COMPOSITIONS COMPRISING BACTERIAL STRAINS

TECHNICAL FIELD

This invention is in the field of compositions comprising bacterial strains isolated from the mammalian digestive tract and the use of such compositions in the treatment of disease.

BACKGROUND TO THE INVENTION

The human intestine is thought to be sterile *in utero*, but it is exposed to a large variety of maternal and environmental microbes immediately after birth. Thereafter, a dynamic period of microbial colonization and succession occurs, which is influenced by factors such as delivery mode, environment, diet and host genotype, all of which impact upon the composition of the gut microbiota, particularly during early life. Subsequently, the microbiota stabilizes and becomes adult-like [1]. The human gut microbiota contains more than 500-1000 different phylotypes belonging essentially to two major bacterial divisions, the Bacteroidetes and the Firmicutes [2]. The successful symbiotic relationships arising from bacterial colonization of the human gut have yielded a wide variety of metabolic, structural, protective and other beneficial functions. The enhanced metabolic activities of the colonized gut ensure that otherwise indigestible dietary components are degraded with release of by-products providing an important nutrient source for the host. Similarly, the immunological importance of the gut microbiota is well-recognized and is exemplified in germfree animals which have an impaired immune system that is functionally reconstituted following the introduction of commensal bacteria [3-5].

Dramatic changes in microbiota composition have been documented in gastrointestinal disorders such as inflammatory bowel disease (IBD). For example, the levels of *Clostridium* cluster XIVa bacteria are reduced in IBD patients whilst numbers of *E. coli* are increased, suggesting a shift in the balance of symbionts and pathobionts within the gut [6-9]. Interestingly, this microbial dysbiosis is also associated with imbalances in T effector cell populations.

In recognition of the potential positive effect that certain bacterial strains may have on the animal gut, various strains have been proposed for use in the treatment of various diseases (see, for example, [10-13]). Also, certain strains, including mostly *Lactobacillus* and *Bifidobacterium* strains, have been proposed for use in treating various inflammatory and autoimmune diseases that are not directly linked to the intestines (see [14] and [15] for reviews). However, the relationship between different diseases and different bacterial strains, and the precise effects of particular bacterial strains on the gut and at a systemic level and on any particular types of diseases, are poorly characterised. For example, certain *Enterococcus* species have been implicated in causing cancer [16].

There is a requirement in the art for new methods of treating diseases. There is also a requirement for the potential effects of gut bacteria to be characterised so that new therapies using gut bacteria can be developed.

SUMMARY OF THE INVENTION

The inventors have developed new therapies for treating and preventing diseases. In particular, the inventors have developed new therapies for treating and preventing cancer. In particular, the inventors have identified that bacterial strains of the species *Enterococcus gallinarum* can be effective for treating and preventing cancer. As described in the examples, oral administration of compositions comprising *Enterococcus gallinarum* may reduce tumor size in mouse models of cancer.

In preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *Enterococcus gallinarum*, for use in a method of treating or preventing cancer, such as breast, lung or liver cancer. The inventors have identified that treatment with compositions comprising a bacterial strain of the species *Enterococcus gallinarum* can reduce tumour growth in mouse models of breast, lung and liver cancer. In certain embodiments, the composition is for use in a method of reducing tumour size or preventing tumour growth in the treatment of cancer. Compositions using *Enterococcus gallinarum* may be particularly effective for reducing tumour size or preventing tumour growth in the treatment of cancer.

In preferred embodiments of the invention, the bacterial strain in the composition is of *Enterococcus gallinarum*. Closely related strains may also be used, such as bacterial strains that have a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Enterococcus gallinarum*. Preferably, the bacterial strain has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:1 or 2. Preferably, the sequence identity is to SEQ ID NO:2. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:2.

Accordingly, the invention also provides a composition comprising a bacterial strain that has a 16s rRNA sequence that is at least 95% identical to the 16s rRNA sequence of a bacterial strain of *Enterococcus gallinarum* for use in a method of treating or preventing cancer. In particular, the invention provides a composition comprising a bacterial strain that has a 16s rRNA sequence that is at least 95% identical to SEQ ID NO: 2 for use in a method of treating or preventing cancer. In some embodiments, the bacterial strain in the composition is not of *Enterococcus gallinarum*. In some embodiments, the bacterial strain in the composition is not of *Enterococcus gallinarum*, but is a closely related strain.

In certain embodiments, the composition of the invention is for oral administration. Oral administration of the strains of the invention can be effective for treating cancer. Also, oral administration is convenient for patients and practitioners and allows delivery to and / or partial or total colonisation of the intestine.

In certain embodiments, the composition of the invention comprises one or more pharmaceutically acceptable excipients or carriers.

In certain embodiments, the composition of the invention comprises a bacterial strain that has been lyophilised. Lyophilisation is an effective and convenient technique for preparing stable compositions that allow delivery of bacteria.

In certain embodiments, the invention provides a food product comprising the composition as described above.

In certain embodiments, the invention provides a vaccine composition comprising the composition as described above.

Additionally, the invention provides a method of treating or preventing cancer, comprising administering a composition comprising a bacterial strain of the species *Enterococcus gallinarum*.

In developing the above invention, the inventors have identified and characterised a bacterial strain that is particularly useful for therapy. The *Enterococcus gallinarum* strain of the invention is shown to be effective for treating cancer. Therefore, in another aspect, the invention provides a cell of the *Enterococcus gallinarum* strain deposited under accession number NCIMB 42488, or a derivative thereof. The invention also provides compositions comprising such cells, or biologically pure cultures of such cells. The invention also provides a cell of the *Enterococcus gallinarum* strain deposited under accession number NCIMB 42488, or a derivative thereof, for use in therapy, in particular for cancer. Similarly, the invention provides a cell of a bacterial strain that has a 16s rRNA sequence that is at least 95% identical to SEQ ID NO: 2, or a derivative thereof. The invention also provides compositions comprising such cells, or biologically pure cultures of such cells. The invention also provides a cell of a bacterial strain that has a 16s rRNA sequence that is at least 95% identical to SEQ ID NO:2, or a derivative thereof, for use in therapy, in particular for treating or preventing cancer.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: Mouse model of breast cancer – tumor volume.

Figure 2: Mouse model of lung cancer – tumour volume.

Figure 3: Mouse model of liver cancer – liver weight.

Figure 4A: Cytokine levels (pg/ml) in immature dendritic cells (No bacteria).

Figure 4B: Cytokine levels (pg/ml) in immature dendritic cells after the addition of LPS.

Figure 4C: Cytokine levels (pg/ml) in immature dendritic cells after the addition of MRX518.

Figure 4D: Cytokine levels (pg/ml) in immature dendritic cells after the addition of MRX518 and LPS.

Figure 5A: Cytokine levels in THP-1 cells (No bacteria).

Figure 5B: Cytokine levels in THP-1 cells after addition of bacterial sediment.

Figure 5C: Cytokine levels in THP-1 cells after the addition of MRX518 alone or in combination with LPS.

DISCLOSURE OF THE INVENTION

Bacterial strains

The compositions of the invention comprise a bacterial strain of the species *Enterococcus gallinarum*. The examples demonstrate that bacteria of this species are useful for treating or preventing cancer.

The invention also provides compositions comprising a bacterial strain that has a 16s rRNA sequence that is at least 95% identical to the 16s rRNA sequence of a bacterial strain of *Enterococcus gallinarum* for use in therapy, for example, for use in a method of treating or preventing cancer. In particular, the invention also provides compositions comprising a bacterial strain that has a 16s rRNA sequence that is at least 95% identical to SEQ ID NO: 2 for use in therapy, for example, for use in a method of treating or preventing cancer. In some embodiments, the bacterial strain in the composition is not of *Enterococcus gallinarum,* but is a closely related strain.

The invention provides an *Enterococcus gallinarum* for use in therapy, for example, for use in treating or preventing cancer. Similarly, the invention provides a composition comprising a bacterial strain of the species *Enterococcus gallinarum,* for use in therapy, for example, for use in treating or preventing cancer. In certain embodiments, the compositions of the invention comprise a bacterial strain that has a 16s rRNA sequence that is at least 95% identical to SEQ ID NO: 2, for example which is a *Enterococcus gallinarum,* and do not contain any other bacterial genus. In certain embodiments, the compositions of the invention comprise a single strain of a bacterial strain that has a 16s rRNA sequence that is at least 95% identical to SEQ ID NO: 2, for example, which is an *Enterococcus gallinarum,* and do not contain any other bacterial strain or species.

Enterococcus gallinarum forms coccoid cells, mostly in pairs or short chains. It is nonmotile and colonies on blood agar or nutrient agar are circular and smooth. *Enterococcus gallinarum* reacts with Lancefield group D antisera. The type strain of *Enterococcus gallinarum* is F87/276 = PB21 = ATCC 49573 = CCUG 18658 = CIP 103013 = JCM 8728 = LMG 13129 = NBRC 100675 = NCIMB 702313 (formerly NCDO 2313) = NCTC 12359 [17]. The GenBank accession number for a 16S rRNA gene sequence of *Enterococcus gallinarum* is AF039900 (disclosed herein as SEQ ID NO:1). An exemplary *Enterococcus gallinarum* strain is described in [17].

The *Enterococcus gallinarum* bacterium deposited under accession number NCIMB 42488 was tested in the Examples and is also referred to herein as strain MRX518. References to MRX518 and MRx0518 are used interchangeably. A 16S rRNA sequence for the MRX518 strain that was tested is provided in SEQ ID NO:2. Strain MRX518 was deposited with the international depositary authority NCIMB, Ltd. (Ferguson Building, Aberdeen, AB21 9YA, Scotland) by 4D Pharma Research Ltd. (Life

Sciences Innovation Building, Aberdeen, AB25 2ZS, Scotland) on 16th November 2015 as "*Enterococcus sp*" and was assigned accession number NCIMB 42488.

The genome of strain MRX518 comprises a chromosome and plasmid. A chromosome sequence for strain MRX518 is provided in SEQ ID NO:3. A plasmid sequence for strain MRX518 is provided in SEQ ID NO:4. These sequences were generated using the PacBio RS II platform.

Bacterial strains closely related to the strain tested in the examples are also expected to be effective for treating or preventing cancer. In certain embodiments, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Enterococcus gallinarum*. Preferably, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:1 or 2. Preferably, the sequence identity is to SEQ ID NO:2. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:2.

Bacterial strains that are biotypes of the bacterium deposited under accession number 42488 are also expected to be effective for treating or preventing cancer. A biotype is a closely related strain that has the same or very similar physiological and biochemical characteristics.

Strains that are biotypes of the bacterium deposited under accession number NCIMB 42488 and that are suitable for use in the invention may be identified by sequencing other nucleotide sequences for the bacterium deposited under accession number NCIMB 42488. For example, substantially the whole genome may be sequenced and a biotype strain for use in the invention may have at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity across at least 80% of its whole genome (e.g. across at least 85%, 90%, 95% or 99%, or across its whole genome). For example, in some embodiments, a biotype strain has at least 98% sequence identity across at least 98% of its genome or at least 99% sequence identity across 99% of its genome. Other suitable sequences for use in identifying biotype strains may include hsp60 or repetitive sequences such as BOX, ERIC, $(GTG)_5$, or REP or [18]. Biotype strains may have sequences with at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of the bacterium deposited under accession number NCIMB 42488. In some embodiments, a biotype strain has a sequence with at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of strain MRX518 deposited as NCIMB 42488 and comprises a 16S rRNA sequence that is at least 99% identical (e.g. at least 99.5% or at least 99.9% identical) to SEQ ID NO:2. In some embodiments, a biotype strain has a sequence with at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of strain MRX518 deposited as NCIMB 42488 and has the 16S rRNA sequence of SEQ ID NO:2.

In certain embodiments, the bacterial strain for use in the invention has a chromosome with sequence identity to SEQ ID NO:3. In preferred embodiments, the bacterial strain for use in the invention has a chromosome with at least 90% sequence identity (e.g. at least 92%, 94%, 95%, 96%, 97%, 98%, 99%

or 100% sequence identity) to SEQ ID NO:3 across at least 60% (e.g. at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of SEQ ID NO:3. For example, the bacterial strain for use in the invention may have a chromosome with at least 90% sequence identity to SEQ ID NO:3 across 70% of SEQ ID NO:3, or at least 90% sequence identity to SEQ ID NO:3 across 80% of SEQ ID NO:3, or at least 90% sequence identity to SEQ ID NO:3 across 90% of SEQ ID NO:3, or at least 90% sequence identity to SEQ ID NO:3 across 100% of SEQ ID NO:3, or at least 95% sequence identity to SEQ ID NO:3 across 70% of SEQ ID NO:3, or at least 95% sequence identity to SEQ ID NO:3 across 80% of SEQ ID NO:3, or at least 95% sequence identity to SEQ ID NO:3 across 90% of SEQ ID NO:3, or at least 95% sequence identity to SEQ ID NO:3 across 100% of SEQ ID NO:3, or at least 98% sequence identity to SEQ ID NO:3 across 70% of SEQ ID NO:3, or at least 98% sequence identity to SEQ ID NO:3 across 80% of SEQ ID NO:3, or at least 98% sequence identity to SEQ ID NO:3 across 90% of SEQ ID NO:3, or at least 98% identity to SEQ ID NO:3 across 95% of SEQ ID NO:3, or at least 98% sequence identity to SEQ ID NO:3 across 100% of SEQ ID NO:3, or at least 99.5% sequence identity to SEQ ID NO:3 across 90% of SEQ ID NO:3, or at least 99.5% identity to SEQ ID NO:3 across 95% of SEQ ID NO:3, or at least 99.5% identity to SEQ ID NO:3 across 98% of SEQ ID NO:3, or at least 99.5% sequence identity to SEQ ID NO:3 across 100% of SEQ ID NO:3.

In certain embodiments, the bacterial strain for use in the invention has a plasmid with sequence identity to SEQ ID NO:4. In preferred embodiments, the bacterial strain for use in the invention has a plasmid with at least 90% sequence identity (e.g. at least 92%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity) to SEQ ID NO:4 across at least 60% (e.g. at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of SEQ ID NO:4. For example, the bacterial strain for use in the invention may have a plasmid with at least 90% sequence identity to SEQ ID NO:4 across 70% of SEQ ID NO:4, or at least 90% sequence identity to SEQ ID NO:4 across 80% of SEQ ID NO:4, or at least 90% sequence identity to SEQ ID NO:4 across 90% of SEQ ID NO:4, or at least 90% sequence identity to SEQ ID NO:4 across 100% of SEQ ID NO:4, or at least 95% sequence identity to SEQ ID NO:4 across 70% of SEQ ID NO:4, or at least 95% sequence identity to SEQ ID NO:4 across 80% of SEQ ID NO:4, or at least 95% sequence identity to SEQ ID NO:4 across 90% of SEQ ID NO:4, or at least 95% sequence identity to SEQ ID NO:4 across 100% of SEQ ID NO:4, or at least 98% sequence identity to SEQ ID NO:4 across 70% of SEQ ID NO:4, or at least 98% sequence identity to SEQ ID NO:4 across 80% of SEQ ID NO:4, or at least 98% sequence identity to SEQ ID NO:4 across 90% of SEQ ID NO:4, or at least 98% sequence identity to SEQ ID NO:4 across 100% of SEQ ID NO:4.

In certain embodiments, the bacterial strain for use in the invention has a chromosome with sequence identity to SEQ ID NO:3 and a plasmid with sequence identity to SEQ ID NO:4.

In certain embodiments, the bacterial strain for use in the invention has a chromosome with sequence identity to SEQ ID NO:3, for example as described above, and a 16S rRNA sequence with sequence identity to any of SEQ ID NO:1 or 2, for example as described above, preferably with a 16s rRNA sequence that is at least 99% identical to SEQ ID NO: 2, more preferably which comprises the 16S

rRNA sequence of SEQ ID NO:2, and optionally comprises a plasmid with sequence identity to SEQ ID NO:4, as described above.

In certain embodiments, the bacterial strain for use in the invention has a chromosome with sequence identity to SEQ ID NO:3, for example as described above, and optionally comprises a plasmid with sequence identity to SEQ ID NO:4, as described above, and is effective for treating or preventing cancer.

In certain embodiments, the bacterial strain for use in the invention has a chromosome with sequence identity to SEQ ID NO:3, for example as described above, and a 16S rRNA sequence with sequence identity to any of SEQ ID NOs: 1 or 2, for example as described above, and optionally comprises a plasmid with sequence identity to SEQ ID NO:4, as described above, and is effective for treating or preventing cancer.

In certain embodiments, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 99%, 99.5% or 99.9% identical to the 16s rRNA sequence represented by SEQ ID NO: 2 (for example, which comprises the 16S rRNA sequence of SEQ ID NO:2) and a chromosome with at least 95% sequence identity to SEQ ID NO:3 across at least 90% of SEQ ID NO:3, and optionally comprises a plasmid with sequence identity to SEQ ID NO:4, as described above, and which is effective for treating or preventing cancer.

In certain embodiments, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 99%, 99.5% or 99.9% identical to the 16s rRNA sequence represented by SEQ ID NO: 2 (for example, which comprises the 16S rRNA sequence of SEQ ID NO:2) and a chromosome with at least 98% sequence identity (e.g. at least 99% or at least 99.5% sequence identity) to SEQ ID NO:3 across at least 98% (e.g. across at least 99% or at least 99.5%) of SEQ ID NO:3, and optionally comprises a plasmid with sequence identity to SEQ ID NO:4, as described above, and which is effective for treating or preventing cancer.

In certain embodiments, the bacterial strain for use in the invention is a *Enterococcus gallinarum* and has a 16s rRNA sequence that is at least 99%, 99.5% or 99.9% identical to the 16s rRNA sequence represented by SEQ ID NO: 2 (for example, which comprises the 16S rRNA sequence of SEQ ID NO:2) and a chromosome with at least 98% sequence identity (e.g. at least 99% or at least 99.5% sequence identity) to SEQ ID NO:3 across at least 98% (e.g. across at least 99% or at least 99.5%) of SEQ ID NO:3, and optionally comprises a plasmid with sequence identity to SEQ ID NO:4, as described above, and which is effective for treating or preventing cancer.

Alternatively, strains that are biotypes of the bacterium deposited under accession number NCIMB 42488 and that are suitable for use in the invention may be identified by using the accession number NCIMB 42488 deposit and restriction fragment analysis and/or PCR analysis, for example by using fluorescent amplified fragment length polymorphism (FAFLP) and repetitive DNA element (rep)-PCR

fingerprinting, or protein profiling, or partial 16S or 23s rDNA sequencing. In preferred embodiments, such techniques may be used to identify other *Enterococcus gallinarum* strains.

In certain embodiments, strains that are biotypes of the bacterium deposited under accession number NCIMB 42488 and that are suitable for use in the invention are strains that provide the same pattern as the bacterium deposited under accession number NCIMB 42488 when analysed by amplified ribosomal DNA restriction analysis (ARDRA), for example when using Sau3AI restriction enzyme (for exemplary methods and guidance see, for example,[19]). Alternatively, biotype strains are identified as strains that have the same carbohydrate fermentation patterns as the bacterium deposited under accession number NCIMB 42488. In some embodiments, the carbohydrate fermentation pattern is determined using the API 50 CHL panel (bioMérieux). In some embodiments, the bacterial strain used in the invention is:

(i) positive for fermentation of at least one of (e.g. at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or all of): L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, N-acetylglucosamine, amygdalin, arbutin, salicin, D-cellobiose, D-maltose, sucrose, D-trehalose, gentiobiose, D-tagatose and potassium gluconate; and/or

(ii) intermediate for fermentation of at least one of (e.g. at least 2, 3, 4 or all of): D-mannitol, Methyl-αD-glycopyranoside, D-lactose, starch, and L-fucose;

preferably as determined by API 50 CHL analysis (preferably using the API 50 CHL panel from bioMérieux).

Other *Enterococcus gallinarum* strains that are useful in the compositions and methods of the invention, such as biotypes of the bacterium deposited under accession number NCIMB 42488, may be identified using any appropriate method or strategy, including the assays described in the examples. For instance, strains for use in the invention may be identified by culturing in anaerobic YCFA and/or administering the bacteria to the type II collagen-induced arthritis mouse model and then assessing cytokine levels. In particular, bacterial strains that have similar growth patterns, metabolic type and/or surface antigens to the bacterium deposited under accession number NCIMB 42488 may be useful in the invention. A useful strain will have comparable immune modulatory activity to the NCIMB 42488 strain. In particular, a biotype strain will elicit comparable effects on the cancer disease models to the effects shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples.

In some embodiments, the bacterial strain used in the invention is:

(i) Positive for at least one of (e.g. at least 2, 3, 4, 5, 6, 7 or all of): mannose fermentation, glutamic acid decarboxylase, arginine arylamidase, phenylalanine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, histidine arylamidase and serine arylamidase; and/or

(ii) Intermediate for at least one of (e.g. at least 2 or all of): β-galactosidase-6-phosphate, β-glucosidase and N-acetyl-β-glucosaminidase; and/or

(iii) Negative for at least one of (e.g. at least 2, 3, 4, 5, 6 or all of): Raffinose fermentation, Proline arylamidase, Leucyl glycine arylamidase, Leucine arylamidase, Alanine arylamidase, Glycine arylamidase and Glutamyl glutamic acid arylamidase,

preferably as determined by an assay of carbohydrate, amino acid and nitrate metabolism, and optionally an assay of alkaline phosphatase activity, more preferably as determined by Rapid ID 32A analysis (preferably using the Rapid ID 32A system from bioMérieux).

In some embodiments, the bacterial strain used in the invention is:

(i) Negative for at least one of (e.g. at least 2, 3, or all 4 of) glycine arylamidase, raffinose fermentation, proline arylamidase, and leucine arylamidase, for example, as determined by an assay of carbohydrate, amino acid and nitrate metabolism, preferably as determined by Rapid ID 32A analysis (preferably using the Rapid ID 32A system from bioMérieux); and/or

(ii) Intermediate positive for fermentation of L-fucose, preferably as determined by API 50 CHL analysis (preferably using the API 50 CHL panel from bioMérieux).

In some embodiments, the bacterial strain used in the invention is an extracellular ATP producer, for example one which produces 6-6.7 ng/µl (for example, 6.1-6.6 ng/µl or 6.2-6.5 ng/µl or 6.33 ± 0.10 ng/µl) of ATP as measured using the ATP Assay Kit (Sigma-Aldrich, MAK190). Bacterial extracellular ATP can have pleiotropic effects including activation of T cell-receptor mediated signalling (Schenk et al., 2011), promotion of intestinal Th17 cell differentiation (Atarashi et al., 2008) and induction of secretion of the pro-inflammatory mediator IL-1β by activating the NLRP3 inflammasome (Karmarkar et al., 2016). Accordingly, a bacterial strain which is an extracellular ATP producer is useful for treating or preventing cancer.

In some embodiments, the bacterial strain for use in the invention comprises one or more of the following three genes: Mobile element protein; Xylose ABC transporter, permease component; and FIG00632333: hypothetical protein. For example, in certain embodiments, the bacterial strain for use in the invention comprises genes encoding Mobile element protein and Xylose ABC transporter, permease component; Mobile element protein and FIG00632333: hypothetical protein; Xylose ABC transporter, permease component and FIG00632333: hypothetical protein; or Mobile element protein, Xylose ABC transporter, permease component, and FIG00632333: hypothetical protein.

A particularly preferred strain of the invention is the *Enterococcus gallinarum* strain deposited under accession number NCIMB 42488. This is the exemplary MRX518 strain tested in the examples and shown to be effective for treating disease. Therefore, the invention provides a cell, such as an isolated cell, of the *Enterococcus gallinarum* strain deposited under accession number NCIMB 42488, or a derivative thereof. The invention also provides a composition comprising a cell of the *Enterococcus*

gallinarum strain deposited under accession number NCIMB 42488, or a derivative thereof. The invention also provides a biologically pure culture of the *Enterococcus gallinarum* strain deposited under accession number NCIMB 42488. The invention also provides a cell of the *Enterococcus gallinarum* strain deposited under accession number NCIMB 42488, or a derivative thereof, for use in therapy, in particular for the diseases described herein. A derivative of the strain deposited under accession number NCIMB 42488 may be a daughter strain (progeny) or a strain cultured (subcloned) from the original.

A derivative of a strain of the invention may be modified, for example at the genetic level, without ablating the biological activity. In particular, a derivative strain of the invention is therapeutically active. A derivative strain will have comparable immune modulatory activity to the original NCIMB 42488 strain. In particular, a derivative strain will elicit comparable effects on the cancer disease models to the effects shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples. A derivative of the NCIMB 42488 strain will generally be a biotype of the NCIMB 42488 strain.

References to cells of the *Enterococcus gallinarum* strain deposited under accession number NCIMB 42488 encompass any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number NCIMB 42488, and such cells are encompassed by the invention. Thus, in some embodiments, reference to cells of the *Enterococcus gallinarum* strain deposited under accession number NCIMB 42488 refers only to the MRX518 strain deposited under NCIMB 42488 and does not refer to a bacterial strain that was not deposited under NCIMB 42488. In some embodiments, reference to cells of the *Enterococcus gallinarum* strain deposited under accession number NCIMB 42488 refers to cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number NCIMB 42488, but which are not the strain deposited under NCIMB 42488.

In preferred embodiments, the bacterial strains in the compositions of the invention are viable and capable of partially or totally colonising the intestine.

Treating cancer

In preferred embodiments, the compositions of the invention are for use in treating or preventing cancer. The examples demonstrate that administration of the compositions of the invention can lead to a reduction in tumour growth in a number of tumour models.

In certain embodiments, treatment with the compositions of the invention results in a reduction in tumour size or a reduction in tumour growth. In certain embodiments, the compositions of the invention are for use in reducing tumour size or reducing tumour growth. The compositions of the invention may be effective for reducing tumour size or growth. In certain embodiments, the compositions of the invention are for use in patients with solid tumours. In certain embodiments, the compositions of the invention are for use in reducing or preventing angiogenesis in the treatment of cancer. The

compositions of the invention may have an effect on the immune or inflammatory systems, which have central roles in angiogenesis. In certain embodiments, the compositions of the invention are for use in preventing metastasis.

In certain embodiments, the compositions of the invention are for use in treating or preventing breast cancer. The examples demonstrate that the compositions of the invention may be effective for treating breast cancer. In certain embodiments, the compositions of the invention are for use in reducing tumour size, reducing tumour growth, or reducing angiogenesis in the treatment of breast cancer. In preferred embodiments the cancer is mammary carcinoma. In preferred embodiments the cancer is stage IV breast cancer.

In certain embodiments, the compositions of the invention are for use in treating or preventing lung cancer. The examples demonstrate that the compositions of the invention may be effective for treating lung cancer. In certain embodiments, the compositions of the invention are for use in reducing tumour size, reducing tumour growth, or reducing angiogenesis in the treatment of lung cancer. In preferred embodiments the cancer is lung carcinoma.

In certain embodiments, the compositions of the invention are for use in treating or preventing liver cancer. The examples demonstrate that the compositions of the invention may be effective for treating liver cancer. In certain embodiments, the compositions of the invention are for use in reducing tumour size, reducing tumour growth, or reducing angiogenesis in the treatment of liver cancer. In preferred embodiments the cancer is hepatoma (hepatocellular carcinoma).

In certain embodiments, the compositions of the invention are for use in treating or preventing colon cancer. The examples demonstrate that the compositions of the invention have an effect on colon cancer cells and may be effective for treating colon cancer. In certain embodiments, the compositions of the invention are for use in reducing tumour size, reducing tumour growth, or reducing angiogenesis in the treatment of colon cancer. In preferred embodiments the cancer is colorectal adenocarcinoma.

In some embodiments, the cancer is of the intestine. In some embodiments, the cancer is of a part of the body which is not the intestine. In some embodiments, the cancer is not cancer of the intestine. In some embodiments, the cancer is not colorectal cancer. In some embodiments, the cancer is not cancer of the small intestine. In some embodiments, the treating or preventing occurs at a site other than at the intestine. In some embodiments, the treating or preventing occurs at the intestine and also at a site other than at the intestine.

In certain embodiments, the compositions of the invention are for use in treating or preventing carcinoma. The examples demonstrate that the compositions of the invention may be effective for treating numerous types of carcinoma. In certain embodiments, the compositions of the invention are for use in treating or preventing non-immunogenic cancer. The examples demonstrate that the compositions of the invention may be effective for treating non-immunogenic cancers.

The therapeutic effects of the compositions of the invention on cancer may be mediated by a pro-inflammatory mechanism. Examples 2, 4 and 5 demonstrate that the expression of a number of pro-inflammatory cytokines may be increased following administration of MRX518. Inflammation can have a cancer-suppressive effect [20] and pro-inflammatory cytokines such as TNFα are being investigated as cancer therapies [21]. The up-regulation of genes such as TNF shown in the examples may indicate that the compositions of the invention may be useful for treating cancer via a similar mechanism. The up-regulation of CXCR3 ligands (CXCL9, CXCL10) and IFNγ-inducible genes (IL-32) may indicate that the compositions of the invention elicit an IFNγ-type response. IFNγ is a potent macrophage-activating factor that can stimulate tumirocidal activity [22], and CXCL9 and CXCL10, for example, also have anti-cancer effects [23-25]. Therefore, in certain embodiments, the compositions of the invention are for use in promoting inflammation in the treatment of cancer. In preferred embodiments, the compositions of the invention are for use in promoting Th1 inflammation in the treatment of cancer. Th1 cells produce IFNγ and have potent anti-cancer effects [20]. In certain embodiments, the compositions of the invention are for use in treating an early-stage cancer, such as a cancer that has not metastasized, or a stage 0 or stage 1 cancer. Promoting inflammation may be more effective against early-stage cancers [20]. In certain embodiments, the compositions of the invention are for use in promoting inflammation to enhance the effect of a second anti-cancer agent. In certain embodiments, the treatment or prevention of cancer comprises increasing the level of expression of one or more cytokines. For example, in certain embodiments, the treatment or prevention of cancer comprises increasing the level of expression of one or more of IL-1β, IL-6 and TNF-α, for example, IL-1β and IL-6, IL-1β and TNF-α, IL-6 and TNF-α or all three of IL-1β, IL-6 and TNF-α. Increases in levels of expression of any of IL-1β, IL-6 and TNF-α are known to be indicative of efficacy in treatment of cancer.

Examples 4 and 5 demonstrate that when a bacterial strain as described herein is used in combination with lipopolysaccharide (LPS), there is a synergistic increase in IL-1β. LPS is known to elicit a pro-inflammatory effect. Thus, in certain embodiments, the treatment or prevention comprises using a bacterial strain as described herein in combination with an agent that upregulates IL-1β. In certain embodiments, the treatment or prevention comprises using a bacterial strain as described herein in combination with LPS. Accordingly, a composition of the invention may additionally comprise an agent that upregulates IL-1β. Accordingly, a composition of the invention may additionally comprise LPS.

In certain embodiments, the compositions of the invention are for use in treating a patient that has previously received chemotherapy. In certain embodiments, the compositions of the invention are for use in treating a patient that has not tolerated a chemotherapy treatment. The compositions of the invention may be particularly suitable for such patients.

In certain embodiments, the compositions of the invention are for preventing relapse. The compositions of the invention may be suitable for long-term administration. In certain embodiments, the compositions of the invention are for use in preventing progression of cancer.

In certain embodiments, the compositions of the invention are for use in treating non-small-cell lung carcinoma. In certain embodiments, the compositions of the invention are for use in treating small-cell lung carcinoma. In certain embodiments, the compositions of the invention are for use in treating squamous-cell carcinoma. In certain embodiments, the compositions of the invention are for use in treating adenocarcinoma. In certain embodiments, the compositions of the invention are for use in treating glandular tumors, carcinoid tumors, or undifferentiated carcinomas.

In certain embodiments, the compositions of the invention are for use in treating hepatoblastoma, cholangiocarcinoma, cholangiocellular cystadenocarcinoma or liver cancer resulting from a viral infection.

In certain embodiments, the compositions of the invention are for use in treating invasive ductal carcinoma, ductal carcinoma in situ or invasive lobular carcinoma.

In further embodiments, the compositions of the invention are for use in treating or preventing acute lymphoblastic leukemia (ALL), acute myeloid leukemia, adrenocortical carcinoma, basal-cell carcinoma, bile duct cancer, bladder cancer, bone tumor, osteosarcoma/malignant fibrous histiocytoma, brainstem glioma, brain tumor, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, breast cancer, bronchial adenomas/carcinoids, Burkitt's lymphoma, carcinoid tumor, cervical cancer, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-cell lymphoma, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (GIST), germ cell tumor, glioma, childhood visual pathway and hypothalamic, Hodgkin lymphoma, melanoma, islet cell carcinoma, Kaposi sarcoma, renal cell cancer, laryngeal cancer, leukaemias, lymphomas, mesothelioma, neuroblastoma, non-Hodgkin lymphoma, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, parathyroid cancer, pharyngeal cancer, pituitary adenoma, plasma cell neoplasia, prostate cancer, renal cell carcinoma, retinoblastoma, sarcoma, testicular cancer, thyroid cancer, or uterine cancer.

The compositions of the invention may be particularly effective when used in combination with further therapeutic agents. The immune-modulatory effects of the compositions of the invention may be effective when combined with more direct anti-cancer agents. Therefore, in certain embodiments, the invention provides a composition comprising a bacterial strain of the species *Enterococcus gallinarum* and an anticancer agent. In preferred embodiments the anticancer agent is an immune checkpoint inhibitor, a targeted antibody immunotherapy, a CAR-T cell therapy, an oncolytic virus, or a cytostatic drug. In preferred embodiments, the composition comprises an anti-cancer agent selected from the

group consisting of: Yervoy (ipilimumab, BMS); Keytruda (pembrolizumab, Merck); Opdivo (nivolumab, BMS); MEDI4736 (AZ/MedImmune); MPDL3280A (Roche/Genentech); Tremelimumab (AZ/MedImmune); CT-011 (pidilizumab, CureTech); BMS-986015 (lirilumab, BMS); MEDI0680 (AZ/MedImmune); MSB-0010718C (Merck); PF-05082566 (Pfizer); MEDI6469 (AZ/MedImmune); BMS-986016 (BMS); BMS-663513 (urelumab, BMS); IMP321 (Prima Biomed); LAG525 (Novartis); ARGX-110 (arGEN-X); PF-05082466 (Pfizer); CDX-1127 (varlilumab; CellDex Therapeutics); TRX-518 (GITR Inc.); MK-4166 (Merck); JTX-2011 (Jounce Therapeutics); ARGX-115 (arGEN-X); NLG-9189 (indoximod, NewLink Genetics); INCB024360 (Incyte); IPH2201 (Innate Immotherapeutics/AZ); NLG-919 (NewLink Genetics); anti-VISTA (JnJ); Epacadostat (INCB24360, Incyte); F001287 (Flexus/BMS); CP 870893 (University of Pennsylvania); MGA271 (Macrogenix); Emactuzumab (Roche/Genentech); Galunisertib (Eli Lilly); Ulocuplumab (BMS); BKT140/BL8040 (Biokine Therapeutics); Bavituximab (Peregrine Pharmaceuticals); CC 90002 (Celgene); 852A (Pfizer); VTX-2337 (VentiRx Pharmaceuticals); IMO-2055 (Hybridon, Idera Pharmaceuticals); LY2157299 (Eli Lilly); EW-7197 (Ewha Women's University, Korea); Vemurafenib (Plexxikon); Dabrafenib (Genentech/GSK); BMS-777607 (BMS); BLZ945 (Memorial Sloan-Kettering Cancer Centre); Unituxin (dinutuximab, United Therapeutics Corporation); Blincyto (blinatumomab, Amgen); Cyramza (ramucirumab, Eli Lilly); Gazyva (obinutuzumab, Roche/Biogen); Kadcyla (ado-trastuzumab emtansine, Roche/Genentech); Perjeta (pertuzumab, Roche/Genentech); Adcetris (brentuximab vedotin, Takeda/Millennium); Arzerra (ofatumumab, GSK); Vectibix (panitumumab, Amgen); Avastin (bevacizumab, Roche/Genentech); Erbitux (cetuximab, BMS/Merck); Bexxar (tositumomab-I131, GSK); Zevalin (ibritumomab tiuxetan, Biogen); Campath (alemtuzumab, Bayer); Mylotarg (gemtuzumab ozogamicin, Pfizer); Herceptin (trastuzumab, Roche/Genentech); Rituxan (rituximab, Genentech/Biogen); volociximab (Abbvie); Enavatuzumab (Abbvie); ABT-414 (Abbvie); Elotuzumab (Abbvie/BMS); ALX-0141 (Ablynx); Ozaralizumab (Ablynx); Actimab-C (Actinium); Actimab-P (Actinium); Milatuzumab-dox (Actinium); Emab-SN-38 (Actinium); Naptumonmab estafenatox (Active Biotech); AFM13 (Affimed); AFM11 (Affimed); AGS-16C3F (Agensys); AGS-16M8F (Agensys); AGS-22ME (Agensys); AGS-15ME (Agensys); GS-67E (Agensys); ALXN6000 (samalizumab, Alexion); ALT-836 (Altor Bioscience); ALT-801 (Altor Bioscience); ALT-803 (Altor Bioscience); AMG780 (Amgen); AMG 228 (Amgen); AMG820 (Amgen); AMG172 (Amgen); AMG595 (Amgen); AMG110 (Amgen); AMG232 (adecatumumab, Amgen); AMG211 (Amgen/MedImmune); BAY20-10112 (Amgen/Bayer); Rilotumumab (Amgen); Denosumab (Amgen); AMP-514 (Amgen); MEDI575 (AZ/MedImmune); MEDI3617 (AZ/MedImmune); MEDI6383 (AZ/MedImmune); MEDI551 (AZ/MedImmune); Moxetumomab pasudotox (AZ/MedImmune); MEDI565 (AZ/MedImmune); MEDI0639 (AZ/MedImmune); MEDI0680 (AZ/MedImmune); MEDI562 (AZ/MedImmune); AV-380 (AVEO); AV203 (AVEO); AV299 (AVEO); BAY79-4620 (Bayer); Anetumab ravtansine (Bayer); vantictumab (Bayer); BAY94-9343 (Bayer); Sibrotuzumab (Boehringer Ingleheim); BI-836845 (Boehringer Ingleheim); B-701 (BioClin); BIIB015 (Biogen); Obinutuzumab (Biogen/Genentech); BI-505 (Bioinvent); BI-1206 (Bioinvent);

TB-403 (Bioinvent); BT-062 (Biotest) BIL-010t (Biosceptre); MDX-1203 (BMS); MDX-1204 (BMS); Necitumumab (BMS); CAN-4 (Cantargia AB); CDX-011 (Celldex); CDX1401 (Celldex); CDX301 (Celldex); U3-1565 (Daiichi Sankyo); patritumab (Daiichi Sankyo); tigatuzumab (Daiichi Sankyo); nimotuzumab (Daiichi Sankyo); DS-8895 (Daiichi Sankyo); DS-8873 (Daiichi Sankyo); DS-5573 (Daiichi Sankyo); MORab-004 (Eisai); MORab-009 (Eisai); MORab-003 (Eisai); MORab-066 (Eisai); LY3012207 (Eli Lilly); LY2875358 (Eli Lilly); LY2812176 (Eli Lilly); LY3012217(Eli Lilly); LY2495655 (Eli Lilly); LY3012212 (Eli Lilly); LY3012211 (Eli Lilly); LY3009806 (Eli Lilly); cixutumumab (Eli Lilly); Flanvotumab (Eli Lilly); IMC-TR1 (Eli Lilly); Ramucirumab (Eli Lilly); Tabalumab (Eli Lilly); Zanolimumab (Emergent Biosolution); FG-3019 (FibroGen); FPA008 (Five Prime Therapeutics); FP-1039 (Five Prime Therapeutics); FPA144 (Five Prime Therapeutics); catumaxomab (Fresenius Biotech); IMAB362 (Ganymed); IMAB027 (Ganymed); HuMax-CD74 (Genmab); HuMax-TFADC (Genmab); GS-5745 (Gilead); GS-6624 (Gilead); OMP-21M18 (demcizumab, GSK); mapatumumab (GSK); IMGN289 (ImmunoGen); IMGN901 (ImmunoGen); IMGN853 (ImmunoGen); IMGN529 (ImmunoGen); IMMU-130 (Immunomedics); milatuzumab-dox (Immunomedics); IMMU-115 (Immunomedics); IMMU-132 (Immunomedics); IMMU-106 (Immunomedics); IMMU-102 (Immunomedics); Epratuzumab (Immunomedics); Clivatuzumab (Immunomedics); IPH41 (Innate Immunotherapeutics); Daratumumab (Janssen/Genmab); CNTO-95 (Intetumumab, Janssen); CNTO-328 (siltuximab, Janssen); KB004 (KaloBios); mogamulizumab (Kyowa Hakko Kirrin); KW-2871 (ecromeximab, Life Science); Sonepcizumab (Lpath); Margetuximab (Macrogenics); Enoblituzumab (Macrogenics); MGD006 (Macrogenics); MGF007 (Macrogenics); MK-0646 (dalotuzumab, Merck); MK-3475 (Merck); Sym004 (Symphogen/Merck Serono); DI17E6 (Merck Serono); MOR208 (Morphosys); MOR202 (Morphosys); Xmab5574 (Morphosys); BPC-1C (ensituximab, Precision Biologics); TAS266 (Novartis); LFA102 (Novartis); BHQ880 (Novartis/Morphosys); QGE031 (Novartis); HCD122 (lucatumumab, Novartis); LJM716 (Novartis); AT355 (Novartis); OMP-21M18 (Demcizumab, OncoMed); OMP52M51 (Oncomed/GSK); OMP-59R5 (Oncomed/GSK); vantictumab (Oncomed/Bayer); CMC-544 (inotuzumab ozogamicin, Pfizer); PF-03446962 (Pfizer); PF-04856884 (Pfizer); PSMA-ADC (Progenics); REGN1400 (Regeneron); REGN910 (nesvacumab, Regeneron/Sanofi); REGN421 (enoticumab, Regeneron/Sanofi); RG7221, RG7356, RG7155, RG7444, RG7116, RG7458, RG7598, RG7599, RG7600, RG7636, RG7450, RG7593, RG7596, DCDS3410A, RG7414 (parsatuzumab), RG7160 (imgatuzumab), RG7159 (obintuzumab), RG7686, RG3638 (onartuzumab), RG7597 (Roche/Genentech); SAR307746 (Sanofi); SAR566658 (Sanofi); SAR650984 (Sanofi); SAR153192 (Sanofi); SAR3419 (Sanofi); SAR256212 (Sanofi), SGN-LIV1A (lintuzumab, Seattle Genetics); SGN-CD33A (Seattle Genetics); SGN-75 (vorsetuzumab mafodotin, Seattle Genetics); SGN-19A (Seattle Genetics) SGN-CD70A (Seattle Genetics); SEA-CD40 (Seattle Genetics); ibritumomab tiuxetan (Spectrum); MLN0264 (Takeda); ganitumab (Takeda/Amgen); CEP-37250 (Teva); TB-403 (Thrombogenic); VB4-845 (Viventia); Xmab2512 (Xencor); Xmab5574 (Xencor); nimotuzumab (YM Biosciences); Carlumab (Janssen); NY-ESO TCR (Adaptimmune); MAGE-A-10 TCR

(Adaptimmune); CTL019 (Novartis); JCAR015 (Juno Therapeutics); KTE-C19 CAR (Kite Pharma); UCART19 (Cellectis); BPX-401 (Bellicum Pharmaceuticals); BPX-601 (Bellicum Pharmaceuticals); ATTCK20 (Unum Therapeutics); CAR-NKG2D (Celyad); Onyx-015 (Onyx Pharmaceuticals); H101 (Shanghai Sunwaybio); DNX-2401 (DNAtrix); VCN-01 (VCN Biosciences); Colo-Ad1 (PsiOxus Therapeutics); ProstAtak (Advantagene); Oncos-102 (Oncos Therapeutics); CG0070 (Cold Genesys); Pexa-vac (JX-594, Jennerex Biotherapeutics); GL-ONC1 (Genelux); T-VEC (Amgen); G207 (Medigene); HF10 (Takara Bio); SEPREHVIR (HSV1716, Virttu Biologics); OrienX010 (OrienGene Biotechnology); Reolysin (Oncolytics Biotech); SVV-001 (Neotropix); Cacatak (CVA21, Viralytics); Alimta (Eli Lilly), cisplatin, oxaliplatin, irinotecan, folinic acid, methotrexate, cyclophosphamide, 5-fluorouracil, Zykadia (Novartis), Tafinlar (GSK), Xalkori (Pfizer), Iressa (AZ), Gilotrif (Boehringer Ingelheim), Tarceva (Astellas Pharma), Halaven (Eisai Pharma), Veliparib (Abbvie), AZD9291 (AZ), Alectinib (Chugai), LDK378 (Novartis), Genetespib (Synta Pharma), Tergenpumatucel-L (NewLink Genetics), GV1001 (Kael-GemVax), Tivantinib (ArQule); Cytoxan (BMS); Oncovin (Eli Lilly); Adriamycin (Pfizer); Gemzar (Eli Lilly); Xeloda (Roche); Ixempra (BMS); Abraxane (Celgene); Trelstar (Debiopharm); Taxotere (Sanofi); Nexavar (Bayer); IMMU-132 (Immunomedics); E7449 (Eisai); Thermodox (Celsion); Cometriq (Exellxis); Lonsurf (Taiho Pharmaceuticals); Camptosar (Pfizer); UFT (Taiho Pharmaceuticals); and TS-1 (Taiho Pharmaceuticals).

In some embodiments, the one or more bacterial strains having a 16s rRNA sequence that is at least 95% identical to SEQ ID NO:2, for example which is an *Enterococcus gallinarum*, is/are the only therapeutically active agent(s) in a composition of the invention. In some embodiments, the bacterial strain(s) in the composition is/are the only therapeutically active agent(s) in a composition of the invention.

Modes of administration

Preferably, the compositions of the invention are to be administered to the gastrointestinal tract in order to enable delivery to and / or partial or total colonisation of the intestine with the bacterial strain of the invention. Generally, the compositions of the invention are administered orally, but they may be administered rectally, intranasally, or via buccal or sublingual routes.

In certain embodiments, the compositions of the invention may be administered as a foam, as a spray or a gel.

In certain embodiments, the compositions of the invention may be administered as a suppository, such as a rectal suppository, for example in the form of a theobroma oil (cocoa butter), synthetic hard fat (e.g. suppocire, witepsol), glycero-gelatin, polyethylene glycol, or soap glycerin composition.

In certain embodiments, the composition of the invention is administered to the gastrointestinal tract via a tube, such as a nasogastric tube, orogastric tube, gastric tube, jejunostomy tube (J tube), percutaneous endoscopic gastrostomy (PEG), or a port, such as a chest wall port that provides access to the stomach, jejunum and other suitable access ports.

The compositions of the invention may be administered once, or they may be administered sequentially as part of a treatment regimen. In certain embodiments, the compositions of the invention are to be administered daily.

In certain embodiments of the invention, treatment according to the invention is accompanied by assessment of the patient's gut microbiota. Treatment may be repeated if delivery of and / or partial or total colonisation with the strain of the invention is not achieved such that efficacy is not observed, or treatment may be ceased if delivery and / or partial or total colonisation is successful and efficacy is observed.

In certain embodiments, the composition of the invention may be administered to a pregnant animal, for example a mammal such as a human in order to reduce the likelihood of cancer developing in her child *in utero* and / or after it is born.

The compositions of the invention may be administered to a patient that has been diagnosed with cancer, or that has been identified as being at risk of a cancer. The compositions may also be administered as a prophylactic measure to prevent the development of cancer in a healthy patient.

The compositions of the invention may be administered to a patient that has been identified as having an abnormal gut microbiota. For example, the patient may have reduced or absent colonisation by *Enterococcus gallinarum*.

The compositions of the invention may be administered as a food product, such as a nutritional supplement.

Generally, the compositions of the invention are for the treatment of humans, although they may be used to treat animals including monogastric mammals such as poultry, pigs, cats, dogs, horses or rabbits. The compositions of the invention may be useful for enhancing the growth and performance of animals. If administered to animals, oral gavage may be used.

Compositions

Generally, the composition of the invention comprises bacteria. In preferred embodiments of the invention, the composition is formulated in freeze-dried form. For example, the composition of the invention may comprise granules or gelatin capsules, for example hard gelatin capsules, comprising a bacterial strain of the invention.

Preferably, the composition of the invention comprises lyophilised bacteria. Lyophilisation of bacteria is a well-established procedure and relevant guidance is available in, for example, references [26-28].

Alternatively, the composition of the invention may comprise a live, active bacterial culture.

In some embodiments, the bacterial strain in the composition of the invention has not been inactivated, for example, has not been heat-inactivated. In some embodiments, the bacterial strain in the composition of the invention has not been killed, for example, has not been heat-killed. In some

embodiments, the bacterial strain in the composition of the invention has not been attenuated, for example, has not been heat-attenuated. For example, in some embodiments, the bacterial strain in the composition of the invention has not been killed, inactivated and/or attenuated. For example, in some embodiments, the bacterial strain in the composition of the invention is live. For example, in some embodiments, the bacterial strain in the composition of the invention is viable. For example, in some embodiments, the bacterial strain in the composition of the invention is capable of partially or totally colonising the intestine. For example, in some embodiments, the bacterial strain in the composition of the invention is viable and capable of partially or totally colonising the intestine.

In some embodiments, the composition comprises a mixture of live bacterial strains and bacterial strains that have been killed.

In preferred embodiments, the composition of the invention is encapsulated to enable delivery of the bacterial strain to the intestine. Encapsulation protects the composition from degradation until delivery at the target location through, for example, rupturing with chemical or physical stimuli such as pressure, enzymatic activity, or physical disintegration, which may be triggered by changes in pH. Any appropriate encapsulation method may be used. Exemplary encapsulation techniques include entrapment within a porous matrix, attachment or adsorption on solid carrier surfaces, self-aggregation by flocculation or with cross-linking agents, and mechanical containment behind a microporous membrane or a microcapsule. Guidance on encapsulation that may be useful for preparing compositions of the invention is available in, for example, references [29] and [30].

The composition may be administered orally and may be in the form of a tablet, capsule or powder. Encapsulated products are preferred because *Enterococcus gallinarum* are anaerobes. Other ingredients (such as vitamin C, for example), may be included as oxygen scavengers and prebiotic substrates to improve the delivery and / or partial or total colonisation and survival *in vivo*. Alternatively, the probiotic composition of the invention may be administered orally as a food or nutritional product, such as milk or whey based fermented dairy product, or as a pharmaceutical product.

The composition may be formulated as a probiotic.

A composition of the invention includes a therapeutically effective amount of a bacterial strain of the invention. A therapeutically effective amount of a bacterial strain is sufficient to exert a beneficial effect upon a patient. A therapeutically effective amount of a bacterial strain may be sufficient to result in delivery to and / or partial or total colonisation of the patient's intestine.

A suitable daily dose of the bacteria, for example for an adult human, may be from about 1×10^3 to about 1×10^{11} colony forming units (CFU); for example, from about 1×10^7 to about 1×10^{10} CFU; in another example from about 1×10^6 to about 1×10^{10} CFU.

In certain embodiments, the composition contains the bacterial strain in an amount of from about 1 x 10^6 to about 1 x 10^{11} CFU/g, respect to the weight of the composition; for example, from about 1 x 10^8 to about 1 x 10^{10} CFU/g. The dose may be, for example, 1 g, 3g, 5g, and 10g.

Typically, a probiotic, such as the composition of the invention, is optionally combined with at least one suitable prebiotic compound. A prebiotic compound is usually a non-digestible carbohydrate such as an oligo- or polysaccharide, or a sugar alcohol, which is not degraded or absorbed in the upper digestive tract. Known prebiotics include commercial products such as inulin and transgalacto-oligosaccharides.

In certain embodiments, the probiotic composition of the present invention includes a prebiotic compound in an amount of from about 1 to about 30% by weight, respect to the total weight composition, (e.g. from 5 to 20% by weight). Carbohydrates may be selected from the group consisting of: fructo- oligosaccharides (or FOS), short-chain fructo-oligosaccharides, inulin, isomalt-oligosaccharides, pectins, xylo-oligosaccharides (or XOS), chitosan-oligosaccharides (or COS), beta-glucans, arable gum modified and resistant starches, polydextrose, D-tagatose, acacia fibers, carob, oats, and citrus fibers. In one aspect, the prebiotics are the short-chain fructo-oligosaccharides (for simplicity shown herein below as FOSs-c.c); said FOSs-c.c. are not digestible carbohydrates, generally obtained by the conversion of the beet sugar and including a saccharose molecule to which three glucose molecules are bonded.

The compositions of the invention may comprise pharmaceutically acceptable excipients or carriers. Examples of such suitable excipients may be found in the reference [31]. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art and are described, for example, in reference [32]. Examples of suitable carriers include lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol and the like. Examples of suitable diluents include ethanol, glycerol and water. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, or in addition to, the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s). Examples of suitable binders include starch, gelatin, natural sugars such as glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweeteners, natural and synthetic gums, such as acacia, tragacanth or sodium alginate, carboxymethyl cellulose and polyethylene glycol. Examples of suitable lubricants include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Preservatives, stabilizers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

The compositions of the invention may be formulated as a food product. For example, a food product may provide nutritional benefit in addition to the therapeutic effect of the invention, such as in a

nutritional supplement. Similarly, a food product may be formulated to enhance the taste of the composition of the invention or to make the composition more attractive to consume by being more similar to a common food item, rather than to a pharmaceutical composition. In certain embodiments, the composition of the invention is formulated as a milk-based product. The term "milk-based product" means any liquid or semi-solid milk- or whey- based product having a varying fat content. The milk-based product can be, e.g., cow's milk, goat's milk, sheep's milk, skimmed milk, whole milk, milk recombined from powdered milk and whey without any processing, or a processed product, such as yoghurt, curdled milk, curd, sour milk, sour whole milk, butter milk and other sour milk products. Another important group includes milk beverages, such as whey beverages, fermented milks, condensed milks, infant or baby milks; flavoured milks, ice cream; milk-containing food such as sweets.

In certain embodiments, the compositions of the invention contain a single bacterial strain or species and do not contain any other bacterial strains or species. Such compositions may comprise only *de minimis* or biologically irrelevant amounts of other bacterial strains or species. Such compositions may be a culture that is substantially free from other species of organism. Thus, in some embodiments, the invention provides a composition comprising one or more strains from the species *Enterococcus gallinarum*, which does not contain bacteria from any other species or which comprises only *de minimis* or biologically irrelevant amounts of bacteria from another species for use in therapy.

In some embodiments, the compositions of the invention comprise more than one bacterial strain or species. For example, in some embodiments, the compositions of the invention comprise more than one strain from within the same species (e.g. more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40 or 45 strains), and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise less than 50 strains from within the same species (e.g. less than 45, 40, 35, 30, 25, 20, 15, 12, 10, 9, 8, 7, 6, 5, 4 or 3 strains), and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise 1-40, 1-30, 1-20, 1-19, 1-18, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 strains from within the same species and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise more than one species from within the same genus (e.g. more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, 23, 25, 30, 35 or 40 species), and, optionally, do not contain bacteria from any other genus. In some embodiments, the compositions of the invention comprise less than 50 species from within the same genus (e.g. less than 50, 45, 40, 35, 30, 25, 20, 15, 12, 10, 8, 7, 6, 5, 4 or 3 species), and, optionally, do not contain bacteria from any other genus. In some embodiments, the compositions of the invention comprise 1-50, 1-40, 1-30, 1-20, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 species from within the same genus and, optionally, do not contain bacteria from any other genus. The invention comprises any combination of the foregoing.

In some embodiments, the composition comprises a microbial consortium. For example, in some embodiments, the composition comprises the bacterial strain having a 16s rRNA sequence that is at least 95% identical to SEQ ID NO:2, for example, which is an *Enterococcus gallinarum*, as part of a microbial consortium. For example, in some embodiments, the bacterial strain is present in combination with one or more (e.g. at least 2, 3, 4, 5, 10, 15 or 20) other bacterial strains from other genera with which it can live symbiotically *in vivo* in the intestine. For example, in some embodiments, the composition comprises a bacterial strain having a 16s rRNA sequence that is at least 95% identical to SEQ ID NO:2, for example, which is an *Enterococcus gallinarum,* in combination with a bacterial strain from a different genus. In some embodiments, the microbial consortium comprises two or more bacterial strains obtained from a faeces sample of a single organism, e.g. a human. In some embodiments, the microbial consortium is not found together in nature. For example, in some embodiments, the microbial consortium comprises bacterial strains obtained from faeces samples of at least two different organisms. In some embodiments, the two different organisms are from the same species, e.g. two different humans, e.g. two different human infants. In some embodiments, the two different organisms are an infant human and an adult human. In some embodiments, the two different organisms are a human and a non-human mammal.

In some embodiments, the composition of the invention additionally comprises a bacterial strain that has the same safety and therapeutic efficacy characteristics as strain MRX518, but which is not MRX518 deposited as NCIMB 42488, or which is not an *Enterococcus gallinarum*.

In some embodiments in which the composition of the invention comprises more than one bacterial strain, species or genus, the individual bacterial strains, species or genera may be for separate, simultaneous or sequential administration. For example, the composition may comprise all of the more than one bacterial strain, species or genera, or the bacterial strains, species or genera may be stored separately and be administered separately, simultaneously or sequentially. In some embodiments, the more than one bacterial strains, species or genera are stored separately but are mixed together prior to use.

In some embodiments, the bacterial strain for use in the invention is obtained from human infant faeces. In some embodiments in which the composition of the invention comprises more than one bacterial strain, all of the bacterial strains are obtained from human infant faeces or if other bacterial strains are present they are present only in *de minimis* amounts. The bacteria may have been cultured subsequent to being obtained from the human infant faeces and being used in a composition of the invention.

As mentioned above, in some embodiments, the one or more bacterial strains having a 16s rRNA sequence that is at least 95% identical to SEQ ID NO:2, for example which is an *Enterococcus gallinarum*, is/are the only therapeutically active agent(s) in a composition of the invention. In some embodiments, the bacterial strain(s) in the composition is/are the only therapeutically active agent(s) in a composition of the invention.

The compositions for use in accordance with the invention may or may not require marketing approval.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is lyophilised. In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is spray dried. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is live. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is viable. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is capable of partially or totally colonising the intestine. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is viable and capable of partially or totally colonising the intestine.

In some cases, the lyophilised or spray dried bacterial strain is reconstituted prior to administration. In some cases, the reconstitution is by use of a diluent described herein.

The compositions of the invention can comprise pharmaceutically acceptable excipients, diluents or carriers.

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain as used in the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a disorder when administered to a subject in need thereof; and wherein the disorder is breast cancer. In preferred embodiments the cancer is mammary carcinoma. In preferred embodiments the cancer is stage IV breast cancer.

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain as used in the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a disorder when administered to a subject in need thereof; and wherein the disorder is lung cancer. In preferred embodiments the cancer is lung carcinoma.

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain as used in the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a disorder when administered to a subject in need thereof; and wherein the disorder is liver cancer. In preferred embodiments the cancer is hepatoma (hepatocellular carcinoma).

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a disorder when administered to a subject in need

thereof; and wherein the disorder is colon cancer. In preferred embodiments the cancer is colorectal adenocarcinoma.

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a disorder when administered to a subject in need thereof; and wherein the disorder is carcinoma.

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a disorder when administered to a subject in need thereof; and wherein the disorder is a non-immunogenic cancer.

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a disorder when administered to a subject in need thereof; and wherein the disorder is selected from the group consisting of non-small-cell lung carcinoma, small-cell lung carcinoma, squamous-cell carcinoma, adenocarcinoma, glandular tumors, carcinoid tumors undifferentiated carcinomas.

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a disorder when administered to a subject in need thereof; and wherein the disorder is selected from the group consisting of hepatoblastoma, cholangiocarcinoma, cholangiocellular cystadenocarcinoma or liver cancer resulting from a viral infection.

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a disorder when administered to a subject in need thereof; and wherein the disorder is selected from the group consisting of invasive ductal carcinoma, ductal carcinoma in situ or invasive lobular carcinoma.

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a disorder when administered to a subject in need thereof; and wherein the disorder is selected from the group consisting of acute lymphoblastic leukemia (ALL), acute myeloid leukemia, adrenocortical carcinoma, basal-cell carcinoma, bile duct cancer, bladder cancer, bone tumor, osteosarcoma/malignant fibrous histiocytoma, brainstem glioma, brain tumor, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, breast cancer, bronchial adenomas/carcinoids, Burkitt's lymphoma, carcinoid tumor, cervical cancer, chronic lymphocytic

leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-cell lymphoma, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (GIST), germ cell tumor, glioma, childhood visual pathway and hypothalamic, Hodgkin lymphoma, melanoma, islet cell carcinoma, Kaposi sarcoma, renal cell cancer, laryngeal cancer, leukaemias, lymphomas, mesothelioma, neuroblastoma, non-Hodgkin lymphoma, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, parathyroid cancer, pharyngeal cancer, pituitary adenoma, plasma cell neoplasia, prostate cancer, renal cell carcinoma, retinoblastoma, sarcoma, testicular cancer, thyroid cancer, or uterine cancer.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the amount of the bacterial strain is from about 1×10^3 to about 1×10^{11} colony forming units per gram with respect to a weight of the composition.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered at a dose of 1 g, 3 g, 5 g or 10 g.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered by a method selected from the group consisting of oral, rectal, subcutaneous, nasal, buccal, and sublingual.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a carrier selected from the group consisting of lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol and sorbitol.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a diluent selected from the group consisting of ethanol, glycerol and water.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising an excipient selected from the group consisting of starch, gelatin, glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweetener, acacia, tragacanth, sodium alginate, carboxymethyl cellulose, polyethylene glycol, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate and sodium chloride.

In certain embodiments, the invention provides the above pharmaceutical composition, further comprising at least one of a preservative, an antioxidant and a stabilizer.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a preservative selected from the group consisting of sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein when the composition is stored in a sealed container at about 4◦C or about 25◦C and the container is placed

in an atmosphere having 50% relative humidity, at least 80% of the bacterial strain as measured in colony forming units, remains after a period of at least about: 1 month, 3 months, 6 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years.

In some embodiments, the composition of the invention is provided in a sealed container comprising a composition as described herein. In some embodiments, the sealed container is a sachet or bottle. In some embodiments, the composition of the invention is provided in a syringe comprising a composition as described herein.

The composition of the present invention may, in some embodiments, be provided as a pharmaceutical formulation. For example, the composition may be provided as a tablet or capsule. In some embodiments, the capsule is a gelatine capsule ("gel-cap").

In some embodiments, the compositions of the invention are administered orally. Oral administration may involve swallowing, so that the compound enters the gastrointestinal tract, and/or buccal, lingual, or sublingual administration by which the compound enters the blood stream directly from the mouth.

Pharmaceutical formulations suitable for oral administration include solid plugs, solid microparticulates, semi-solid and liquid (including multiple phases or dispersed systems) such as tablets; soft or hard capsules containing multi- or nano-particulates, liquids (e.g. aqueous solutions), emulsions or powders; lozenges (including liquid-filled); chews; gels; fast dispersing dosage forms; films; ovules; sprays; and buccal/mucoadhesive patches.

In some embodiments the pharmaceutical formulation is an enteric formulation, i.e. a gastro-resistant formulation (for example, resistant to gastric pH) that is suitable for delivery of the composition of the invention to the intestine by oral administration. Enteric formulations may be particularly useful when the bacteria or another component of the composition is acid-sensitive, e.g. prone to degradation under gastric conditions.

In some embodiments, the enteric formulation comprises an enteric coating. In some embodiments, the formulation is an enteric-coated dosage form. For example, the formulation may be an enteric-coated tablet or an enteric-coated capsule, or the like. The enteric coating may be a conventional enteric coating, for example, a conventional coating for a tablet, capsule, or the like for oral delivery. The formulation may comprise a film coating, for example, a thin film layer of an enteric polymer, e.g. an acid-insoluble polymer.

In some embodiments, the enteric formulation is intrinsically enteric, for example, gastro-resistant without the need for an enteric coating. Thus, in some embodiments, the formulation is an enteric formulation that does not comprise an enteric coating. In some embodiments, the formulation is a capsule made from a thermogelling material. In some embodiments, the thermogelling material is a cellulosic material, such as methylcellulose, hydroxymethylcellulose or hydroxypropylmethylcellulose (HPMC). In some embodiments, the capsule comprises a shell that

does not contain any film forming polymer. In some embodiments, the capsule comprises a shell and the shell comprises hydroxypropylmethylcellulose and does not comprise any film forming polymer (e.g. see [33]). In some embodiments, the formulation is an intrinsically enteric capsule (for example, Vcaps® from Capsugel).

In some embodiments, the formulation is a soft capsule. Soft capsules are capsules which may, owing to additions of softeners, such as, for example, glycerol, sorbitol, maltitol and polyethylene glycols, present in the capsule shell, have a certain elasticity and softness. Soft capsules can be produced, for example, on the basis of gelatine or starch. Gelatine-based soft capsules are commercially available from various suppliers. Depending on the method of administration, such as, for example, orally or rectally, soft capsules can have various shapes, they can be, for example, round, oval, oblong or torpedo-shaped. Soft capsules can be produced by conventional processes, such as, for example, by the Scherer process, the Accogel process or the droplet or blowing process.

Culturing methods

The bacterial strains for use in the present invention can be cultured using standard microbiology techniques as detailed in, for example, references [34-36].

The solid or liquid medium used for culture may be YCFA agar or YCFA medium. YCFA medium may include (per 100ml, approximate values): Casitone (1.0 g), yeast extract (0.25 g), $NaHCO_3$ (0.4 g), cysteine (0.1 g), K_2HPO_4 (0.045 g), KH_2PO_4 (0.045 g), NaCl (0.09 g), $(NH_4)_2SO_4$ (0.09 g), $MgSO_4 \cdot 7H_2O$ (0.009 g), $CaCl_2$ (0.009 g), resazurin (0.1 mg), hemin (1 mg), biotin (1 μg), cobalamin (1 μg), *p*-aminobenzoic acid (3 μg), folic acid (5 μg), and pyridoxamine (15 μg).

Bacterial strains for use in vaccine compositions

The inventors have identified that the bacterial strains of the invention are useful for treating or preventing cancer. This is likely to be a result of the effect that the bacterial strains of the invention have on the host immune system. Therefore, the compositions of the invention may also be useful for preventing cancer, when administered as vaccine compositions. In certain such embodiments, the bacterial strains of the invention are viable. In certain such embodiments, the bacterial strains of the invention are capable of partially or totally colonising the intestine. In certain such embodiments, the bacterial strains of the invention are viable and capable of partially or totally colonising the intestine. In other certain such embodiments, the bacterial strains of the invention may be killed, inactivated or attenuated. In certain such embodiments, the compositions may comprise a vaccine adjuvant. In certain embodiments, the compositions are for administration via injection, such as via subcutaneous injection.

General

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, references [37] and [38-44], *etc.*

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* is optional and means, for example, *x*±10%.

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

References to a percentage sequence identity between two nucleotide sequences means that, when aligned, that percentage of nucleotides are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of ref. [45]. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in ref. [46].

Unless specifically stated, a process or method comprising numerous steps may comprise additional steps at the beginning or end of the method, or may comprise additional intervening steps. Also, steps may be combined, omitted or performed in an alternative order, if appropriate.

Various embodiments of the invention are described herein. It will be appreciated that the features specified in each embodiment may be combined with other specified features, to provide further embodiments. In particular, embodiments highlighted herein as being suitable, typical or preferred may be combined with each other (except when they are mutually exclusive).

MODES FOR CARRYING OUT THE INVENTION

Example 1 – Efficacy of bacterial inocula in mouse models of cancer

Summary

This study tested the efficacy of compositions comprising bacterial strains according to the invention in four tumor models.

Materials

Test substance - Bacterial strain #MRX518.

Reference substance - Anti-CTLA-4 antibody (clone: 9H10, catalog: BE0131, isotype: Syrian Hamster IgG1, Bioxcell).

Test and reference substances vehicles - Bacterial culture medium (Yeast extract, Casitone, Fatty Acid medium (YCFA)). Each day of injection to mice, antibody was diluted with PBS (ref: BE14-516F, Lonza, France).

Treatment doses - Bacteria: $2x10^8$ in 200 µL. The a-CTLA-4 was injected at 10 mg/kg/inj. Anti-CTLA-4 was administered at a dose volume of 10 mL/kg/adm (i.e. for one mouse weighing 20 g, 200 µL of test substance will be administered) according to the most recent body weight of mice.

Routes of administration - Bacterial inoculum was administered by oral gavage (per os, PO) via a cannula. Cannulas were decontaminated every day. Anti-CTLA-4 was injected into the peritoneal cavity of mice (Intraperitoneally, IP).

Culture conditions of bacterial strain - The culture conditions for the bacterial strain were as follows:

- Pipette 10 mL of YCFA (from the prepared 10 mL E&O lab bottles) into Hungate tubes
- Seal the tubes and flush with CO_2 using a syringe input and exhaust system
- Autoclave the Hungate tubes
- When cooled, inoculate the Hungate tubes with 1 mL of the glycerol stocks
- Place the tubes in a static 37°C incubator for about 16 hours.
- The following day, take 1 mL of this subculture and inoculate 10 mL of YCFA (pre-warmed flushed Hungate tubes again, all in duplicate)
- Place them in a static 37°C incubator for 5 to 6h

Cancer cell line and culture conditions -

The cell lines that were used are detailed in the table below:

Cell line	Type	Mouse strain	Origin
EMT-6	Breast carcinoma	BALB/c	ATCC
LL/2 (LLC1)	Lung carcinoma	C57BL/6	ATCC CRL1642
Hepa1-6	Hepatocellular carcinoma	C57BL/6	IPSEN INNOVATION

The EMT-6 cell line was established from a transplantable murine mammary carcinoma that arose in a BALB/cCRGL mouse after implantation of a hyperplastic mammary alveolar nodule [47].

The LL/2 (LLC1) cell line was established from the lung of a C57BL mouse bearing a tumor resulting from an implantation of primary Lewis lung carcinoma [48].

The Hepa 1-6 cell line is a derivative of the BW7756 mouse hepatoma that arose in a C57/L mouse [49].

Cell culture conditions - All cell lines were grown as monolayer at 37°C in a humidified atmosphere (5% CO_2, 95% air). The culture medium and supplement are indicated in the table below:

Cell line	Culture medium	Supplement
EMT6	RPMI 1640 containing 2mM L-glutamine (ref: BE12-702F, Lonza)	10% fetal bovine serum (ref: #3302, Lonza)
LL/2 (LLC1)	RPMI 1640 containing 2mM L-glutamine (ref: BE12-702F, Lonza)	10% fetal bovine serum (ref: #3302, Lonza)
Hepa1-6	DMEM (ref:11960-044, Gibco)	10% fetal bovine serum (ref: #3302, Lonza) 2mM L-Glutamine penicillin-streptomycin (Sigma G-6784)

For experimental use, adherent tumor cells were detached from the culture flask by a 5 minute treatment with trypsin-versene (ref: BE17-161E, Lonza), in Hanks' medium without calcium or magnesium (ref: BE10-543F, Lonza) and neutralized by addition of complete culture medium. The cells were counted in a hemocytometer and their viability will be assessed by 0.25% trypan blue exclusion assay.

Use of animals -

Healthy female Balb/C (BALB/cByJ) mice, of matching weight and age, were obtained from CHARLES RIVER (L'Arbresles) for the EMT6 model experiments.

Healthy female C57BL/6 (C57BLl6J) mice, of matching weight and age, were obtained from CHARLES RIVER (L'Arbresles) for the LL/2(LLC1) and the Hepa1-6 model experiments.

Animals were maintained in SPF health status according to the FELASA guidelines, and animal housing and experimental procedures according to the French and European Regulations and NRC Guide for the Care and Use of Laboratory Animals were followed [50,51]. Animals were maintained in housing rooms under controlled environmental conditions: Temperature: 22 ± 2°C, Humidity 55 ± 10%, Photoperiod (12h light/12h dark), HEPA filtered air, 15 air exchanges per hour with no recirculation. Animal enclosures were provided with sterile and adequate space with bedding material, food and water, environmental and social enrichment (group housing) as described: 900 cm^2 cages (ref: green, Tecniplast) in ventilated racks, Epicea bedding (SAFE),10 kGy Irradiated diet (A04-10, SAFE), Complete food for immuno-competent rodents - R/M-H Extrudate, water from water bottles.

Experimental design and treatments

Antitumor activity, EMT6 model

Treatment schedule - The start of first dosing was considered as D0. On D0, non-engrafted mice were randomized according to their individual body weight into groups of 9/8 using Vivo manager® software (Biosystemes, Couternon, France). On D0, the mice received vehicle (culture medium) or

bacterial strain. On D14, all mice were engrafted with EMT-6 tumor cells as described below. On D24, mice from the positive control group received anti-CTLA-4 antibody treatments.

The treatment schedule is summarized in the table below:

Group	No. Animals	Treatment	Dose	Route	Treatment Schedule
1	8	Untreated	-	-	-
2	8	Vehicle (media)	-	PO	Q1Dx42
3	9	Bacterial strain #1 (MRX518)	2x108 bacteria	PO	Q1Dx42
4	8	Anti-CTLA4	10 mg/kg	IP	TWx2

The monitoring of animals was performed as described below.

Induction of EMT6 tumors in animals - On D14, tumors were induced by subcutaneous injection of $1x10^6$ EMT-6 cells in 200 µL RPMI 1640 into the right flank of mice.

Euthanasia - Each mouse was euthanized when it reached a humane endpoint as described below, or after a maximum of 6 weeks post start of dosing.

Antitumor activity, LL/2 (LLC1) model

Treatment schedule - The start of first dosing was considered as D0. On D0, non-engrafted mice were randomized according to their individual body weight into 7 groups of 9/8 using Vivo manager® software (Biosystemes, Couternon, France). On D0, the mice will received vehicle (culture medium) or bacterial strain. On D14, all mice were engrafted with LL/2 tumor cells as described below. On D27, mice from the positive control group received anti-CTLA-4 antibody treatments.

The treatment schedule is summarized in the table below:

Group	No. Animals	Treatment	Dose	Route	Treatment Schedule
1	8	Untreated	-	-	-
2	9	Vehicle (media)	-	PO	Q1Dx42
3	9	Bacterial strain #1 (MRX518)	$2x10^8$ bacteria	PO	Q1Dx42
4	8	Anti-CTLA4	10 mg/kg	IP	TWx2

The monitoring of animals was performed as described below.

Induction of LL/2 (LLC1) tumors in animals - On D14, tumors were induced by subcutaneous injection of $1x10^6$ LL/2 (LLC1) cells in 200 µL RPMI 1640 into the right flank of mice.

Euthanasia - Each mouse was euthanized when it reached a humane endpoint as described below, or after a maximum of 6 weeks post start of dosing.

Antitumor activity, Hepa1-6 model

Treatment schedule - The start of first dosing was considered as D0. On D0, non-engrafted mice were randomized according to their individual body weight into 7 groups of 9 using Vivo manager® software (Biosystemes, Couternon, France). On D0, the mice received vehicle (culture medium) or bacterial strain. On D14, all mice were engrafted with Hepa 1-6 tumor cells as described below. On D16, mice from the positive control group received anti-CTLA-4 antibody treatments.

The treatment schedule is summarized in the table below:

Group	No. Animals	Treatment	Dose	Route	Treatment Schedule
1	9	Untreated	-	-	-
2	9	Vehicle (media)	-	PO	Q1Dx42
6	9	Bacterial strain #4 (MRX518)	$2x10^8$ bacteria	PO	Q1Dx42
7	9	Anti-CTLA4	10 mg/kg	IP	TWx2

The monitoring of animals was performed as described below.

Orthotopic induction of Hepa 1-6 tumor cells in animals by intrasplenic injection - On D14, one million ($1x10^6$) Hepa 1-6 tumor cells in 50 µL RPMI 1640 medium were transplanted via intra-splenic injection into mice. Briefly, a small left subcostal flank incision was made and the spleen was exteriorized. The spleen was exposed on a sterile gauze pad, and injected under visual control with the cell suspension with a 27-gauge needle. After the cell inoculation, the spleen was excised.

Euthanasia - Each mouse was euthanized when it reached a humane endpoint as described in section below, or after a maximum of 6 weeks post start of dosing.

Evaluation of tumor burden at euthanasia - At the time of termination, livers were collected and weighed.

Animal monitoring

Clinical monitoring - The length and width of the tumor was measured twice a week with callipers and the volume of the tumor was estimated by this formula [52]:

$$\text{Tumor volume} = \frac{\text{width}^2 \times length}{2}$$

Humane endpoints [53]: Signs of pain, suffering or distress: pain posture, pain face mask, behaviour; Tumor exceeding 10% of normal body weight, but non-exceeding 2000 mm^3; Tumors interfering with

ambulation or nutrition; Ulcerated tumor or tissue erosion; 20% body weight loss remaining for 3 consecutive days; Poor body condition, emaciation, cachexia, dehydration; Prolonged absence of voluntary responses to external stimuli; Rapid laboured breathing, anaemia, significant bleeding; Neurologic signs: circling, convulsion, paralysis; Sustained decrease in body temperature; Abdominal distension.

Anaesthesia - Isoflurane gas anesthesia were used for all procedures: surgery or tumor inoculation, i.v. injections, blood collection. Ketamine and Xylazine anesthesia were used for stereotaxia surgical procedure.

Analgesia - Carprofen or multimodal carprofen/buprenorphine analgesia protocol were adapted to the severity of surgical procedure. Non-pharmacological care was provided for all painful procedures. Additionally, pharmacological care not interfering with studies (topic treatment) were provided at the recommendation of the attending veterinarian.

Euthanasia - Euthanasia of animals was performed by gas anesthesia over-dosage (Isoflurane) followed by cervical dislocation or exsanguination.

Results

Antitumor activity, EMT6 model

The results are shown in Figure 1. Treatment with the bacterial strain of the invention led to a clear reduction in tumour volume relative to both the negative controls. The positive control also led to a reduction in tumour volume, as would be expected.

Antitumor activity, LL/2 (LLC1) model

The results are shown in Figure 2. Treatment with the bacterial strain of the invention led to a clear reduction in tumour volume relative to both the negative controls.

Antitumor activity, Hepa1-6 model

The results are shown in Figure 3. The untreated negative control does not appear as would be expected, because liver weight was lower in this group than the other groups. However, the vehicle negative control and the positive control groups both appear as would be expected, because mice treated with vehicle alone had larger livers than mice treated with anti-CTLA4 antibodies, reflecting a greater tumour burden in the vehicle negative control group. Treatment with the bacterial strain of the invention led to a clear reduction in liver weight (and therefore tumour burden) relative to the mice in the vehicle negative control group.

These data indicate that strain MRX518 may be useful for treating or preventing cancer, and in particular for reducing tumour volume in breast, lung and liver cancers.

Example 2 – PCR gene analysis

A pure culture of bacteria MRX518 was studied in a PCR gene analysis. There were two arms to the experiment: 1) MRX518 was co-cultured with human colonic cells (CaCo2) to investigate the effects of the bacteria on the host, and 2) MRX518 was co-cultured on CaCo2 cells that were stimulated with IL1 to mimic the effect of the bacteria in an inflammatory environment. The effects in both scenarios were evaluated through gene expression analysis. The results are shown below:

Gene	**Fold change**	**Function**
CXCL3	28412.73	CXCR2 ligand,
CXCL2	135.42	CXCR2 ligand, 90% homology with CXCL1.
CXCL9	34.76	CXCR3 ligand, primarily thought of as Th1 cell chemoattractant (inducible by IFN-g)
IL8	31.81	Cytokine, chemoattractant (especially neutrophils), many receptors including CXCR1 and CXCR2/
CXCL1	16.48	CXCR2 ligand, stimulates cell proliferation as well as migration, overexpression is neuroprotective in EAE.
CD40	14.33	Co-stimulatory molecule, route of T cell dependent DC activation.
TNF	13.50	Major proinflammatory cytokine
IL17C	12.18	Promotes antibacterial response from epthielium, synergistic with IL-22,
CXCL10	10.66	Close homology with CXCL9, think also CXCR3 ligand?
HSPA1B	10.19	Heat shock protein
NFKBIA	8.87	NFkB signalling; PI3K
JUN	7.61	Antibacterial response; GPCR signalling.
TNFAIP3	6.63	TNF signalling
DUSP1	6.36	Anti-inflammatory phosphatase, inactivates MAPKs
JUNB	5.36	Transcription factor, JAK-STAT signalling
BIRC3	4.86	Adherens junctions, tight junctions

DUSP2	4.59	Anti-inflammatory, inactivates MAPK.
IL32	4.29	Proinflammatory cytokine, induced by IFN-g, IL-18
DUSP5	3.12	Anti-inflammatory, inactivates MAPK
FOS	3.03	Transcription factors, TLR signalling, forms part of AP-1
GADD45B	2.89	Cell growth and proliferation
CLDN4	2.61	Tight junctions
ADM	2.57	NFkB signalling
KLF10	2.49	Cell arrest, TGF-b singllaing.
DEFB4A	-2.34	Antimicrobial peptide
APBA1	-2.53	Signalling
IGFBP1	-2.72	Signalling pathway
IL28B	-2.73	IFN-lambda, antiviral immune defence,
IL10	-3.38	Anti-inflammatory cytokine
NR4A1	-5.57	Nuclear receptor, anti-inflammatory, regulator of T cell proliferation. T helper cell differentiation
NOD2	-14.98	PRR, inflammasome activator, promotes autophagy
INOS	-26.88	Proinflammatory, generator of nitric oxide

These data appear to show two gene expression signatures - CXCR1/2 ligands (CXCL3, CXCL2, CXCL1, IL-8), which is associated with pro-inflammatory cell migration, and CXCR3 ligands (CXCL9,CXCL10), which is more specifically indicative of IFN-γ-type responses, also supported by IL-32, which is IFN-γ-inducible.

Example 3 – Stability testing

A composition described herein containing at least one bacterial strain described herein is stored in a sealed container at 25˚C or 4˚C and the container is placed in an atmosphere having 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90% or 95% relative humidity. After 1 month, 2 months, 3 months, 6 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years, at least 50%, 60%, 70%, 80% or 90% of the bacterial strain shall remain as measured in colony forming units determined by standard protocols.

Example 4 – *cytokine production in immature dendritic cells induced by MRX518 compared to MRX518 + LPS*

Summary

This study tested the effect of the bacterial strain MRX518 alone and in combination with lipopolysaccharide (LPS) on cytokine production in immature dendritic cells.

A monocyte population was isolated from peripheral blood mononuclear cells (PBMCs). The monocyte cells were subsequently differentiated into immature dendritic cells. The immature dendritic cells were plated out at 200,000 cells/well and incubated with MRX518 at a final concentration of 10^7/ml, with the optional addition of LPS at a final concentration of 100ng/ml. The negative control involved incubating the cells with RPMI media alone and positive controls incubated the cells with LPS at a final concentration of 100ng/ml. The cytokine content of the cells was then analysed.

Results

The results of these experiments can be seen in Figures 4a-d. The addition of MRX518 alone leads to a substantial increase in the level of cytokines IL-6 and TNF-α compared to the negative control (Figure 4a and c). The addition of LPS (positive control) leads to an increase in the level of IL-6 and TNF-α compared to the negative control but not IL-1β (Figure 4b). A combination of MRX518 and LPS led to a synergistic increase in the level of IL-1β produced (Figure 4d).

Conclusion

MRX518 has the ability to induce higher IL-6 and TNF-α cytokine production in immature dendritic cells. The combination LPS and MRX518 can increase the levels of cytokines IL-1β in immature dendritic cells. These data indicate that MRX518 alone or in combination with LPS can increase inflammatory cytokines IL-1β, IL-6 and TNF-α, which promotes inflammation that can suppress cancer. Treatment with MRX518 alone or in combination with can induce cytokines that can limit tumour growth.

Example 5 – *cytokine production in THP-1 cells induced by MRX518 compared to MRX518 + LPS*

Summary

This study tested the effect of bacterial strain MRX518 alone and in combination with LPS on cytokine production in THP-1 cells, a model cell line for monocytes and macrophages.

THF-1 cells were differentiated into M0 medium for 48h with 5ng/mL phorbol-12-myristate-13-acetate (PMA). These cells were subsequently incubated with MRX518 at a final concentration of 10^8/ml, with or without the addition of LPS at a final concentration of 100ng/ml. The bacteria were then washed off and the cells allowed to incubate under normal growing conditions for 24 h. The cells were then spun down and the resulting supernatant was analysed for cytokine content.

Results

The results of these experiments can be seen in Figures 5a-c. The addition of MRX518 without LPS leads to an increase in the cytokine levels of IL-1β, IL-6 and TNF-α compared to the no bacterial and the bacterial sediment controls. The addition of LPS and MRX518 leads to a synergistic increase in the production of cytokines.

Conclusion

MRX518 has the ability to induce cytokine production in THP-1 cells, which can be synergistically increased with the addition of LPS. These data indicate that MRX518 alone or in combination with LPS can increase inflammatory cytokines IL-1β, IL-6 and TNF-α, which promotes inflammation that can suppress cancer. Treatment with MRX518 alone or in combination with can induce cytokines that can limit tumour growth.

Sequences

SEQ ID NO:1 (*Enterococcus gallinarum* 16S rRNA gene - AF039900)

```
   1 taatacatgc aagtcgaacg ctttttcttt caccggagct tgctccaccg aaagaaaaag
  61 agtggcgaac gggtgagtaa cacgtgggta acctgcccat cagaagggga taacacttgg
 121 aaacaggtgc taataccgta taacactatt ttccgcatgg aagaaagttg aaaggcgctt
 181 ttgcgtcact gatggatgga cccgcggtgc attagctagt tggtgaggta acggctcacc
 241 aaggccacga tgcatagccg acctgagagg gtgatcggcc acactgggac tgagacacgg
 301 cccagactcc tacgggaggc agcagtaggg aatcttcggc aatggacgaa agtctgaccg
 361 agcaacgccg cgtgagtgaa gaaggttttc ggatcgtaaa actctgttgt tagagaagaa
 421 caaggatgag agtagaacgt tcatcccttg acggtatcta accagaaagc cacggctaac
 481 tacgtgccag cagccgcggt aatacgtagg tggcaagcgt tgtccggatt tattgggcgt
 541 aaagcgagcg caggcggttt cttaagtctg atgtgaaagc ccccggctca accggggagg
 601 gtcattggaa actgggagac ttgagtgcag aagaggagag tggaattcca tgtgtagcgg
 661 tgaaatgcgt agatatatgg aggaacacca gtggcgaagg cggctctctg gtctgtaact
 721 gacgctgagg ctcgaaagcg tggggagcga acaggattag ataccctggt agtccacgcc
 781 gtaaacgatg agtgctaagt gttggagggt ttccgccctt cagtgctgca gcaaacgcat
 841 taagcactcc gcctggggag tacgaccgca aggttgaaac tcaaaggaat tgacgggggc
 901 ccgcacaagc ggtggagcat gtggtttaat tcgaagcaac gcgaagaacc ttaccaggtc
 961 ttgacatcct ttgaccactc tagagataga gcttcccctt cgggggcaaa gtgacaggtg
1021 gtgcatggtt gtcgtcagct cgtgtcgtga gatgttgggt taagtcccgc aacgagcgca
1081 acccttattg ttagttgcca tcatttagtt gggcactcta gcgagactgc cggtgacaaa
1141 ccggaggaag gtggggatga cgtcaaatca tcatgccccc tatgacctgg gctacacacg
1201 tgctacaatg ggaagtacaa cgagttgcga agtcgcgagg ctaagctaat ctcttaaagc
1261 ttctctcagt tcggattgta ggctgcaact cgcctacatg aagccggaat cgctagtaat
```

```
1321 cgcggatcag cacgccgcgg tgaatacgtt cccgggcctt gtacacaccg cccgtcacac
1381 cacgagagtt tgtaacaccc gaagtcggtg aggtaacctt tttggagcca gccgcctaag
1441 gtgggataga tgattggggt gaagtcgtaa caaggtagcc gtatcggaag gtgcggctgg
1501 atcacc
```

SEQ ID NO:2 (consensus 16S rRNA sequence for *Enterococcus gallinarum* strain MRX518)

```
TGCTATACATGCAGTCGAACGCTTTTTCTTTCACCGGAGCTTGCTCCACCGAAAGAAAAAGAGTGGCGAACGGGTGA
GTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGAAACAGGTGCTAATACCGTATAACACTATTTTC
CGCATGGAAGAAAGTTGAAAGGCGCTTTTGCGTCACTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAGGTA
ACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGAC
TCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAG
GTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAGAACGTTCATCCCTTGACGGTATCTAA
CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGC
GTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGG
GAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCAGT
GGCGAAGGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGG
TAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCAAACGCATTAAGCA
CTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG
GTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTCCCCTT
CGGGGGCAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTATTGTTAGTTGCCATCATTTAGTTGGGCACTCTAGCGAGACTGCCGGTGACAAACCGGAGGAAGGTGG
GGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGAAGTACAACGAGTTGCGAA
GTCGCGAGGCTAAGCTAATCTCTTAAAGCTTCTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCCGGA
ATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGA
GAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTTGGAGCCAGCCGCCTAAGGTG
```

SEQ ID NO:3 (strain MRX518 chromosome sequence) – see electronic sequence listing.

SEQ ID NO:4 (strain MRX518 plasmid sequence) – see electronic sequence listing.

REFERENCES

[1] Spor *et al.* (2011) *Nat Rev Microbiol.* 9(4):279-90.
[2] Eckburg *et al.* (2005) *Science.* 10;308(5728):1635-8.
[3] Macpherson *et al.* (2001) *Microbes Infect.* 3(12):1021-35
[4] Macpherson *et al.* (2002) *Cell Mol Life Sci.* 59(12):2088-96.
[5] Mazmanian *et al.* (2005) *Cell* 15;122(1):107-18.
[6] Frank *et al.* (2007) *PNAS* 104(34):13780-5.
[7] Scanlan *et al.* (2006) *J Clin Microbiol.* 44(11):3980-8.
[8] Kang *et al.* (2010) *Inflamm Bowel Dis.* 16(12):2034-42.
[9] Machiels *et al.* (2013) *Gut.* 63(8):1275-83.
[10] WO 2013/050792
[11] WO 03/046580
[12] WO 2013/008039
[13] WO 2014/167338
[14] Goldin and Gorbach (2008) *Clin Infect Dis.* 46 Suppl 2:S96-100.
[15] Azad *et al.* (2013) *BMJ.* 347:f6471.
[16] Strickertsson *et al.* (2014) *Genes.* 5(3): 726-738.

[17] Collins *et al.* (1984) *Int J Syst Evol Microbiol.* 34: 220-223.
[18] Masco *et al.* (2003) *Systematic and Applied Microbiology,* 26:557-563.
[19] Srůtková *et al.* (2011) *J. Microbiol. Methods*, 87(1):10-6.
[20] Haabeth *et al.* (2012) *OncoImmunology* 1(1):1146-1152.
[21] Lejeune *et al.* (2006) *Cancer Immun.* 6:6
[22] Pace *et al.* (1983) *PNAS.* 80:8782-6.
[23] Sgadari *et al.* (1996) *PNAS.* 93:13791-6.
[24] Arenberg et al. (1996) *J. Exp. Med.* 184:981-92.
[25] Sgadari *et al.* (1997) *Blood.* 89:2635-43.
[26] Miyamoto-Shinohara *et al.* (2008) *J. Gen. Appl. Microbiol.*, 54, 9–24.
[27] Cryopreservation and Freeze-Drying Protocols, ed. by Day and McLellan, Humana Press.
[28] Leslie *et al.* (1995) *Appl. Environ. Microbiol.* 61, 3592–3597.
[29] Mitropoulou *et al.* (2013) *J Nutr Metab.* (2013) 716861.
[30] Kailasapathy *et al.* (2002) *Curr Issues Intest Microbiol.* 3(2):39-48.
[31] Handbook of Pharmaceutical Excipients, 2nd Edition, (1994), Edited by A Wade and PJ Weller
[32] Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985)
[33] US 2016/0067188
[34] Handbook of Microbiological *Media, Fourth Edition (*2010) Ronald Atlas, CRC Press.
[35] *Maintaining Cultures for Biotechnology and Industry* (1996) Jennie C. Hunter-Cevera, Academic Press
[36] Strobel (2009) *Methods Mol Biol.* 581:247-61.
[37] Gennaro (2000) *Remington: The Science and Practice of Pharmacy.* 20th edition, ISBN: 0683306472.
[38] *Molecular Biology Techniques: An Intensive Laboratory Course*, (Ream *et al.*, eds., 1998, Academic Press).
[39] *Methods In Enzymology* (S. Colowick and N. Kaplan, eds., Academic Press, Inc.)
[40] *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell, eds, 1986, Blackwell Scientific Publications)
[41] Sambrook *et al.* (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edition (Cold Spring Harbor Laboratory Press).
[42] *Handbook of Surface and Colloidal Chemistry* (Birdi, K.S. ed., CRC Press, 1997)
[43] Ausubel *et al.* (eds) (2002) *Short protocols in molecular biology*, 5th edition (Current Protocols).
[44] *PCR (Introduction to Biotechniques Series*), 2nd ed. (Newton & Graham eds., 1997, Springer Verlag)
[45] *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30
[46] Smith & Waterman (1981) *Adv. Appl. Math.* 2: 482-489.
[47] Rockwell *et al.*, (1972) *J Natl Cancer Inst.* 49:735–49.
[48] Bertram and Janik (1980) *Cancer Lett.* 11:63–73.
[49] Darlington (1987) *Meth Enzymol.* 151:19–38.
[50] Principe d'éthique de l'expérimentation animale, Directive n°2010/63 CEE 22nd September 2010, Décrêt n°2013-118 1st February 2013.
[51] Guide for the Care and Use of Laboratory Animals: Eighth Edition. The National Academies Press; 2011
[52] Simpson-Herren and Lloyd (1970) *Cancer Chemother Rep.* 54:143–74.
[53] Workman et al. (2010) *Br. J. Cancer.* 102:1555–77.

CLAIMS

1. A composition comprising a bacterial strain of the species *Enterococcus gallinarum* for use in therapy.

2. The composition of claim 1 for use in a method of treating or preventing cancer.

3. The composition of claim 1 or claim 2 which does not contain bacteria from any other species, or which comprises only de minimis or biologically irrelevant amounts of bacteria from another species.

4. The composition of any one of the preceding claims, wherein the composition is for use in a method of treating or preventing lung cancer, breast cancer, liver cancer or colon cancer.

5. The composition of any one of the preceding claims, wherein the composition is for use in a method of reducing tumour size, reducing tumour growth, preventing metastasis or preventing angiogenesis.

6. The composition of any one of claims 1-5, wherein the bacterial strain has the 16s rRNA sequence represented by SEQ ID NO:2.

7. The composition of any one of the preceding claims, wherein the composition is for oral administration, wherein the composition comprises one or more pharmaceutically acceptable excipients or carriers, and/or wherein the bacterial strain is lyophilised.

8. The composition of any one of the preceding claims, wherein the bacterial strain is capable of partially or totally colonising the intestine.

9. The composition of any one of the preceding claims, wherein the composition comprises a single strain of *Enterococcus gallinarum*.

10. The composition of any one of the preceding claim, which comprises the *Enterococcus gallinarum* bacterial strain as part of a microbial consortium.

11. A food product or a vaccine composition comprising the composition of any one of the preceding claims, for the use of any one of the preceding claims.

12. A cell or biologically pure culture of the *Enterococcus gallinarum* strain deposited under accession number NCIMB 42488.

13. A composition comprising the cell of claim 12, optionally comprising a pharmaceutically acceptable carrier or excipient.

14. A cell of the *Enterococcus gallinarum* strain deposited under accession number NCIMB 42488 for use in therapy, optionally wherein the cell is for use in a method defined in any one of claims 1-5.