail without the LF82 strain.

EXAMPLE 7

***In vivo* assay of a cocktail of phages on the LF82 strain.**

In vivo replication of bacteriophages (cocktail of P2+P6+P8+CLB_P2) in the gut of mice was evaluated as follows:

20 mice were dispatched into two (2) groups of ten (10) mice each:

Group 1: LF82SK-colonized mice

Group 2: LF82SK-colonized mice + phages

Streptomycin (5 g/L) was added to drinking water of all animals 3 days before day 0 and kept along the experiment.

At day 0, LF82SK was given to mice of both groups in order to allow the strain to colonize mice's gut.

At day 3, bacteriophages (cocktail of P2+P6+P8+CLB_P2 at 10^8 pfu/mL each) were given to mice of Group 2 by gavage.

At day 4 and 7, 5 mice of each group were sacrificed to evaluate the number of bacteria and bacteriophages in the ileum, in the colon and in the feces. 100 µl of ileal and colonic homogenates from the two groups were taken to extract whole DNA using Maxwell® 16 Tissue DNA purification kit from Promega.

Results:

Level of LF82 in stools:

At day 4 and 7 levels of LF82 were:

in group 1: 7 10^9 ; 1 10^9 cfu/g

in group 2: 8 10^7 ; 5 10^8 cfu/g

Level of Phages in stools:

At day 4 and 7 levels of Phages were:

in group 1: none

in group 2: 5 10^9; 6 10^9 pfu/g

In the presence of the phage cocktail the level of LF82 in stools was significantly lower than in their absence showing that the phage cocktail was able to infect LF82 inside mice's gut.

Level of LF82 in organs:

at day 4 levels of LF82 were:

in ileum of group 1: 100% of bacteria are E. coli (LF82)

in ileum of group 2: 20% of bacteria are E. coli (LF82)

in colon of group 1: 40% of bacteria are E. coli (LF82)

in colon of group 2: 2% of bacteria are E. coli (LF82)

at day 7 levels of LF82 were:

in ileum of group 1: 100% of bacteria are E. coli (LF82)

in ileum of group 2: 50% of bacteria are E. coli (LF82)

in colon of group 1: 25% of bacteria are E. coli (LF82)

in colon of group 2: 10% of bacteria are E. coli (LF82)

Level of Phages in organs:

at day 4 levels of Phages were:

in ileum of group 1: none

in ileum of group 2: 7 10^8 pfu/g

in colon of group 1: none

in colon of group 2: 5 10^{10} pfu/g

at day 7 levels of LF82 were:

in ileum of group 1: none

in ileum of group 2: 7 10^8 pfu/g

in colon of group 1: none

in colon of group 4: 2 10^8 pfu/g

At day 2 and 5 the level of LF82 was reduced in both ileum and colon in the group treated by phages. This shows that phages infect LF82 in gut sections and not only in stools. Concomitantly, the level of phages at day 7 stays as high as at day 2 showing that phage can last several days in the gut after a unique initial administration.

EXAMPLE 8

***In vivo* assay of the infectivity of bacteriophages**

In vivo assay of the infectivity of bacteriophages (cocktail of P2+P6+P8) in CEACAM6 mice infected with LF82SK was evaluated as follows:

48 mice were dispatched into three (4) groups as follows:

Group 1: non-colonized mice (8 mice)

Group 2: non-colonized mice + phages (12 mice)

Group 3: LF82SK-colonized mice (16 mice)

Group 4: LF82SK-colonized mice + phages (12 mice)

DSS (dextran sulfate) 0.25% was introduced in the drinking water 3 days before day 0 and kept along the experiment.

Streptomycin (5mg) was administrated by oral gavage to all animals 1 day before day 0.

At day 0, LF82SK was administered to mice of Group 3 and 4 in order to allow the strain to colonize mice's gut.

At day 1, phages (cocktail of P2+P6+P8 at 10^7 pfu/mL each) were administered once to each mouse of Group 2 and 4 by oral gavage in CMC. This kind of administration has many advantages: known quantity of bacteriophage administration and immediate gastric acidity neutralization.

At day 1, 4 mice from Group 3 were sacrificed to evaluate the number of bacteria in the ileum, in the colon and in the feces before the administration of phages.

At day 2, respectively 4, 6, 6 and 6 mice from Groups 1, 2, 3 and 4 were sacrificed to evaluate the number of bacteria and bacteriophages in the ileum, in the colon and in the feces.

At day 5, respectively 4, 6, 6 and 6 mice from Groups 1, 2, 3 and 4 were sacrificed to evaluate the number of bacteria and bacteriophages in the ileum, in the colon and in the feces.

100 µl of ileal, colon and feces homogenates from the four groups were taken to extract whole DNA using Maxwell® 16 Tissue DNA purification kit from Promega.

Weight, stool consistency and presence of fecal blood were monitored daily.

DNA extracted from ileal sections was used to run quantitative PCR using one set of primers (SEQ ID NO: 44-45) to amplify a specific gene (pMT1) from LF82. Results were expressed as the number of copies of this gene per gram of tissues.

LF82 pMT1 F (SEQ ID NO:44)	CCATTCATGCAGCAGCTCTTT
LF82 pMT1 R (SEQ ID NO:45)	ATCGGACAACATTAGCGGTGT

Results:

Values represent the median values obtained for each group of mice.

In group 1, neither LF82 nor Phages were detected along the experiment.

Level of LF82 in stools:

At day 1: the level of LF82 in Groups 3 and 4 were 5 10^9 and 6 10^9 cfu/g resp.

At day 2, 3 and 5 levels of LF82 were:

in group 3: 3 10^9 ; 5 10^8 ; 5 10^7 cfu/g

in group 4: 5 10^5 ; 5 10^5 ; 5 10^3 cfu/g

Level of Phages in stools:

At day 2, 3 and 5 levels of Phages were:

in group 2: 5 10^5 pfu/g; not detected; not detected

in group 4: 1 10^9; 1 10^7; 5 10^6 pfu/g

In the presence of phages the level of LF82 in stools was significantly lower than in their absence. Concomitantly, the level of phages was significantly higher in mice colonised by LF82 than in LF82-free mice. Both data confirmed that phages can infect LF82 in the gut.

Level of LF82 in organs:

at day 2 levels of LF82 were:

in ileum of group 3: 2 10^6 copies of pMT1/g

in ileum of group 4: 8 10^4 copies of pMT1/g

in colon of group 3: 2 10^7 copies of pMT1/g

in colon of group 4: 1 10^5 copies of pMT1/g

at day 5 levels of LF82 were:

in ileum of group 3: 5 10^4 copies of pMT1/g

in ileum of group 4: 8 10^4 copies of pMT1/g

in colon of group 3: 6 10^6 copies of pMT1/g

in colon of group 4: 2 10^5 copies of pMT1/g

Level of Phages in organs:

at day 2 levels of Phages were:

in ileum of group 2: not detected

in ileum of group 4: 8 10^5 pfu/g

in colon of group 2: 5 10^4 pfu/g

in colon of group 4: 5 10^6 pfu/g

at day 5 levels of LF82 were:

in ileum of group 2: not detected

in ileum of group 4: not detected

in colon of group 2: not detected

in colon of group 4: 2 10^4 pfu/g

At day 2, the level of LF82 was reduced in both ileum and colon in the group treated by phages. This shows that phages infected LF82 in gut sections and not only in stools. Concomitantly, the level of phages was significantly higher in mice colonised by LF82 than in LF82-free mice.

At day 5, the level of LF82 in ileum was too weak to see a difference between the two groups while in colon samples the level of LF82 was still reduced in the group that received phages compared to the groups that did not. Concomitantly, we could only detect phages in colon of mice colonised by LF82. This shows that the effect of phages in reducing LF82 can last several days after the initial administration.

Despite high colonisation level of LF82 observed in this experiment, no sign of colitis was observed in any of the groups.

EMBODIMENTS

The present invention in particular relates to the following embodiments:

1. A pharmaceutical composition comprising:
   (i) at least one bacteriophage strain(s) capable of producing a lytic infection in an adherent-invasive *Escherichia coli* strain; and
   (ii) a pharmaceutically acceptable carrier;
   for use in the treatment of inflammatory bowel disease.

2. A composition according to embodiment 1 wherein the adherent-invasive *Escherichia coli* strain is present in one or more of the intestinal parts (small and large) of the subject.

3. A composition according to embodiment 1 wherein the adherent-invasive *Escherichia coli* strain is LF82, 07081, 07082, 07076 or 06075.

4. A composition according to any one of embodiments 1 to 3 wherein the inflammatory bowel disease is Crohn's disease or ulcerative colitis or recurrence of ileal lesions after surgery, for example surgery for the removal of at least a part of the small intestine in CD patients.

5. A composition according to any one of embodiments 1 to 4 wherein the composition comprises at least one of the bacteriophage strain P1 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4694 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P1, the bacteriophage strain P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4695 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P2, the bacteriophage strain P3 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4696 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P3, the bacteriophage strain P4 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4697 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P4, the bacteriophage strain P5 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-

4698 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P5, the bacteriophage strain P6 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4699 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P6, the bacteriophage strain P8 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4700 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P8 and the bacteriophage strain CLB_P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4675 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain CLB_P2.

6. A composition according to any one of embodiments 1 to 5 wherein the composition is for oral administration.

7. Bacteriophage strain P1 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4694 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

8. Bacteriophage strain P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4695 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

9. Bacteriophage strain P3 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4696 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

10. Bacteriophage strain P4 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4697 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

11. Bacteriophage strain P5 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4698 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

12. Bacteriophage strain P6 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4699 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

13. Bacteriophage strain P8 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4700 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

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0-1	**Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)**	
0-1-1	Prepared Using	PCT Online Filing Version 3.5.000.235 MT/FOP 20020701/0.20.5.20
0-2	**International Application No.**	
0-3	**Applicant's or agent's file reference**	171371aa/isc

1	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
1-1	**page**	2
1-2	**line**	14
1-3	**Identification of deposit**	
1-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
1-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
1-3-3	Date of deposit	15 November 2012 (15.11.2012)
1-3-4	Accession Number	CNCM I-4694
1-5	**Designated States for Which Indications are Made**	All designations
2	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
2-1	**page**	2
2-2	**line**	20
2-3	**Identification of deposit**	
2-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
2-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
2-3-3	Date of deposit	15 November 2012 (15.11.2012)
2-3-4	Accession Number	CNCM I-4695
2-5	**Designated States for Which Indications are Made**	All designations

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3	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
3-1	**page**	2
3-2	**line**	26
3-3	**Identification of deposit**	
3-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
3-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
3-3-3	Date of deposit	15 November 2012 (15.11.2012)
3-3-4	Accession Number	CNCM I-4696
3-5	**Designated States for Which Indications are Made**	All designations
4	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
4-1	**page**	2
4-2	**line**	32
4-3	**Identification of deposit**	
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4-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
4-3-3	Date of deposit	15 November 2012 (15.11.2012)
4-3-4	Accession Number	CNCM I-4697
4-5	**Designated States for Which Indications are Made**	All designations
5	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
5-1	**page**	3
5-2	**line**	4
5-3	**Identification of deposit**	
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5-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
5-3-3	Date of deposit	15 November 2012 (15.11.2012)
5-3-4	Accession Number	CNCM I-4698
5-5	**Designated States for Which Indications are Made**	All designations

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6-2	**line**	10
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6-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
6-3-3	Date of deposit	15 November 2012 (15.11.2012)
6-3-4	Accession Number	CNCM I-4699
6-5	**Designated States for Which Indications are Made**	All designations
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7-1	**page**	3
7-2	**line**	16
7-3	**Identification of deposit**	
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7-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
7-3-3	Date of deposit	15 November 2012 (15.11.2012)
7-3-4	Accession Number	CNCM I-4700
7-5	**Designated States for Which Indications are Made**	All designations
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8-1	**page**	3
8-2	**line**	22
8-3	**Identification of deposit**	
8-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
8-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
8-3-3	Date of deposit	27 September 2012 (27.09.2012)
8-3-4	Accession Number	CNCM I-4675
8-5	**Designated States for Which Indications are Made**	All designations

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9	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
9-1	**page**	4
9-2	**line**	2
9-3	**Identification of deposit**	
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9-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
9-3-3	Date of deposit	25 January 2013 (25.01.2013)
9-3-4	Accession Number	CNCM I-4712
9-5	***Designated States for Which* Indications are Made**	All designations
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10-1	**page**	4
10-2	**line**	2
10-3	**Identification of deposit**	
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10-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
10-3-3	Date of deposit	25 January 2013 (25.01.2013)
10-3-4	Accession Number	CNCM I-4713
10-5	**Designated States for Which Indications are Made**	All designations
11	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
11-1	**page**	4
11-2	**line**	3
11-3	**Identification of deposit**	
11-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
11-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
11-3-3	Date of deposit	25 January 2013 (25.01.2013)
11-3-4	Accession Number	CNCM I-4714
11-5	**Designated States for Which Indications are Made**	All designations

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12	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
12-1	**page**	4
12-2	**line**	3
12-3	**Identification of deposit**	
12-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
12-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
12-3-3	Date of deposit	25 January 2013 (25.01.2013)
12-3-4	Accession Number	CNCM I-4715
12-5	***Designated States for Which Indications are Made***	All designations
13	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
13-1	**page**	4
13-2	**line**	3
13-3	**Identification of deposit**	
13-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
13-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
13-3-3	Date of deposit	25 January 2013 (25.01.2013)
13-3-4	Accession Number	CNCM I-4716
13-5	**Designated States for Which Indications are Made**	All designations
14	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
14-1	**page**	5
14-2	**line**	7
14-3	**Identification of deposit**	
14-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
14-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
14-3-3	Date of deposit	15 March 2013 (15.03.2013)
14-3-4	Accession Number	CNCM I-4723
14-5	**Designated States for Which Indications are Made**	All designations

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0-1	**Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)**	
0-1-1	Prepared Using	PCT Online Filing Version 3.5.000.235 MT/FOP 20020701/0.20.5.20
0-2	**International Application No.**	EP2014058840
0-3	**Applicant's or agent's file reference**	171371aa/isc

1	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
1-1	**page**	2
1-2	**line**	14
1-3	**Identification of deposit**	
1-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
1-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
1-3-3	Date of deposit	15 November 2012 (15.11.2012)
1-3-4	Accession Number	CNCM I-4694
1-5	**Designated States for Which Indications are Made**	All designations
2	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
2-1	**page**	2
2-2	**line**	20
2-3	**Identification of deposit**	
2-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
2-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
2-3-3	Date of deposit	15 November 2012 (15.11.2012)
2-3-4	Accession Number	CNCM I-4695
2-5	**Designated States for Which Indications are Made**	All designations
3	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
3-1	**page**	2
3-2	**line**	26
3-3	**Identification of deposit**	
3-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
3-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
3-3-3	Date of deposit	15 November 2012 (15.11.2012)
3-3-4	Accession Number	CNCM I-4696
3-5	**Designated States for Which Indications are Made**	All designations

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4	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
4-1	**page**	2
4-2	**line**	32
4-3	**Identification of deposit**	
4-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
4-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
4-3-3	Date of deposit	15 November 2012 (15.11.2012)
4-3-4	Accession Number	CNCM I-4697
4-5	**Designated States for Which *Indications are Made***	All designations
5	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
5-1	**page**	3
5-2	**line**	4
5-3	**Identification of deposit**	
5-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
5-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
5-3-3	*Date of deposit*	15 November 2012 (15.11.2012)
5-3-4	Accession Number	CNCM I-4698
5-5	**Designated States for Which Indications are Made**	All designations
6	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
6-1	**page**	3
6-2	**line**	10
6-3	**Identification of deposit**	
6-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
6-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
6-3-3	Date of deposit	15 November 2012 (15.11.2012)
6-3-4	Accession Number	CNCM I-4699
6-5	**Designated States for Which Indications are Made**	All designations

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7	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
7-1	**page**	3
7-2	**line**	16
7-3	**Identification of deposit**	
7-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
7-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
7-3-3	Date of deposit	15 November 2012 (15.11.2012)
7-3-4	Accession Number	CNCM I-4700
7-5	**Designated States for Which *Indications are Made***	All designations
8	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
8-1	**page**	3
8-2	**line**	22
8-3	**Identification of deposit**	
8-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
8-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
8-3-3	Date of deposit	27 September 2012 (27.09.2012)
8-3-4	Accession Number	CNCM I-4675
8-5	**Designated States for Which Indications are Made**	All designations
9	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
9-1	**page**	4
9-2	**line**	2
9-3	**Identification of deposit**	
9-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
9-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
9-3-3	Date of deposit	25 January 2013 (25.01.2013)
9-3-4	Accession Number	CNCM I-4712
9-5	**Designated States for Which Indications are Made**	All designations

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10	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
10-1	**page**	4
10-2	**line**	2
10-3	**Identification of deposit**	
10-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
10-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
10-3-3	Date of deposit	25 January 2013 (25.01.2013)
10-3-4	Accession Number	CNCM I-4713
10-5	**Designated States for Which *Indications are Made***	All designations
11	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
11-1	**page**	4
11-2	**line**	3
11-3	**Identification of deposit**	
11-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
11-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
11-3-3	*Date of deposit*	25 January 2013 (25.01.2013)
11-3-4	Accession Number	CNCM I-4714
11-5	**Designated States for Which Indications are Made**	All designations
12	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
12-1	**page**	4
12-2	**line**	3
12-3	**Identification of deposit**	
12-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
12-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
12-3-3	Date of deposit	25 January 2013 (25.01.2013)
12-3-4	Accession Number	CNCM I-4715
12-5	**Designated States for Which Indications are Made**	All designations

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13	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
13-1	**page**	4
13-2	**line**	3
13-3	**Identification of deposit**	
13-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
13-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
13-3-3	Date of deposit	25 January 2013 (25.01.2013)
13-3-4	Accession Number	CNCM I-4716
13-5	**Designated States for Which *Indications are Made***	All designations
14	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
14-1	**page**	5
14-2	**line**	7
14-3	**Identification of deposit**	
14-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
14-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
14-3-3	*Date of deposit*	15 March 2013 (15.03.2013)
14-3-4	Accession Number	CNCM I-4723
14-5	**Designated States for Which Indications are Made**	All designations

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CLAIMS

1. Use of at least one bacteriophage strain capable of producing a lytic infection in an adherent-invasive *Escherichia coli* strain in the manufacture of a medicament for treating inflammatory bowel disease in a subject in need thereof, wherein the medicament is to be administered to the subject.

2. The use according to claim 1 wherein the adherent-invasive *Escherichia coli* strain is present in one or more of the intestinal parts (small and large) of the subject.

3. The use according to claim 1 wherein the adherent-invasive *Escherichia coli* strain is LF82, 07081, 07082, 07076 or 06075.

4. The use according to any one of claims 1 to 3 wherein the inflammatory bowel disease is Crohn's disease.

5. The use according to any one of claims 1 to 4 wherein the inflammatory bowel disease is ulcerative colitis.

6. The use according to any one of claims 1 to 5 wherein the at least one bacteriophage strain comprises the bacteriophage strain P1 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4694 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P1.

7. The use according to any one of claims 1 to 6 wherein the at least one bacteriophage strain comprises the bacteriophage strain P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4695 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P2.

8. The use according to any one of claims 1 to 7 wherein the at least one bacteriophage strain comprises the bacteriophage strain P3 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4696 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P3.

9. The use according to any one of claims 1 to 8 wherein the at least one bacteriophage strain comprises the bacteriophage strain P4 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4697 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P4.

10. The use according to any one of claims 1 to 9 wherein the at least one bacteriophage strain comprises the bacteriophage strain P5 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4698 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P5.

11. The use according to any one of claims 1 to 10 wherein the at least one bacteriophage strain comprises the bacteriophage strain P6 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4699 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P6.

12. The use according to any one of claims 1 to 11 wherein the at least one bacteriophage strain comprises the bacteriophage strain P8 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4700 or a variant thereof, wherein the variant has

the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P8.

13. The use according to any one of claims 1 to 12 wherein the at least one bacteriophage strain comprises the bacteriophage strain CLB_P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4675 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain CLB_P2.

14. The use according to any one of claims 1 to 13, wherein the at least one bacteriophage strain comprises

- the bacteriophage strain P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4695 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P2;
- the bacteriophage strain P8 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4700 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P8; and
- the bacteriophage strain CLB_P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4675 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain CLB_P2.

15. The use according to any one of claims 1 to 14, wherein the administration is oral administration.

16. The use according to any one of claims 1 to 15, wherein the inflammatory bowel disease is recurrence of ileal lesions after surgery.

17. The use according to claim 16, wherein the surgery is to remove at least a part of the small intestine in CD patients.

18. Bacteriophage strain P1 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4694 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

19. Bacteriophage strain P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4695 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

20. Bacteriophage strain P3 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4696 or a variant thereof, wherein the variant has the same lytic activity preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

21. Bacteriophage strain P4 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4697 or a variant thereof, wherein the variant has the same lytic activity,

preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

22. Bacteriophage strain P5 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4698 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

23. Bacteriophage strain P6 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4699 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

24. Bacteriophage strain P8 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4700 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain.

25. A pharmaceutical composition comprising a combination of two or more of
- the bacteriophage strain P1 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4694 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P1;
- the bacteriophage strain P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4695 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P2;
- the bacteriophage strain P3 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4696 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P3;
- the bacteriophage strain P4 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4697 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P4;
- the bacteriophage strain P5 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4698 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P5;
- the bacteriophage strain P6 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4699 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P6;
- the bacteriophage strain P8 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM

I-4700 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P8;

- the bacteriophage strain CLB_P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4675 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain CLB_P2.

26. The pharmaceutical composition according to claim 25 comprising:

- the bacteriophage strain P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4695 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P2;
- the bacteriophage strain P8 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4700 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain P8; and
- the bacteriophage strain CLB_P2 deposited with the French National Collection of Microorganisms at the Institut Pasteur under Accession Number CNCM I-4675 or a variant thereof, wherein the variant has the same lytic activity, preferably the same lytic activity and the same phenotypic characteristics as said bacteriophage strain CLB_P2.

27. The pharmaceutical composition according to any one of claims 25 or 26, wherein said pharmaceutical composition is for the treatment of inflammatory bowel disease.

28. The pharmaceutical composition according to any one of claims 25 to 27, said pharmaceutical composition comprising a pharmaceutically acceptable carrier.

29. The pharmaceutical composition for use according to any one of claims 27 or 28, wherein the inflammatory bowel disease is Crohn's disease.

30. The pharmaceutical composition for use according to any one of claims 27 to 29, wherein the pharmaceutical composition is for oral administration.