

## RECOMBINANT BACTERIA AND USES THEREOF

### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore patent application No. 5 10201901828T, filed 28 February 2019, the contents of it being hereby incorporated by reference in its entirety for all purposes.

### FIELD OF THE INVENTION

[0002] The present invention relates to microbiology and molecular biology, in particular 10 the development of recombinant bacterium, and the use of recombinant bacterium in treating cancer.

### BACKGROUND OF THE INVENTION

[0003] Colorectal cancer, or cancer of the colon and rectum, is the third most common 15 neoplasm worldwide and the most commonly diagnosed cancer in Singapore.

[0004] Surgery is the main form of treatment for colorectal cancer. However, surgery removes bulk tumor, leaving behind microscopic residual disease which ultimately results in recrudescence.

[0005] For Stages II and III, radiation therapy may be added to help kill the cancer and 20 shrink the tumor. Radiation may also be used in Stage IV to improve symptoms and prolong life. However, the use of radiation therapy as a single modality in the definitive treatment of colorectal cancer has met with little success despite efforts to increase the total dose to the tumor and reduce the amount of irradiated normal tissue. Radiation therapy is rarely helpful in patients with colorectal cancer that is unsuitable for resection, as the usual reason for 25 nonresectability is the lack of anatomic localization of the cancer. Radiation therapy is sometimes used to relieve localized obstruction, particularly in the region of the cardia, and for patients with chronically bleeding cancers that cannot be resected. The median survival for patients treated with radiation is around 12 months, and long-term survivors are few.

[0006] Many chemotherapeutic drugs have been tried in the past as single agents for the 30 palliation of colorectal cancer, but the results were generally disappointing. Nevertheless, the role of chemotherapy in the management of colorectal cancer is continually evolving. Active chemotherapeutic agents include 5-fluorouracil, IMC-C225 (cetuximab), leucovorin,

irinotecan, oxaliplatin, Camptosar (Pharmacia & Upjohn), and Celebrex. Oftentimes, chemotherapy with radiation in adjunct to surgery is used. In general, chemotherapy can achieve long-term survival rates of up to 15% to 20%, even in patients with recurrent or metastatic disease. Unfortunately, the high initial response rate to first line chemotherapy  
5 does not appear to translate into a survival benefit. Moreover, there are many undesirable side effects associated with chemotherapy such as temporary hair loss, mouth sores, anemia (decreased numbers of red blood cells that may cause fatigue, dizziness, and shortness of breath), leukopenia (decreased numbers of white blood cells that may lower resistance to infection), thrombocytopenia (decreased numbers of platelets that may lead to easy bleeding  
10 or bruising), and gastrointestinal symptoms like nausea, vomiting, and diarrhea.

[0007] While surgery, chemotherapeutic agents, and radiation are useful in the treatment of colorectal cancer, there is a continued need to find better treatment modalities and approaches to manage the disease that are more effective and less toxic, especially when clinical oncologists are giving increased attention to the quality of life of cancer patients.

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### SUMMARY OF THE INVENTION

[0008] In one aspect, there is provided a recombinant, probiotic lactic acid bacterium, wherein the bacterium comprises a non-replicating plasmid vector comprising (a) a tumor suppressor gene or anti-inflammatory gene operably linked to a first promoter that directs  
20 expression of the tumor suppressor gene or anti-inflammatory gene in a mammalian cell, and (b) an adhesin gene operably linked to a second promoter that directs expression of the adhesin gene in the bacterium.

[0009] In another aspect, there is provided a method of treating cancer or reducing inflammation in a subject, wherein the method comprises administering a pharmaceutically  
25 effective amount of the recombinant, probiotic lactic acid bacterium of the present invention into the gastrointestinal tract of the subject, and wherein the cancer or inflammation is a cancer or inflammation of the gastrointestinal tract.

[0010] In another aspect, there is provided a method of treating cancer in a subject, wherein the method comprises administering a pharmaceutically effective amount of a  
30 recombinant, probiotic lactic acid bacterium, wherein the bacterium comprises a non-replicating plasmid vector comprising an adhesin gene operably linked to a promoter that directs expression of the adhesin gene in the bacterium.

### BRIEF DESCRIPTION OF THE DRAWINGS

[0011] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0012] **Fig. 1** provides illustration of examples of the recombinant bacteria of the present invention, where a single non-replicating recombinant plasmid comprises both the adhesin gene and the tumor suppressor/anti-inflammatory gene.

[0013] **Fig. 2** shows results of survival data analysis using data available from Marisa L *et al* (**Fig. 2A**) and The Cancer Genome Atlas (**Fig. 2B**) based on Kaplan-Meier method. The Y-axis in each graph represents the probability of relapse-free survival (RFS), and the X-axis in each graph represents the survival time in months. RFS probability in patient cohort from Marisa L *et al.* trended marginally higher ( $P = 0.1$ ) for patients with tumour that had high DUSP10 expression (**Fig. 2A** left panel). After sample stratification for ERK expression, it was found that high DUSP10 in tumours was associated with approximately 20% improvement in survival probability of patients with high ERK expression (**Fig. 2A** right panel). Improvement in patient survival was more striking in the TCGA cohort (**Fig. 2B**). Survival of patients with high DUSP10 expression was increased by approximately 20% up to 60 months with or without ERK2 stratification. Together, the data in **Fig. 2** suggests that DUSP10 expression may be functionally important for control of tumour development that resulted in better survival in the patients.

[0014] **Fig. 3** shows effects of DUSP10 over expression on the growth of Caco2 and DLD1 xenograft tumours. **Fig. 3A** are representative images showing Caco2 and DLD1 xenograft tumours developed in immunocompromised mice after 18 days post-administration. **Fig. 3B** shows bar charts summarizing the size and weight of the respective xenografts. Mean  $\pm$  standard deviations are shown. \* and \*\* represent  $P < 0.05$  and  $P < 0.01$  respectively (Mann-Whitney test). The results show that DUSP10 overexpression reduced tumour growth in vivo.

[0015] **Fig. 4** shows the effects of of DUSP10 over expression on metastatic colonisation of HCT116 cells to the liver in an intra-splenic injection mouse model. **Fig. 4A** shows representative images (left panel) and H&E micrographs (right panel) showing metastatic lesions (red arrows) developed in liver of immunocompromised mice after intra-splenic

injection of either DUSP10 overexpression or control HCT116 cells. **Fig. 4B** shows bar charts summarizing quantitative measurement of liver weight, total tumour load per liver and largest tumour size. Mean  $\pm$  standard deviations are shown. \*\* and \*\*\* represent  $P < 0.01$  and  $P < 0.001$  respectively (Mann-Whitney test). The results show that overexpression of DUSP10 reduced metastatic colonisation of HCT116 cells to the liver in an intra-splenic injection mouse model.

**Fig. 5** depicts a schematic diagram showing DNA construct developed for transformation into *L. lactis* for expression of bacterial fibronectin-binding protein (derived from *S. aureus*) and human DUSP10 (not drawn to scale).

**Fig. 6** shows the effects of *L. lactis*-Fnb-DUSP10 infection in Caco2 cells. **Fig. 6A** is a bar chart showing the mRNA level, and **Fig. 6B** is a western blot imaging showing the protein level, of DUSP10 in uninfected and various *L. lactis* infected Caco2 cells (MOI = 1500). **Fig. 6C** is a bar chart showing the percentage of Caco2 proliferation after 3 days of daily infection with various *L. lactis* bacteria. 1 = uninfected cells, 2 = cells infected with *L. lactis* bacteria with control vector, 3 = cells infected with *L. lactis* bacteria with Fnb only, 4 = cells infected with *L. lactis* bacteria with DUSP10. Mean  $\pm$  standard deviations are shown. \*\* and \*\*\* represent  $P < 0.01$  and  $P < 0.001$  respectively (1-way ANOVA). The results demonstrate that Caco2 cells infected with *L. lactis*-Fnb-DUSP10 showed increased DUSP10 expression and resulted in suppression of cell proliferation.

**Fig. 7** shows the effects of *L. lactis*-Fnb-DUSP10 on colon tumour load in mice. **Fig. 7A** shows representative images of colon tumours found in AOM/DSS induced CRC mice with or without various *L. lactis* treatment through oral gavage. Top and bottom dot plots in **Fig. 7B** and **Fig. 7C** show the number of tumours per colon and average tumour size from each group of mice respectively. Mean percentage reduction in tumour load and size compared to untreated control is shown under each dot plot. Mean  $\pm$  standard deviations and 1-way ANOVA (\*) P values are shown. The results show that treatment with *L. lactis*-Fnb-DUSP10 for 7 had successfully suppressed intestinal tumour development in the AOM/DSS induced CRC mouse model.

**Fig. 8** shows the effect of *L. lactis*-Fnb-DUSP10 on caecal transplanted Ls174T tumour growth. **Fig. 8A** shows representative image of caecum containing tumour (black arrows). **Fig. 8B** is a bar chart showing weight of caecum in untreated and treated mice. Mean  $\pm$  standard deviations are shown. \* $P < 0.05$  (Student's T test). The results show that the

size of the transplanted Ls174T xenografts was smaller in mice treated with *L. lactis*-Fnb-DUSP10.

[0020] **Fig. 9** shows the effects of *L. lactis*-Fnb-DUSP10 treatment in mice with colonic inflammation. **Fig. 9A** shows representative H&E (Haemotoxylin and Eosin) micrographs for colonic inflammation, **Fig. 9B** is a dot plot showing histological scores for colonic inflammation, **Fig. 9C** shows weight change in mice during 2% DSS and *L. lactis* treatment. **Fig. 9D** shows changes in mRNA expression level of genes involved in inflammation. 1 = Unt; 2= Vect; 3= Fnb; and 4= DUSP10. “Unt”, “Vect”, “Fnb” and “DUSP10” denote; untreated, *Lactis*-vector, *Lactis*-Fnb and *Lactis*-Fnb-DUSP10 treated groups, “PC” and “DC” refer to proximal and distal colon respectively. \*, \*\* and \*\*\* denote P <0.05, <0.01 and <0.001 respectively (One-way ANOVA, Kruskal-Wallis test). Mean  $\pm$  standard deviations were shown in each graph. All statistical comparison were made to untreated control group.

[0021] **Fig. 10** shows the effects of *L. lactis*-Fnb-DUSP10 and/or anti-PDL-1 treatment on colon tumour load in mice. **Fig. 10A** shows representative images of colon tumours found in AOM/DSS induced CRC mice with or without various *L. lactis* and/or anti-PDL-1 treatment. The dot plot in **Fig. 10B** shows the number of tumours per colon from each group of mice. Mean percentage reduction in tumour load compared to untreated control is shown under each dot plot. Mean  $\pm$  standard deviations and 1-way ANOVA (\*) P values are shown. “Unt”, “Vect”, “Lactis-DUSP10”, “anti-PDL-1”, “anti-PDL-1 + Vect” and “anti-PDL-1 + Lactis-DUSP10” denote: untreated, *Lactis*-vector, *Lactis*-Fnb-DUSP10, anti-PDL-1, anti-PDL-1 + *Lactis*-vector, and anti-PDL-1 + *Lactis*-Fnb-DUSP10 treated groups. The results show that *L. lactis*-Fnb-DUSP10 and *L. lactis*-Fnb-DUSP10 + anti-PDL-1 treated groups had successfully suppressed intestinal tumour development in the AOM/DSS induced CRC mouse model.

## 25 DETAILED DESCRIPTION OF THE PRESENT INVENTION

[0022] It has been found by the inventors that delivering non-replicating plasmid vector to the host cells allows better control of the expression of the genetic materials contained in the plasmid vector, thus resulting in improved control of the treatment regime which comprises the expression of the genetic materials in the host cells. Thus, in one aspect, there is provided a recombinant, probiotic lactic acid bacterium, wherein the bacterium comprises a non-replicating plasmid vector comprising (a) a tumor suppressor gene or anti-inflammatory gene operably linked to a first promoter that directs expression of the tumor suppressor gene or

anti-inflammatory gene in a mammalian cell, and (b) an adhesin gene operably linked to a second promoter that directs expression of the adhesin gene in the bacterium.

[0023] The term “recombinant bacterium” as used herein refers to a bacterium containing recombinant genetic materials such as recombinant DNA molecule(s). Recombinant DNA molecules are usually formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic materials from multiple sources, creating sequences that would not otherwise be found in the genome. It is well understood that the DNA of most bacteria is contained in a single circular molecule, called the bacterial chromosome. In addition to the bacterial chromosome, bacteria often contain plasmids, which are small circular DNA molecules that are physically separated from the bacterial chromosome, which can replicate independently. In some examples, the recombinant bacterium contains recombinant DNA molecule(s) in the plasmids.

[0024] The term “probiotic” as used herein refers to non-pathogenic and non-toxigenic microorganisms, in particular bacteria, that perform beneficial functions for the host organism. The gastrointestinal tract of mammalian species harbors a complex microbial ecosystem containing a large number and variety of bacteria. The resident bacterial population in the gastrointestinal tract has a major impact on gastrointestinal function and thereby on the health and wellbeing of the host organism. Among these, some bacteria are opportunistic or considered to be detrimental and cause adverse conditions such as diarrhea, infections, gastroenteritis and endotoxaemia, while some bacteria species are considered as “probiotic”, in that they perform beneficial functions for the host organism.

[0025] The term “lactic acid bacteria” or its grammatical variants as used herein refers to an order of gram-positive, low-GC, acid-tolerant, generally nonsporulating, nonrespiring, either rod-shaped (bacilli) or spherical (cocci) bacteria that share common metabolic and physiological characteristics. These bacteria, usually found in decomposing plants and milk products, produce lactic acid as the major metabolic end product of carbohydrate fermentation. Examples of lactic acid bacteria include *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Sporolactobacillus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*.

[0026] *Lactococcus* is a genus of lactic acid bacteria. They are gram-positive, catalase-negative, non-motile cocci that are found singly, in pairs, or in chains. *Lactococcus* are known as homofermenters, which means that they produce a single product, lactic acid in this

case, as the major or only product of glucose fermentation. Twelve species of *Lactococcus* are currently recognized: *L. chungangensis*, *L. formosensis*, *L. fujiensis*, *Lactococcus hircilactis*, *L. garvieae* (*L. garvieae* subsp. *garvieae*, *L. garvieae* subsp. *bovis*), *L. lactis* (*L. lactis* subsp. *cremoris*, *L. lactis* subsp. *hordniae*, *L. lactis* subsp. *lactis*, *L. lactis* subsp. *tructae*), *L. laudensis*, *L. nasutitermitis*, *L. piscium*, *L. plantarum*, *L. raffinolactis* and *L. taiwanensis*.

[0027] In one specific example, the recombinant, probiotic lactic acid bacterium is of the *L. lactis* species. *L. lactis* is a Gram-positive bacterium used extensively in the production of buttermilk and cheese. *L. lactis* cells are cocci that group in pairs and short chains, and, depending on growth conditions, appear ovoid with a typical length of 0.5 - 1.5  $\mu\text{m}$ . *L. lactis* does not produce spores (nonsporulating) and are not motile (nonmotile). *L. lactis* produce lactic acid from sugars. The capability to produce lactic acid is one of the reasons why *L. lactis* is one of the most important microorganisms in the dairy industry. Based on its history in food fermentation, *L. lactis* has generally recognized as safe (GRAS) status with few case reports of being an opportunistic pathogen. GRAS is a United States Food and Drug Administration (FDA) designation that a chemical or substance added to food is considered safe by experts, and so is exempted from the usual Federal Food, Drug, and Cosmetic Act (FFDCA) food additive tolerance requirements. In some examples, the recombinant, probiotic lactic acid bacterium, or the recombinant lactic acid producing bacterium, or the bacterium of the *Lactococcus* genus as disclosed herein is classified as a GRAS bacterium.

[0028] The term “express” or its grammatical variants as used herein refers to the transcription from DNA to an RNA nucleic acid molecule at least complementary in part to a region of one of the two nucleic acid strands of the gene, followed by the translation from said RNA nucleic acid molecule to give a protein or polypeptide or a portion thereof.

[0029] The term “adhesin” as used herein refers to cell-surface components or appendages of bacteria that facilitate adhesion or adherence to other cells or to surfaces, usually the host cells they are infecting or living in. Adherence is an essential step in bacterial pathogenesis or infection, required for colonizing a new host. In some bacteria, adhesins also function as invasins, which are a class of proteins associated with the penetration of bacteria into host cells. Invasins play a role in promoting entry during the initial stage of bacterial infection. Examples of the adhesin expressed in the recombinant bacteria of the present invention include fibronectin-binding protein and internalin.

[0030] Fibronectin is a multidomain glycoprotein found ubiquitously in human body fluids and extracellular matrices of a variety of human tissues and organs. After secretion, fibronectin molecules bind to transmembrane integrins, which facilitate dimerization and cytoskeletal coupling. The integrin-bound fibronectin is capable of binding to ECM components such as collagen and laminin. Human fibronectin plays a major role in the regulation of cell migration, tissue repair, and adhesion. Fibronectin is also a common target for bacterial adhesins in the gastrointestinal tract. Fibronectin-binding proteins (FnBPs) have been identified in a wide variety of host-associated bacteria. For example, fibronectin-binding protein A (FnBPA) and FnBPB function as adhesins and/or invasins for *Staphylococcus aureus* infection.

[0031] Internalins are surface proteins found on *Listeria monocytogenes*. They exist in two known forms, InlA and InlB. They are used by the bacteria to invade mammalian cells via cadherins transmembrane proteins and Met receptors respectively. In cultured cells, InlA is necessary to facilitate Listeria entry into human epithelial cells, while InlB is necessary for Listeria internalisation in several other cell types, including hepatocytes, fibroblasts, and epithelioid cells. The term “non-replicating plasmid vector” as used herein refers to a plasmid vector that cannot replicate in the mammalian host cells after being delivered to the mammalian host cells. The inventors of the present invention found that use of non-replicating plasmid vectors allows improved control of a treatment regime that involves the delivery of the plasmid vectors into the mammalian host cells. For example, any adverse reaction due to the delivery of the plasmid vectors can be controlled or terminated readily upon discontinuation of the treatment. This is because mammalian host cells infected with the recombinant bacteria of the present invention will not be able to pass on the plasmid vector to their daughter cells, hence any adverse effect will be restricted to the primary cells that have been infected, which will stop naturally at the end of the life-span of the infected cells. The use of non-replicating plasmid vectors also allows fine tuning of the dosage (i.e. the expression of the genes contained in the plasmid vectors) over time to achieve optimal clinical efficacy, which may vary among different patients. The non-replicating plasmid vectors are generally constructed with the bacterial origin of replication (ori), with no other factors such as EBV or SV40 ori and their corresponding antigen components that can facilitating replication in mammalian cells. As such, the resulting construct(s) are able to

replicate in the recombinant bacterium as disclosed herein, but are not able to replicate in the mammalian host cells.

[0032] Expressing adhesin(s) in bacteria allows the bacteria to infect its host cells in the gastrointestinal tract of a host organism, when the bacteria have been administered to the gastrointestinal tract of the host organism. Since probiotic lactic acid bacteria are generally recognized as safe bacteria, the inventors of the present invention postulate that probiotic lactic acid bacteria that express adhesin(s) can be used to deliver genetic materials to the cells of the host organism, in particular the cells in the gastrointestinal tract of the host organism, after the cells in the gastrointestinal tract of the host organism are infected by the bacteria.

[0033] It is preferred that the genetic material being delivered to the cells of the host organism is beneficial to the host organism. For example, when the cells of the host organism being infected by the bacteria are tumor cells, and the genetic material being delivered to the cells encodes for tumor suppressor proteins, then the bacteria can be used for treating tumor in the host organism. In preferred embodiments, the genetic material being delivered to the cells only suppress the growth of the tumor cells but not the growth of the healthy non-tumor cells.

[0034] The term “tumor suppressor gene” or its grammatical variants as used herein refers to a type of gene that makes a protein called a tumor suppressor protein that helps control cell growth. Mutations in tumor suppressor genes may lead to cancer.

[0035] The term “anti-inflammatory gene” or its grammatical variants as used herein refers to a type of gene that makes a protein that helps to prevent inflammation or reduce the symptoms of inflammation.

[0036] In some examples, the tumor suppressor gene or anti-inflammatory gene has tumor-suppressing or anti-inflammatory effects on cells of the gastrointestinal tract.

Examples of tumor suppressor genes include *TP53*, *APC*, *CD95*, *PTEN*, *TRAIL*, *MDA7*, and *PMAIP1*. Examples of anti-inflammatory genes include *IL10*, *IL11*, *IL13*, *IL1-ra*, and *TGFb*. In some examples, anti-inflammatory genes being delivered to the host cells can induce the expression of other anti-inflammatory genes, or suppress the expression of pro-inflammatory genes. Examples of pro-inflammatory genes include *IL1b*, *IL6*, *IL18* and *TNFα*.

[0037] In one specific example, the tumor suppressor gene or anti-inflammatory gene being delivered to the host organism is a *DUSP10* gene. *DUSP10* is a protein coding gene that encodes for DUSP10 (Dual Specificity Phosphatase 10), also known as MAP kinase

phosphatase 5 (MKP5). An exemplary sequence of *DUSP10* gene is set out in SEQ ID NO: 1, and an exemplary sequence of DUSP10 protein is set out in SEQ ID NO: 10.

[0038] Dual specificity protein phosphatases inactivate their target kinases by dephosphorylating both the phosphoserine/threonine and phosphotyrosine residues. They negatively regulate members of the MAP kinase superfamily, which is associated with cellular proliferation and differentiation. Different members of this family of dual specificity phosphatases show distinct substrate specificities for MAP kinases, different tissue distribution and subcellular localization, and different modes of expression induction by extracellular stimuli.

10 [0039] DUSP10 targets the mitogen-activated protein kinases (MAPK, including ERK 1/2), which is downstream of the highly represented EGFR-KRAS-BRAF-MEK-ERK1/2 pathway in colorectal cancer. Multiple gene mutations within this pathway that result in increased ERK1/2 activity are characteristic of colorectal cancer. Since DUSP10 targets the downstream effector ERK1/2, its therapeutic effect is less likely to be affected by the mutations in the upstream effectors of the EGFR-KRAS-BRAF-MEK-ERK1/2 pathway. In addition, inflammatory status/activity of the infected cells could also be controlled, since DUSP10 is a negative regulator of the MAPKs pathway, which is a major intermediate factor for the development of immune responses.

20 [0040] The genetic material being delivered to the cells of the host organism would result in the expression of the gene(s) of interest in the genetic material within the cells of the gastrointestinal tract of the host organism. This means the gene(s) of interest would preferably not be expressed or not be significantly expressed in the bacteria of the present invention in the extracellular/luminal compartment of the gastrointestinal tract of the host organism. Rather, the bacteria are merely operating as a vehicle that delivers the genetic material to the cells of the host organism. The advantage is that the gene product(s), such as the tumor suppressor and/or the anti-inflammatory protein, will be expressed directly in the cells in which the tumor suppressing and/or anti-inflammatory effect is to be exerted. To order to achieve this, gene(s) of interest in the genetic material being delivered should be regulated by a promoter that drives the expression of the gene(s) of interest in the eukaryotic cells, and preferably a promoter that does not drive the expression of the gene(s) of interest in prokaryotic cells such as bacterial cells. As used herein, the term “eukaryote” or “eukaryotic” refers to organisms or cells or tissues derived therefrom belonging to the

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phylogenetic domain Eukarya such as animals (e.g. mammals, insects, reptiles, and birds), ciliates, plants (e.g. monocots, dicots, and algae), fungi, yeasts, flagellates, microsporidia, and protists. The term “prokaryote”, “prokaryotic cell” or “non-eukaryotic organism” refers to organisms including, but not limited to, organisms of the Archaea domain and the Bacteria domain.

[0041] In some preferred examples, the cells of the host organism, in particular mammalian organism, are only transiently transfected by the genetic materials being delivered by the recombinant bacterium. Thus, the first promoter that directs expression of the *DUSP10* gene can be a promoter from mammalian viruses. Examples of such promoters include (i) the polyhedrin promoter from baculovirus (PH promoter), (ii) the enhancer and immediate early promoter from cytomegalovirus (CMV promoter), (iii) the early promoter from simian vacuolating virus 40 (SV40 promoter), (iv) the thymidine kinase promoter from HSV1 (TK promoter), and (v) the 5'LTR promoter from HIV (LTR promoter). Sequences of these exemplary promoters are set out in SEQ ID NOs: 2-6 in **Table 1**. Thus, in some examples, the first promoter that directs expression of the *DUSP10* gene in a mammalian cell is a PH promoter, a CMV promoter, a SV40 promoter, a TK promoter or a LTR promoter.

| SEQ ID NO: | Promoter     | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|------------|--------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2          | PH promoter  | ATCATGGAGATAATTAATAATGATAACCATCTCGCAAATAAA<br>TAAGTATTTTACTGTTTTTCGTAACAGTTTTGTAATAAAAAAA<br>CCTATAAATATTCCGGATTATTCATACCGTCCCACCATCGGG<br>CGCG                                                                                                                                                                                                                                                                                                                 |
| 3          | CMV promoter | TAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATAT<br>GGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTG<br>GCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATG<br>ACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTG<br>ACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGG<br>CAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATT<br>GACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCA<br>GTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA<br>CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCA<br>GTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGAT |

|   |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|---|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|   |                  | TTCCAAGTCTCCACCCCATGACGTCAATGGGAGTTTGT<br>GGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAAC<br>TCCGCCCCATTGACGCAAATGGGCGGTAGGCGGTACGGTG<br>GGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGTCAG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| 4 | SV40<br>promoter | CTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGG<br>CTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATT<br>AGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCA<br>GGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAAC<br>CATAGTCCCGCCCCCTAACTCCGCCCATCCCGCCCCTAACTCC<br>GCCCAGTTCCGCCCATTCTCCGCCCATGGCTGACTAATTTT<br>TTTTATTTATGCAGAGGCCGAGGCCGCTCTGCCTCTGAGCT<br>ATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCT<br>TTTGCAAAAAGCTC                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| 5 | TK<br>promoter   | AAATGAGTCTTCGGACCTCGCGGGGGCCGCTTAAGCGGTGG<br>TTAGGGTTTGTCTGACGCGGGGGGAGGGGGAAGGAACGAA<br>ACACTCTCATTCGGAGGCGGCTCGGGGTTTGGTCTTGGTGG<br>CCACGGGCACGCAGAAGAGCGCCGCGATCCTCTTAAGCACC<br>CCCCCGCCCTCCGTGGAGGCGGGGGTTTGGTCGGCGGGTGG<br>TAACTGGCGGGCCGCTGACTCGGGCGGGTCGCGCGCCCCAG<br>AGTGTGACCTTTTCGGTCTGCTCGCAGACCCCCGGGCGGCG<br>CCGCCGCGGCGGCGACGGGCTCGCTGGGTCCTAGGCTCCAT<br>GGGGACCGTATACGTGGACAGGCTCTGGAGCATCCGCACG<br>ACTGCGGTGATATTACCGGAGACCTTCTGCGGGACGAGCCG<br>GGTCACGCGGCTGACGCGGAGCGTCCGTTGGGCGACAAAC<br>ACCAGGACGGGGCACAGGTACACTATCTTGTACCCGGAGG<br>CGCGAGGGACTGCAGGAGCTTCAGGGAGTGGCGCAGCTGC<br>TTCATCCCCGTGGCCCGTTGCTCGCGTTTGCTGGCGGTGTCC<br>CCGGAAGAAATATATTTGCATGTCTTTAGTTCTATGATGAC<br>ACAAACCCCGCCAGCGTCTTGTGATTGGCGAATTCGAACA<br>CGCAGATGCAGTCGGGGCGGCGCGGTCCCAGGTCCACTTCG<br>CATATTAAGGTGACGCGTGTGGCCTCGAACACCGAGCGACC<br>CTGCAGCGACCCGCTTAA |

|   |                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|---|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 6 | LTR<br>promoter | TGGAAGGGCTAATTCACTCCCAACGAAGACAAGATATCCTT<br>GATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATTA<br>GCAGAACTACACACCAGGGCCAGGGATCAGATATCCACTG<br>ACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGA<br>GAAGTTAGAAGAAGCCAACAAAGGAGAGAACACCAGCTTG<br>TTACACCCTGTGAGCCTGCATGGAATGGATGACCCGGAGAG<br>AGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTC<br>ATCACATGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAAC<br>TGCTGACATCGAGCTTGCTACAAGGGACTTTCCGCTGGGGA<br>CTTCCAGGGAGGCGTGCCCTGGGCGGGACTGGGGAGTGG<br>CGAGCCCTCAGATCCTGCATATAAGCAGCTGCTTTTTGCCTG<br>TACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGC<br>TCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAA<br>AGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTG<br>TGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCA<br>GTGTGGAAAATCTCTAGC |
|---|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**Table 1.** Exemplary sequences of viral promoters

[0042] A plasmid vector comprising a tumor suppressor gene or anti-inflammatory gene is inserted into the probiotic lactic acid bacterium in order for the bacterium to deliver the gene to the cells of the host organism of the bacterium. In order for the tumor suppressor gene or anti-inflammatory gene to be expressed and hence exert its effect on the cells or the host organism, the tumor suppressor gene or anti-inflammatory gene is operably linked to the first promoter that directs expression of the gene in a host cell, in particular a mammalian cell.

[0043] The term “operably link” or its grammatically variants as used herein means that a gene of interest, a linker (if any), and a promoter are joined as part of the same nucleic acid molecule, suitably positioned and oriented for transcription of the gene of interest to be initiated from the promoter. In some examples, it means the tumor suppressor gene or anti-inflammatory gene, the first promoter, and optionally a linker, are joined as part of the plasmid vector, suitably positioned and oriented for transcription of the gene to be initiated from the first promoter.

[0044] The term “vector” as used herein refers to a DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated and/or expressed. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Expression vectors for use in mammalian cells ordinarily include an origin of replication, a promoter located in front of the gene to be expressed, along with any necessary ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional terminator sequences.

[0045] The term “plasmid” as used herein refers to a small DNA molecule within a cell that is physically separated from a chromosomal DNA and can replicate independently. They are most commonly found as small circular, double-stranded DNA molecules in bacteria. In nature, plasmids often carry genes that may benefit the survival of the organism, for example antibiotic resistance. While the chromosomes are big and contain all the essential genetic information for living under normal conditions, plasmids usually are very small and contain only additional genes that may be useful to the organism under certain situations or particular conditions.

[0046] Specific initiation signals may also be required for efficient translation of exogenous nucleic acid coding sequences. These signals include the ATG initiation codon and adjacent sequences. Exogenous translational control signals, including the ATG initiation codon, may additionally need to be provided in the vector. One of ordinary skill in the art would readily be capable of determining this need and providing the necessary signals. It is well known that the initiation codon must be in-frame (or in-phase) with the reading frame of the desired coding sequence to ensure translation of the entire insert. Exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements or transcription terminators. In eukaryotic expression, an appropriate polyadenylation site (e.g., 5'-AATAAA-3') can also be incorporated into the transcriptional unit if not contained within the original cloned segment. Typically, the poly A addition site is placed about 30 to 2000 nucleotides downstream of the termination site of the protein at a position prior to transcription termination.

[0047] The adhesin in the recombinant bacterium can be expressed from a plasmid vector comprising an adhesin gene, or a gene encoding for an adhesion, wherein adhesin gene and a gene encoding for an adhesion can be used interchangeable in the present disclosure. Thus, in

some examples, the recombinant bacterium as disclosed in comprises a plasmid vector comprising an adhesin gene operably linked to a second promoter that directs expression of the adhesin gene in the bacterium. In another example, the recombinant bacterium as disclosed in comprises a plasmid vector comprising an adhesin gene operably linked to a second promoter that directs expression of a gene encoding for an adhesin in the bacterium. Fibronectin-binding protein is an example of adhesins. Non-limiting examples of fibronectin-binding protein include fibronectin-binding protein A (FnBPA) (SEQ ID NO: 12) and fibronectin-binding protein B (FnBPB) (SEQ ID NO: 13), wherein FnBPA and FnBPB had similar functions in terms of their binding ability to fibronectin. An exemplary sequence of the fibronectin-binding protein A gene is set out in SEQ ID NO: 7, and an exemplary sequence of the fibronectin-binding protein B gene is set out in SEQ ID NO: 11. In some preferred examples, the second promoter does not direct expression of the adhesin gene in a host cell, in particular a mammalian host cell. For example, the second promoter can be a prokaryotic promoter. A prokaryotic promoter typically comprises two short sequences at -10 and -35 positions upstream from the transcription start site. The sequence at -10 is called the Pribnow box, or the -10 element, and usually consists of the six nucleotides TATAAT. The Pribnow box is essential to start transcription in prokaryotes. The other sequence at -35 is called the -35 element, and usually comprises the six nucleotides TTGACA. In one specific example, the prokaryotic promoter used to drive the expression of the adhesin gene is the erm promoter. An exemplary sequence of the erm promoter is set out in SEQ ID NO: 8. In some examples, inducible promoter is used. An example of an inducible promoter that can be used in *Lactococcus* is the nisin promoter. An exemplary sequence of the nisin promoter is set out in SEQ ID NO: 9.

[0048] The plasmid vector comprising the tumor suppressor gene or anti-inflammatory gene can be the same plasmid vector as the plasmid vector comprising the adhesin gene, provided that expressions of the tumor suppressor gene or anti-inflammatory gene and the adhesin gene are directed by different promoters, such that the tumor suppressor gene or anti-inflammatory gene will only be expressed in the host cells after the host cells have been infected by the bacterium, and that the adhesin gene will be expressed in the bacterium. It is preferred that the host cells are cells of a mammalian organism. Thus, in some examples, the recombinant bacterium comprises a plasmid vector comprising a tumor suppressor gene or anti-inflammatory gene operably linked to a first promoter that directs expression of the

tumor suppressor gene or anti-inflammatory gene in a mammalian cell, and an adhesin gene operably linked to a second promoter that directs expression of the adhesin gene in the bacterium. In another example, the recombinant bacterium comprises a plasmid vector comprising a tumor suppressor gene or anti-inflammatory gene operably linked to a first promoter that directs expression of the tumor suppressor gene or anti-inflammatory gene in a mammalian cell, and an adhesin gene operably linked to a second promoter that directs expression of a gene encoding for an adhesin in the bacterium.

[0049] The plasmid vector comprising the tumor suppressor gene or anti-inflammatory gene can be a different plasmid vector as the plasmid vector comprising the adhesin gene or a gene encoding for an adhesin, provided that expressions of the tumor suppressor gene or anti-inflammatory gene and the adhesin gene are directed by different promoters, such that the tumor suppressor gene or anti-inflammatory gene will only be expressed in the host cells after the host cells have been infected by the bacterium, and that the adhesin gene or gene encoding for an adhesin will be expressed in the bacterium. It is preferred that the host cells are cells of a mammalian organism. Thus, in some examples, the recombinant bacterium comprises a first plasmid vector comprising a tumor suppressor gene or anti-inflammatory gene operably linked to a first promoter that directs expression of the tumor suppressor gene or anti-inflammatory gene in a mammalian cell, and a second plasmid vector comprising a adhesin gene operably linked to a second promoter that directs expression of the adhesin gene in the bacterium. In another example, the recombinant bacterium comprises a first plasmid vector comprising a tumor suppressor gene or anti-inflammatory gene operably linked to a first promoter that directs expression of the tumor suppressor gene or anti-inflammatory gene in a mammalian cell, and a second plasmid vector comprising a gene encoding for an adhesin operably linked to a second promoter that directs expression of the gene encoding for an adhesin in the bacterium.

[0050] It is to be noted that the terms “first” and “second” as used do not indicate or imply the relative positions or significance of the promoters and/or the plasmid vectors. When a plasmid vector comprises both the first promoter and the second promoter, it is understood that the first promoter and the second promoter are different, in order for the two promoters to direct the expression of the adhesin gene or the gene encoding for an adhesin, and the tumor suppressor gene or anti-inflammatory gene respectively. Similarly, when the recombinant bacterium comprises both the first plasmid vector and the second plasmid

vector, it is understood that the first plasmid vector and the second plasmid vector are different, and they contain the expression cassettes for the adhesin gene or the gene encoding for an adhesin, and the tumor suppressor gene or anti-inflammatory gene respectively.

[0051] The recombinant bacterium of the present invention can be constructed using  
5 methods known in the art, which generally involve the construction of the plasmid vector(s), the transformation of the resultant plasmid vector(s) into the wild-type probiotic lactic acid bacteria, the identification and/or isolation of the bacteria that have been successfully transformed.

[0052] The construction of the plasmid vector(s) can be done by modifying a  
10 commercially available vector backbone that can be replicated in probiotic lactic acid bacteria, in particular lactococcal vector backbones. Examples of such vector backbone include, pTRKH3-ermGFP (Plasmid #27169 from addgene), pCD4, pHP003, pSRQ700, pSRQ800, pSRQ900, pK214, pAH90, pAH33, pAH82, pCIS3, pIL105, pWV01, pCI305, pCRL1127, pMN5, pBL1, pDR1-1B, pDR1-1, pS7b, pMRC01, pCRL291.1, pNZ4000,  
15 pBM02, pCL2.1, pWV02 (reviewed in FEMS Microbiology Review (2006) 30:243), pNZ8008, pNZ8148, pNZ8149, pNZ8150, pNZ9530 and derivatives of pSH71. The vector backbone will be digested using a restriction enzyme to linearize the vector backbone and to create sites for DNA ligation. Each of the gene(s) of interest, in this case the adhesin gene and/or the tumor suppressor gene or anti-inflammatory gene, is ligated to the linearized  
20 vector backbone to form the plasmid vector(s) to be transformed into the bacterial cells.

[0053] Transformation of the plasmid vector(s) into the bacterial cells can be carried out using methods known in the art. Such methods generally involve making the bacterial cells passively permeable to DNA by exposing them to conditions that do not normally occur in nature. Commonly used methods include electroporation method and heat-shock method. In  
25 an electroporation method, the bacterial cells are typically briefly shocked with an electric field of 10-20 kV/cm, which is thought to create holes in the cell membrane through which the plasmid vector(s) may enter. After the electric shock, the holes are rapidly closed by the cells' membrane-repair mechanisms. In a heat-shock method, the cells are incubated in a solution containing divalent cations under cold conditions, before being exposed to a heat  
30 pulse (heat shock). The cations partially disrupt the cell membrane, which allows the DNA to enter the host cell. It is suggested that exposing the cells to divalent cations in cold condition may also change or weaken the cell surface structure, making it more permeable to DNA.

The heat-pulse is thought to create a thermal imbalance across the cell membrane, which forces the DNA to enter the cells through either cell pores or the damaged cell wall.

[0054] The vector backbones that are commercially available for use in molecular cloning typically contain one or more antibiotic resistance gene. After the transformation steps have  
5 been carried out, the resultant bacteria will be placed on an antibiotic-containing culture plate to select for the cells that have been successfully transformed. Since bacterial cells that have not been transformed successfully would not have acquired the antibiotic resistance gene, only those that have been successfully transformed would survive on the plate and form colonies. Each colony contains a cluster of identical plasmid-containing bacterial. Several  
10 colonies will usually be selected, and each of them will be checked to identify the colon(ies) containing the desired plasmid vector(s), by polymerase chain reaction (PCR) sequencing and/or restriction digest. A colony of bacteria identified to contain the desired plasmid vector(s) can then be grown in bulk.

[0055] The recombinant bacteria of the present invention can deliver exogenous tumor  
15 suppressor gene or anti-inflammatory gene to eukaryote cells *in vivo* or *in vitro*. The delivery requires contact and internalization of the recombinant bacteria by the target cells. Internalization can occur through one or more different mechanisms. The contacting between the recombinant bacteria and target cells can be induced to occur *in vivo* or *in vitro*. Typically, the contacting occurs *in vivo*.

[0056] Since increased level of expression of tumor suppressor gene or anti-inflammatory  
20 gene can suppress cancer cell proliferation and/or reduce inflammation, the recombinant bacterium of the present invention can be used for the treatment of cancer and/or inflammation. Thus, in one aspect, there is provided a method of treating cancer or reducing inflammation in a subject, wherein the method comprises administering a pharmaceutically  
25 effective amount of the recombinant, probiotic lactic acid bacterium of the present invention into the gastrointestinal tract of the subject, and wherein the cancer or inflammation is a cancer or inflammation of the gastrointestinal tract.

[0057] In some examples, there are provided the recombinant, probiotic lactic acid  
bacterium of the present invention for use in therapy. In some other examples, there are  
30 provided the recombinant, probiotic lactic acid bacterium of the present invention for use in treating cancer or reducing inflammation in a subject, wherein the cancer or inflammation is a cancer or inflammation of the gastrointestinal tract. In some other examples, there are

provided use of the recombinant, probiotic lactic acid bacterium of the present invention in the manufacture of a medicament for treating cancer or reducing inflammation in a subject, wherein the cancer or inflammation is a cancer or inflammation of the gastrointestinal tract.

[0058] In some examples, the method of treating cancer or reducing inflammation in a subject can further comprise administering a pharmaceutically effective amount of immune checkpoint inhibitor. The administration of a pharmaceutically effective amount of immune checkpoint inhibitor can be administered either together or separately with the recombinant bacterium of the present invention. The term “immune checkpoint inhibitor” refers to a type of drug that blocks or inhibits checkpoint proteins. Checkpoint proteins are regulators of the immune system, and they keep immune responses from being too strong, which can sometimes prevent immune cells from killing cancer cells. When these checkpoint proteins are blocked, immune cells can target and/or eradicate cancer cells more effectively. Checkpoint proteins can be produced by cancer cells, or immune cells such as B-cells or T-cells. In some examples, the immune checkpoint inhibitor can be, but not limited to, inhibitors of programmed death ligand 1 (PDL-1), programmed death 1 (PD-1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4).

[0059] In some examples, there are provided the recombinant, probiotic lactic acid bacterium of the present invention and an immune checkpoint inhibitor for use in therapy. In some other examples, there are provided the recombinant, probiotic lactic acid bacterium of the present invention and an immune checkpoint inhibitor for use in treating cancer or reducing inflammation in a subject, wherein the cancer or inflammation is a cancer or inflammation of the gastrointestinal tract. In some other examples, there are provided use of the recombinant, probiotic lactic acid bacterium of the present invention and an immune checkpoint inhibitor in the manufacture of a medicament for treating cancer or reducing inflammation in a subject, wherein the cancer or inflammation is a cancer or inflammation of the gastrointestinal tract.

[0060] The inventors also found that mice that have been treated with recombinant bacteria expressing Fnb without any tumor suppressor genes also showed reduction of tumour load. Thus, in one aspect, there is provided a method of treating cancer in a subject, wherein the method comprises administering a pharmaceutically effective amount of a recombinant, probiotic lactic acid bacterium, wherein the bacterium comprises a non-replicating plasmid vector comprising an adhesin gene operably linked to a promoter that directs expression of

the adhesin gene in the bacterium. In one example, there is provided a method of treating cancer in a subject, wherein the method comprises administering a pharmaceutically effective amount of a recombinant, probiotic lactic acid bacterium, wherein the bacterium comprises a non-replicating plasmid vector comprising a gene encoding for an adhesin operably linked to a promoter that directs expression of the gene encoding for an adhesin in the bacterium.

[0061] The terms “treating” or its grammatical variants means to administer a composition to a subject or a system with an undesired condition or disease (e.g. cancer). The effect of the administration of the composition to the subject can be, but is not limited to, the cessation of a particular symptom of a condition, a reduction or prevention of the symptoms of a condition, a reduction in the severity of the condition, the complete ablation of the condition, a stabilization or delay of the development or progression of a particular event or characteristic, or minimization of the chances that a particular event or characteristic will occur.

[0062] The term “reduce” or “reduction” or grammatical variants thereof refer to a decrease in the specified parameter as compared to the same parameter of a reference. For example, in the context of “reducing inflammation”, “reducing” refers to a decrease in the severity of inflammation, when compared to the severity of an untreated inflammation. In some examples, the severity of inflammation is reduced by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 95% as compared to an untreated inflammation.

[0063] The term “inflammation” as used herein refers to a process by which the body's white blood cells and substances they produce protect the body from infection with foreign organisms, such as bacteria and viruses. The severity of inflammation can be measured by methods known in the art, such as blood tests which measures the level of fasting insulin, Hemoglobin A1C (HbA1c), C-Reactive Protein (CRP), serum ferritin and Red Blood Cell Width (RDW).

[0064] As used herein the term “pharmaceutically effective amount”, “effective amount” or “therapeutically effective amount” means a dosage sufficient to treat, inhibit, or alleviate one or more symptoms of a disease state being treated or to otherwise provide a desired pharmacologic and/or physiologic effect. The precise dosage will vary according to a variety of factors such as subject-dependent variables (e.g., age, immune system health, etc.), the disease, and the treatment being administered. The effect of the pharmaceutically effective

amount can be relative to a control. Such controls are known in the art, and can be, for example the condition of the subject prior to or in the absence of administration of the drug, or drug combination, or in the case of drug combinations, the effect of the combination can be compared to the effect of administration of only one of the drugs. The pharmaceutically effective amount is determinable by means known in the art without undue experimentation, given the teachings contained herein. In some examples, the amount of bacteria should be sufficient to exert a tumor suppressing effect on the subject while still being avirulent to the subject. In some other example, the amount of bacteria should be sufficient to reduce inflammation in the subject while still being avirulent to the subject. The term "avirulent" as used herein refers to previously virulent organism that has been attenuated to a sufficient degree such that the administration of the attenuated organism to the animal host would not cause any detectable or measurable disease. Generally, such avirulence can be shown by a decrease in the LD50, the numbers of colonized organs, or the number of CFUs by a factor of 10, or a factor of 100, or by a factor of 1000 or more.

[0065] The pharmaceutically effective amount will depend upon the particular subject, the bacterial species, the type of cancer/inflammation involved, and the stage and/or severity of the cancer/inflammation. The dose and/or the dose frequency will also vary according to the age, body weight, and response of the individual subject. In general, the total daily dose range is from about  $10^5$  to  $10^{11}$  CFU (colony-forming unit) bacteria per day, or about  $10^8$  to  $5 \times 10^{10}$  CFU bacteria per day, or about  $2 \times 10^9$  CFU bacteria per day in powder form or  $9 \times 10^8$  to  $1 \times 10^{10}$  CFU bacteria per day in liquid preparations, administered in single or divided doses. The length of time for a course of treatment should be at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 7 weeks, at least 10 weeks, at least 13 weeks, at least 15 weeks, at least 20 weeks, at least 6 months, or at least 1 year. It may be necessary to use dosages outside these ranges in some cases as will be apparent to those skilled in the art. In certain examples, the recombinant bacteria can be administered for a period of time until the symptoms are under control, or when the disease has regressed partially or completely. Further, it is noted that the clinician or treating physician will know how and when to interrupt, adjust, or terminate use of the recombinant bacteria as a medicament in conjunction with individual patient response.

[0066] The term "CFU" as used herein refers to colony-forming unit, which is a unit used to estimate the number of viable bacteria or fungal cells in a sample. Viable is defined as the

ability to multiply via binary fission under the controlled conditions. Counting with colony-forming units requires culturing the microbes and counts only viable cells, in contrast with microscopic examination which counts all cells, living or dead. In some examples, one CFU contains approximately  $10^5$  -  $10^7$  cells.

5 [0067] Depending on the subject, the therapeutic benefits for cancer patients range from inhibiting or retarding the growth of the cancer and/or the spread of the cancer to other parts of the body (i.e., metastasis), palliating the symptoms of the cancer, improving the probability of survival of the subject with the cancer, prolonging the life expectancy of the subject, improving the quality of life of the subject, and/or reducing the probability of relapse after a  
10 successful course of treatment (e.g., surgery, chemotherapy or radiation). The effect of the bacteria of the invention on development and progression of cancer can be monitored by any methods known to one skilled in the art, including but not limited to measuring: a) changes in the size and morphology of the tumor using imaging techniques such as a computed tomographic (CT) scan or a sonogram; and b) changes in levels of biological markers of risk  
15 for cancer.

[0068] The term “Inhibit” or its grammatical variants as used herein means hinder or restrain a particular characteristic. It is understood that this is typically in relation to some standard or expected value, i.e., it is relative, but that it is not always necessary for the standard or relative value to be referred to. For example, “inhibits expression” means  
20 hindering, interfering with or restraining the expression or activity of a gene relative to a standard or a control. “Inhibits activity” can also mean to hinder or restrain the synthesis, expression or function of the gene product, such as a protein, relative to a standard or control.

[0069] The term “gastrointestinal tract” (or “GI tract” in short) as used herein refers to the organ system in animals, in particular mammalian animals, which takes in food, digests it to  
25 extract and absorb energy and nutrients, and expels the remaining waste. The GI tract consists of the oral cavity, esophagus, stomach, small intestine, large intestine, caecum, appendix, rectum, anus, liver and gall bladder.

[0070] Cancer of the GI tract refers to cancers that affect any part of the GI tract. Examples of cancer of the gastrointestinal tract include esophageal cancer, gastric cancer,  
30 colorectal cancer, pancreatic cancer, liver cancer, gallbladder & biliary tract cancer, and anal cancer. In one specific example, the cancer is colorectal cancer.

[0071] Colorectal cancer is cancer of the colon and rectum (or the large intestine), which is the last part of the gastrointestinal tract. When food enters the colon, water is absorbed and the food residue is converted into waste (faeces) by bacteria. The rectum is the terminal part of the colon that stores faeces before it is expelled through the anus. Polyps may form on the inner wall of the colon and rectum. These are benign lumps which are fairly common in people above the age of 50. However, certain types of polyps may develop into cancer. Colorectal cancer often has no symptoms at an early stage. The following signs may be indicated of colorectal cancer: blood in stools, change in bowel habits, abdominal pain or discomfort, anemia, and presence of a lump in the abdomen. Regular screening can detect polyps or colorectal cancer early. The following tests are commonly used, alone or in combination, to detect early stages of colorectal cancer: Faecal Immunochemical Test (FIT), colonoscopy, and flexible sigmoidoscopy. FIT is a preliminary test that detects the presence of small amounts of blood in faeces. Colonoscopy involves the examination of the colon and rectum using a flexible fibre-optic instrument introduced through the anus under sedation. In addition to its diagnostic use, a colonoscopy can be used for treatment, such as removing polyps, biopsying cancerous lumps, and staunching bleeding spots. Flexible sigmoidoscopy examines the internal lining of the lower end of the large intestine. A short, flexible, lighted tube is inserted into the rectum and slowly guided into the sigmoid colon.

[0072] The subject to be administered with the recombinant bacteria of the present invention can be any vertebrate, preferably a mammal, including domestic animals, sport animals, and primates, including humans.

[0073] The recombinant bacteria of the present invention can be administered to the gastrointestinal tract of the subject via any conventional routes of administration, including but not limited to, oral administration, administration by gastric feeding tube, administration by duodenal feeding tube, and rectal administration.

[0074] In some examples, oral administration is the preferred route of administration. The recombinant bacteria of the present invention can be administered orally alone. The recombinant bacteria can also be manufactured as an oral composition. Any dosage form may be employed for providing the subject with an effective dosage of the oral composition. Dosage forms include tablets, capsules, dispersions, suspensions, solutions, and the like. In some examples, compositions suitable for oral administration may be presented as discrete units such as capsules, cachets, or tablets, each containing a predetermined amount of the

recombinant bacteria of the present invention, as a powder or granules or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion, or a water-in-oil liquid emulsion. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation.

[0075] The oral compositions may additionally include binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); binders or fillers (e.g., lactose, pentosan, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets or capsules can be coated by methods well known in the art. The oral compositions may further contain additional ingredients, including but not limited to pharmaceutically acceptable carriers or excipients, vitamins, herbs (including traditional Chinese medicine products), herbal extracts, minerals, amino acids, flavoring agents, coloring agents, and/or preservatives.

The recombinant bacteria can also be added to food which will be consumed by the subject. As known to those skilled in the relevant art, many methods may be used to mix the recombinant bacteria of the invention with food while the bacterial cells remain viable. In some examples, a culture broth comprising bacterial cells is added directly to food just prior to consumption. Dried powders of the bacterial cells can also be reconstituted and added directly to food just prior to consumption. Preferably, the recombinant bacteria of the invention are made and stored under conditions, such as temperature, from about 0°C to 4° C. As used herein, the term “food” broadly refers to any kind of material, liquid or solid, that is used for nourishing an animal, and for sustaining normal or accelerated growth of an animal including humans. Many types of food products or beverages, such as but not limited to, fruit juice, herbal extracts, tea-based beverages, dairy products, soybean product, and rice products, can be used to form nutritional compositions comprising the recombinant bacteria of the invention.

[0076] Since the recombinant bacteria of the present invention are administered orally, the assistance of health professionals in administration of the bacteria is generally not essential.

[0077] In certain subjects, oral administration of the recombinant bacteria of the present invention may not be possible. In such circumstances, alternative administration routes such as administration by gastric feeding tube, administration by duodenal feeding tube, and rectal

administration may be used. When such alternative administration routes are being used, it is preferred that the recombinant bacteria of the present invention be in the form of a liquid preparation.

[0078] Liquid preparations can take the form of solutions or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. The temperature of the liquid used to reconstitute the dried product should in general be less than 65° C. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The recombinant bacteria of the present invention can be used in conjunction or in rotation with other types of treatment modalities, such as but not limited to surgery, chemotherapeutic agents, and radiation.

[0079] In preferred examples, the recombinant bacteria are live when being administered to the gastrointestinal tract of the subject. In some other examples, the recombinant bacteria are inactivated or dead when being administered to the gastrointestinal tract of the subject. The live recombinant bacteria can be either attenuated or un-attenuated. The term "attenuated" and its grammatical variants as used herein refers to the weakening or decreasing of the virulence of the wild-type organism. It follows that the term "un-attenuated" and its grammatical variants as used herein means that the virulence of the organism has not been weakened or decreased as compared to its wild-type organism. In some examples, when the bacteria of the *Lactococcus* genus are not virulent or harmful to its host organism, attenuation of the bacteria is not required.

[0080] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the

present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

5 [0081] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

10 [0082] Other embodiments are within the following claims and non- limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

15

## EXPERIMENTAL SECTION

[0083] The following examples illustrate methods by which aspects of the invention may be practiced or materials that may be prepared which is suitable for the practice of certain embodiments of the invention.

20 [0084] **Example 1 – High DUSP10 expression is associated with better overall survival in CRC patients**

[0085] Recently, an increasing number of studies have shown that dysregulation of DUSPs expression was associated with multiple cancers, and altered function of DUSPs was often reported in association with altered MAPK activities in the tumour. A detailed analysis of published CRC survival datasets from Marisa L *et al.* (PLoS Med. 2013. 10, e1001453) and The Cancer Genome Atlas (TCGA) (Nature 2012. 487, 330-337) was carried out to examine the plausible relationship between DUSP10 levels and CRC patients' survival. As shown in **Fig. 2**, relapse free survival (RFS) probability in patient cohort from Marisa L *et al.* trended marginally higher ( $P = 0.1$ ) for patients with tumour that had high DUSP10 expression (**Fig. 2A**, left panel). After sample stratification for ERK expression, it was found that high DUSP10 in tumours was associated with approximately 20% improvement in survival probability of patients with high ERK expression (**Fig. 2B**, right panel). Improvement in patient survival was more striking in the TCGA cohort (**Fig. 2B**). Survival of

patients with high DUSP10 expression was increased by approximately 20% for up to 60 months with or without ERK2 stratification. Together, these data suggest that DUSP10 expression may be functionally important for control of tumour development that resulted in better survival in the patients.

5 [0086] **Example 2 – DUSP10 overexpression in human CRC cells suppressed cancer cell growth and metastasis**

[0087] To confirm the tumour suppression function of DUSP10, human CRC cell lines Caco2 and DLD1 that stably overexpressing DUSP10 were generated for engraftment as tumour xenografts in immunocompromised mice. Results showed that DUSP10  
10 overexpression reduced tumour growth *in vivo* (**Fig. 3**). A key difference between the two cell lines is the presence of KRAS mutation (G13D) in DLD1, whereas wildtype KRAS is found in Caco2. This suggest that overexpression of DUSP10 may be effective for suppression of tumour cell growth regardless of the KRAS status. Interestingly, overexpression of DUSP10 in HCT116 human CRC cells harbouring Kras mutation (G13D)  
15 resulted in reduced metastasis to the liver (**Fig. 4**). In this experiment, HCT116 cells overexpressing DUSP10 or the control vector were injected into the spleen of immunocompromised NSGS mice for metastasis to occur over 3 weeks. There were approximately two-fold less macroscopic metastatic lesions of smaller size in the liver of mice injected with DUSP10 overexpressed HCT116 cells. KRAS is one of the most  
20 commonly mutated gene that results in resistance to drug therapy and subsequent increased ERK1/2 activation and cellular proliferation. Hence, the findings above demonstrated the potential of DUSP10 overexpression therapy for both KRAS wildtype and mutant tumours and possibility of reduction in metastatic disease progression.

[0088] **Example 3 – Development of fibronectin-binding protein expressing *L. lactis* for the delivery of DUSP10 expression plasmid (*L. Lactis*-Fnb-DUSP10)**  
25

[0089] DNA plasmid harbouring the gene insert for (1) *S. aureus* derived fibronectin-binding protein (Fnb) and (2) human DUSP10 was constructed by modifying the vector backbone, pTRKH3-ermGFP (Addgene) (**Fig. 5**). The resultant DNA plasmid was transformed into *Lactococcus lactis* (ATCC) by electroporation and selected based on  
30 erythromycin resistance. Single, positive clone was subsequently isolated and stored in 20% sterile glycerol until required for use. For all subsequent infection experiments, three different controls were implemented including untreated group, a group that was infected with *L. lactis*

harbouring the vector backbone (“Lactis-vect”) and *L. lactis* harbouring the vector backbone with Fnb gene insert only (Lactis-Fnb).

[0090] **Example 4 – Reduction in tumour cell growth *in vitro* and *in vivo* after treatment with *L. Lactis*-Fnb-DUSP10**

5 [0091] In order to determine the functionality of *L. Lactis*-Fnb-DUSP10 *in vitro*, Caco2 (human CRC cell line) was used for infection with *L. Lactis*-Fnb-DUSP10 or controls (MOI: 1500). As shown in **Fig. 6**, at 24 hours post infection, both mRNA and protein levels of DUSP10 were increased in Caco2 cells infected with *L. Lactis*-Fnb-DUSP10. Functionally, a 20% reduction in Caco2 proliferation was detected after 3 days of daily infection with *L.*  
 10 *Lactis*-Fnb-DUSP10 compared to control uninfected cells (**Fig. 6c**). More importantly, daily treatment through oral gavage of *L. Lactis*-Fnb-DUSP10 (dose =  $1.5 \times 10^9$  CFU bacteria) for 7 weeks had successfully suppressed intestinal tumour development in the AOM/DSS induced CRC mouse model (**Fig. 7**). Tumour load in mice given *L. Lactis*-Fnb-DUSP10 was reduced by ~70% and ~60% compared to untreated mice and mice treated with Lactis-vect.  
 15 Interestingly, mice given Lactis-Fnb also showed ~41% reduction of tumour load, which needs further examination (this may due to the slight induction of DUSP10 after Lactis-Fnb treatment – see **Fig. 6b**). Nevertheless, *L. Lactis*-Fnb-DUSP10 further enhanced the tumour suppression effect by another ~30%. In addition, overall tumour size was ~56% smaller in mice treated with *L. Lactis*-Fnb-DUSP10. The efficacy of *L. Lactis*-Fnb-DUSP10 was also  
 20 determined using a human CRC cell line transplant model. In this pilot experiment, 3x3 mm<sup>2</sup> human Ls174T tumour xenografts were transplanted and established in the caecum of immunocompromised mice for 2 weeks before treatment with *L. Lactis*-Fnb-DUSP10 by oral gavage. As shown in **Fig. 8**, the size of the transplanted Ls174T xenografts was smaller in mice treated with *L. Lactis*-Fnb-DUSP10 for 2 weeks. These *in vivo* results demonstrated that  
 25 *L. Lactis*-Fnb-DUSP10 therapy is highly promising with potential to be integrated with current CRC treatment and should be develop for clinical application.

[0092] **Example 5 – Reduction in colonic inflammation *in vivo* after treatment with *L. Lactis*-Fnb-DUSP10**

[0093] Mice were given 2% DSS (Dextran sulfate sodium) to induce colonic inflammation  
 30 before initiation of *L. lactis*-Fnb-DUSP10 treatment. Different treatments (*L. lactis* with vector control; *L. lactis*-Fnb and *L. lactis*-Fnb-DUSP10) were administered to the mice. As

shown in **Fig. 9**, treating with *L. lactis*-Fnb-DUSP10 has significantly reduced colonic inflammation as compared to the control groups.

[0094] **Example 6 – Reduction in tumour cell growth *in vitro* and *in vivo* after combination treatment with *L. Lactis*-Fnb-DUSP10 and anti-PDL-1**

- 5 [0095] In order to determine whether *L. Lactis*-Fnb-DUSP10 can be used in combination with immune checkpoint inhibitors to treat cancer, mice were treated with a combination of *L. Lactis*-Fnb-DUSP10 and anti-PDL-1. Ten-week old mice were given a single dose of 10mg/kg azoxymethane (AOM) (Sigma Aldrich; MO, USA) via intra-peritoneal injection to induce colon tumours. The mice were allowed to rest for one week before they were
- 10 subjected to 3 cycles of 1% DSS administration for five days with two weeks rest. After 16 weeks post AOM injection, the mice were subjected to the initiation of daily treatments with *L. Lactis* and/or anti-PDL-1 (*L. lactis* with vector control; *L. lactis*-Fnb-DUSP10, anti-PDL-1, anti-PDL-1 + *L. lactis*-vector and anti-PDL-1 + *Lactis*-Fnb-DUSP10) for 4 weeks before the mice were sacrificed and tumour load was determined. Tumour load in mice given anti-
- 15 PDL-1 and anti-PDL-1 + *L. lactis*-vector were reduced by ~68% and ~70% compared to untreated mice and mice treated with *L. lactis*-vector. Tumour load in mice treated with *L. Lactis*-Fnb-DUSP10 were reduced by ~83%. Interestingly, mice given anti-PDL-1 + *Lactis*-Fnb-DUSP10 showed the greatest effect, wherein there is ~90% reduction in tumour load (**Fig.10b**). This shows that the anti-tumour effects of *Lactis*-Fnb-DUSP10 can be further
- 20 enhanced when used in combination with an immune checkpoint inhibitor.

[0096] **Description of SEQ ID**

[0097] **Table 2** below details SEQ ID NOs referenced herein and their corresponding sequences that are not mentioned in Table 1. A brief description of the sequences is also provided.

| SEQ ID NO: | Description | Sequence                                                                                                                                                                                                                                                          |
|------------|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1          | DUSP10 gene | ATGCCTCCGTCTCCTTTAGACGACAGGGTAGTAGTGGCA<br>CTATCTAGGCCCGTCCGACCTCAGGATCTCAACCTTTGTT<br>TAGACTCTAGTTACCTTGGCTCTGCCAACCAGGCAGTAA<br>CAGCCACCCTCCTGTCATCGCCACCACCGTTGTGTCCCTC<br>AAGGCTGCGAATCTGACGTATATGCCCTCATCCAGCGGC<br>TCTGCCCGCTCGCTGAATTGTGGATGCAGCAGTGCCAGCT |

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|  | <p>GCTGCACTGTGGCAACCTACGACAAGGACAATCAGGCCC<br/>AAACCCAAGCCATTGCCGCTGGCACCACCACCACTGCCA<br/>TCGGAACCTCTACCACCTGCCCTGCTAACCAGATGGTCA<br/>ACAATAATGAGAATACAGGCTCTCTAAGTCCATCAAGTG<br/>GGGTGGGCAGCCCTGTGTCAGGGACCCCCAAGCAGCTAG<br/>CCAGCATCAAAATAATCTACCCCAATGACTTGGCCAAAGA<br/>AGATGACCAAATGCAGCAAGAGTCACCTGCCGAGTCAGG<br/>GCCCTGTCATCATTGACTGCAGGCCCTTCATGGAGTACAA<br/>CAAGAGTCACATCCAAGGAGCTGTCCACATTAAGTGTGC<br/>CGATAAGATCAGCCGGCGGAGACTGCAGCAGGGCAAGA<br/>TCACTGTCCTAGACTTGATTTCCTGTAGGGAAGGCAAGG<br/>ACTCTTTCAAGAGGATCTTTTCCAAAGAAATTA<br/>TAGTTTATGATGAGAATACCAATGAACCAAGCCGAGTGA<br/>TGCCCTCCCAGCCACTTCACATAGTCCTCGAGTCCCTGAA<br/>GAGAGAAGGCAAAGAACCTCTGGTGTTGAAAGGTGGACT<br/>TAGTAGTTTTAAGCAGAACCATGAAAACCTCTGTGACAA<br/>CTCCCTCCAGCTCCAAGAGTGCCGGGAGGTGGGGGGCGG<br/>CGCATCCGCGGCCTCGAGCTTGCTACCTCAGCCCATCCCC<br/>ACCACCCCTGACATCGAGAACGCTGAGCTACCCCCATC<br/>TTGCCCTTCCTGTTCCCTTGGAATGAGCAGGATGCTCAGG<br/>ACCTGGACACCATGCAGCGGCTGAACATCGGCTACGTCA<br/>TCAACGTCACCACTCATCTTCCCCTCTACCACTATGAGAA<br/>AGGCCTGTTCAACTACAAGCGGCTGCCAGCCACTGACAG<br/>CAACAAGCAGAACCTGCGGCAGTACTTTGAAGAGGCTTT<br/>TGAGTTCATTGAGGAAGCTCACCAGTGTGGGAAGGGGCT<br/>TCTCATCCACTGCCAGGCTGGGGGTGTCCCGCTCCGCCACC<br/>ATCGTCATCGCTTACTTGATGAAGCACACTCGGATGACC<br/>ATGACTGATGCTTATAAATTTGTCAAAGGCAAACGACCA<br/>ATTATCTCCCCAAACCTTAACTTCATGGGGCAGTTGCTAG<br/>AGTTCGAGGAAGACCTAAACAACGGTGTGACACCGAGA<br/>ATCCTTACACCAAAGCTGATGGGCGTGGAGACGGTTGTG<br/>TGA</p> |
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| 7 | Fibronectin-binding protein A (FnBPA) gene | GCATTTAAAGGGAGATATTATAGTGAAAAACAATCTTAG<br>GTACGGCATTAGAAAACATAAATTGGGAGCAGCATCAGT<br>ATTCTTAGGAACAATGATCGTTGTTGGGATGGGACAAGA<br>TAAAGAAGCTGCAGCATCAGAACAAAAGACAACACTACAG<br>TAGAAGAAAATGGGAATTCAGCTACTGATAATAAAGTAA<br>ACGAAACACAAACAACACTACAACCTAACGTTAATACTATAG<br>ATGAAACACAATCATAACAGCGCAACAGCAACAGAACAA<br>CCGTCAAACGCAACACAAGTAACAACCTGAAAAAGCACC<br>AAAAGCAGTACAAGCACCACAACTGCACAACCAGCAA<br>ATGTAGAAACAGTTAAAGAAGAGGTTAGTTAAGGAAGAA<br>GCGAACCCTCAAGTTAAGGAAACGACACAATCTCAAGAC<br>AATAGCGGAGATCAAAGACAAGTAGATTTAACACCTAAA<br>AAGGCTACACAAAATCAAGCAGCAGAAACACAAGTTGA<br>AGTGGCACAGCCAAGAACGGTATCAGAAAGTAAACCAC<br>GTGTGACAAGATCAGCAGATGTAGCGGAAGCTAAGGAA<br>GCTAGTGACGCGAAAGTGGAAACGGGTACAGATGTGAC<br>AAGTAAAGTTACAGTGGAAAGTGGTTCTATTGAGGCACC<br>TCAAGGAAATAAAGTAGAGCCACATGCTGGTCAACGTGT<br>CGTACTGAAATACAAATTGAAATTCGAAAAGGGTTTACA<br>CAAAGGAGATTATTTTGATTTACCTTGTCTAATAATGTA<br>AATACTTATGGAGTTTCAACAGCTAGAAAAGTACCAGAA<br>ATCAAAAATGGTTCAGTCGTAATGGCGACAGGTCAACTT<br>CTTGGAATGGGAAAATTAGGTATACATTTACAGACTAT<br>ATTGATTATAAAGTAAATGTGACTGCTGATTTAGAAATC<br>AATTTATTTATTGATCCTAAACTGTACAAAGCAATGGAC<br>AACAAACAATAACTTCAACGTTGAATGATAAGGAAACAA<br>AAAACACATTGCCAATAGAATATAATCCAGGAGTAAGTA<br>ATAGCTATGCTAATGTAAATGGATCTATTGAACTTTTGA<br>TAAAGGGAACAATAAATTCATCATGTAGCTTACATAAA<br>ACCACAAAATGGGCATAAATCAGATAGCGTTTCAATTAC<br>TGGTACACTAACTCAAGGTAGCAAAGCTAATGGAAATGT<br>TCCAACGTGTAAGTATATGAAGTCTTAAAGGATGCTAA |
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|  |  | <p>AGAATTACCAGAAAGTGTATATGCAAACATATCAGACTC<br/>TACAATGTTTAAAGATGTAACCTCAGGAAATGAAAGATAA<br/>ATTAAAAGTAGAAAATAATGGGAGTTATAAATTAGACAT<br/>TGAGAAATTAGAAAAAAGTTATGTTATACACTATGATGG<br/>TGAATACTTAAGTGGTTCAGATCAAGTGAATTTTAGAAC<br/>GCATATGTTTGGATATCCAGAACAGCAGTATAAGTACTA<br/>CTATACTCATTTGGGATACCAACTTACATGGGATAATGG<br/>ATTAGTTTATATAGTAACAAAGCAAAAGGTGATGGAAC<br/>GAATGGAACAATAACAGAATCGAATAATATGACATTTGA<br/>TGAAGAATATGGAACCTGGAGTTATTACAGGTCAATATGA<br/>TAAGAATTTAGTAACTACTGTTGAAGAAGAATATGATTC<br/>TTCAACTCTTGACATTGATTACCACACAGCTATAGATGGT<br/>GAAGGTGGTTATGTTGATGGATACATTGAAACAATAGAA<br/>GAAACGGATTTCATCAGCTATTGATATCGATTACCATACTG<br/>CTGTGGATAGCGAAGCGGGTCACGTTGGAGGATACACTG<br/>AGTCCTCTGAGGAATCAAATCCAATTGACTTTGAAGAAT<br/>CTACGCATGAAAATTCAAAACATCACGCTGATGTTGTTG<br/>AATATGAAGAGGATACAAACCCAGGTGGTGGTCAGGTTA<br/>CTACTGAGTCTAACTTAGTTGAATTTGACGAAGAGTCTAC<br/>AAAAGGTATTGTAACTGGCGCAGTGAGCGACCATACAAC<br/>AATTGAAGATACGAAAGAATATACAACCTGAAAGTAATCT<br/>GATTGAACTAGTAGATGAACTACCTGAAGAACATGGTCA<br/>AGCACAAGGACCAATCGAGGAAATTACTGAAAACAATC<br/>ATCATATTTCTCATTTCTGGTTTAGGAACTGAAAATGGTCA<br/>CGGTAATTATGGTGTGATTGAAGAAATCGAAGAAAATAG<br/>CCATGTTGATATTAAGAGTGAATTAGGTTATGAAGGTGG<br/>CCAAAATAGCGGTAACCAGTCATTCGAGGAAGACACAGA<br/>AGAAGATAAACCTAAATATGAACAAGGTGGCAATATCGT<br/>AGATATCGATTTTCGATAGTGTACCTCAAATTCATGGTCAA<br/>AATAAAGGTGATCAGTCATTCGAAGAAGATACAGAGAA<br/>AGACAAACCTAAGTATGAACAAGGTGGTAATATCATTGA<br/>TATCGACTTCGACAGTGTGCCACAAATTCATGGATTCAAT</p> |
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|   |                 | AAGCATAATGAAATTATTGAAGAAGATACAAACAAAGAT<br>AAACCAAATTATCAATTCGGTGGACACAACATTGTTGAT<br>TTTGAAGAAGATACACTTCCGAAAGTAAGCGGTCAAAAT<br>GAAGGTCAACAAACGATTGAAGAAGATACAACGCCGCC<br>AACGCCACCGACACCAGAAGTACCGAGTGAGCCGGAAA<br>CACCAACACCACCGACGCCGGAAGTACCGAGTGAGCCAG<br>AAACACCAACGCCACCGACACCAGAAGTACCAAGTGAG<br>CCGGAAACACCAACACCGCCAACACCAGAGGTACCAAGT<br>GAGCCGGAAACACCAACACCGCCAACACCAGAGGTACC<br>AGTTGAACCTGGTAAACCAGTACCACCTGCTAAAGAAGA<br>ACCTAAAAAACCTTCTAAACCAGTGGAACAAGGTAAAGT<br>AGTAACACCTGTTATTGAAATCAATGAAAAGGTAAAGC<br>AGTGGCACCAACTAAACAAAAACAATCTAAGAAATCTGA<br>ACTACCTGAAACAGGTGGAGAAGAATCAACAAACAAAG<br>GTATGTTGTTTCGGCGGATTATTCAGCATTCTAGGTTTAGC<br>GTTATTACGTAGAAATAAAAAAGAATAACAAAGCATAATT<br>AACAAAAATTGACGGGTTTATTTTCATAAATTACATGAAG<br>TAAGCCTGTTTTTTTTATATTAAATCAAATTTTTAATAGA<br>AAATTAGAGTGTTTTCTGATTGCTTCATTGGTTTATGTCT<br>GATGATTGATAACGAACTGAGAGATAAAGTTAGAATTTT<br>AAACTAGT |
| 8 | erm<br>promoter | CGGTATCGATAAGCTTAGTCTAGAATCGATACGATTTTGA<br>AGTGGCAACAGATAAAAAAAGCAGTTTAAAATTGTTGC<br>TGAACTTTTAAACAAGCAAATACAATCATTGTCGCAAC<br>AGATAGCGACAGAGAAGGCGAAAACATTGCCTGGTCGAT<br>CATTCATAAAGCAAATGCCTTTTCTAAAGATAAACGTA<br>TAAAAGACTATGGATCAATAGTTTAGAAAAAGATGTGAT<br>CCGTAGCGGTTTTCAAAATTTGCAACCAGGAATGAATTA<br>CTATCCCTTTTATCAAGAAGCGCACAAAAAGAAAAACGA<br>AATGATACACCAATCAGTGCAAAAAAAGATATAATGGGA<br>GATAAGACGGTTCGTGTTTCGTGCTGACTTGCACCATATCA<br>TAAAAATCGAAACAGCAAAGAATGGCGGAAACGTAAAA                                                                                                                                                                                                                                                                                                                                                                                                                 |

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|    |                                                         | GAAGTTATGGAAATAAGACTTAGAAGCAAACCTTAAGAGT<br>GTGTTGATAGTGCAGTATCTTAAAATTTTGTATAATAGGA<br>ATTGAAGTTAAATTAGATGCTAAAAATTTGTAATTAAGA<br>AGGAGTGAATT                                                                                                                                                                                                                                                                                                                                                                                                    |
| 9  | nisin<br>promoter                                       | CTCCTGTTTTACAACCGGGTGTACATAGCGAAATACTTGT<br>AATGCGTGGTGATGCACCTGAATCTTCTTCGAAACAGAT<br>ACCAAATCCAAGCTAAAATCTTTTGTACTCATTTTGAGTG<br>CCTCCTTATAATTTATTTTGTAGTTCCTTCGAACGAAATC<br>ATTGTATCTAACAACTTCAGAATTTAATCAGAGCCGTTT<br>ATTATGCTCGCGTTATCGACAATAATATTATTACCAATAC<br>TTTCTCAAGATAGAATTAAGACTGTTTTAGATTTGTTAAT<br>GTTTCTATTGTCAGTATAGTTATAAGACT                                                                                                                                                                                                   |
| 10 | DUSP10<br>protein                                       | MPPSPLDDRVVVALSRPVRPQDLNLCLDSSYLGSANPGSNS<br>HPPVIATTVVSLKAANLTYMPSSSGSARSLNCGCSSASCCTV<br>ATYDKDNQAQTQAIAAGTTTTAIGTSTTCPANQMVNNEN<br>TGSLSPSSGVGSPVSGTPKQLASIKIIPNDLAKKMTKCSKS<br>HLPSQGPVIIDCRPFMEYNKSHIQGAVHINCADKISRRLQQ<br>GKITVLDLISCREGKDSFKRIFSKEIIVYDENTNEPSRVMP SQ<br>PLHIVLESLKREGKEPLVLKGG LSSFKQNHENLCDNSLQLQE<br>CREVGGGASAA SLLPQPIPTTPDIENAELTPILPFLFLGNEQ<br>DAQDLDTMQRLNIGYVINVTTHLPLYHYEKGLFNYKRLPA<br>TDSNKQNL RQYFEEAFEFIEEAHQCGKGLLIHCQAGVSRSA<br>TIVIA YLMKHTRMTMTDAYKFVKGKRPIISP NLNFMGQLLE<br>FEEDLNNGVT PRILTPKLMGVETVV |
| 11 | Fibronectin-<br>binding<br>protein B<br>(FnBPB)<br>gene | GTGAAAAGCAATCTTAGATACGGCATAAGAAAACACAA<br>ATTGGGAGCGGCCTCAGTATTCTTAGGAACAATGATCGT<br>TGTTGGAATGGGACAAGAAAAAGAAGCTGCAGCATCGG<br>AACAAAACAATACTACAGTAGAGGAAAGTGGGAGTTCA<br>GCTACTGAAAGTAAAGCAAGCGAAACACAAACA ACTAC<br>AAATAACGTTAATACAATAGATGAAACACAATCATA CAG<br>CGCGACATCAACTGAGCAACCATCACAATCAACACAAGT<br>AACAA CAGAAGAAGCACCGAAA ACTGTGCAAGCACCAA                                                                                                                                                                                               |

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|  |  | <p>AAGTAGAACTTCGCGAGTTGATTTGCCATCGGAAAAAG<br/>TTGCTGATAAGGAACTACAGGAACTCAAGTTGACATAG<br/>CTCAACCAAGTAACGTCTCAGAAATTAAACCAAGAATGA<br/>AAAGATCAACTGACGTTACAGCAGTTGCAGAGAAAGAA<br/>GTAGTGGAAGAACTAAAGCGACAGGTACAGATGTAAC<br/>AAATAAAGTGGAAGTAGAAGAAGGTAGTGAAATTGTAG<br/>GACATAAACAAGATACGAATGTTGTAAATCCTCATAACG<br/>CAGAAAGAGTAACCTTGAAATATAAATGGAAATTTGGAG<br/>AAGGAATTAAGGCGGGAGATTATTTTGATTTACATTAA<br/>GCGATAATGTTGAACTCATGGTATCTCAACACTGCGTA<br/>AAGTTCCGGAGATAAAAAGTACAGATGGTCAAGTTATGG<br/>CGACAGGAGAAATAATTGGAGAAAGAAAAGTTAGATAT<br/>ACGTTTAAAGAATATGTACAAGAAAAGAAAGATTTAACT<br/>GCTGAATTATCTTTAAATCTATTTATTGATCCTACAACAG<br/>TGACGCAAAAAGGTAACCAAAATGTTGAAGTTAAATTGG<br/>GTGAGACTACGGTTAGCAAAATATTTAATATTCAATATTT<br/>AGGTGGAGTTAGAGATAATTGGGGAGTAACAGCTAATGG<br/>TCGAATTGATACTTTAAATAAAGTAGATGGGAAATTTAG<br/>TCATTTTGCGTACATGAAACCTAACAACCAGTCGTTAAGC<br/>TCTGTGACAGTAACTGGTCAAGTAACTAAAGGAAATAAA<br/>CCAGGGGTAAATAATCCAACAGTTAAGGTATATAAACAC<br/>ATTGGTTCAGACGATTTAGCTGAAAGCGTATATGCAAAG<br/>CTTGATGATGTCAGCAAATTTGAAGATGTGACTGATAAT<br/>ATGAGTTTAGATTTTGATACTAATGGTGGTTATTCTTTAA<br/>ACTTTAATAATTTAGACCAAAGTAAAAATTATGTAATAA<br/>AATATGAAGGGTATTATGATTCAAATGCTAGCAACTTAG<br/>AATTTCAAACACACCTTTTTGGATATTATAACTATTATTA<br/>TACAAGTAATTTAACTTGGA AAAATGGCGTTGCATTTTAC<br/>TCTAATAACGCTCAAGGCGACGGCAAAGATAAACTAAAG<br/>GAACCTATTATAGAACATAGTACTCCTATCGAACTTGAAT<br/>TTAAATCAGAGCCGCCAGTGGAGAAGCATGAATTGACTG<br/>GTACAATCGAAGAAAGTAATGATTCTAAGCCAATTGATT</p> |
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|  |  | TTGAATATCATACAGCTGTTGAAGGTGCAGAAGGTCATG<br>CAGAAGGTACCATTGAAACTGAAGAAGATTCTATTCATG<br>TAGACTTTGAAGAATCGACACATGAAAATTCAAAACATC<br>ATGCTGATGTTGTTGAATATGAAGAAGATACAAACCCAG<br>GTGGTGGTCAGGTTACTACTGAGTCTAACCTAGTTGAATT<br>TGACGAAGATTCTACAAAAGGTATTGTAAGTGGTGCTGT<br>TAGCGATCATACAACAATTGAAGATACGAAAGAATATAC<br>GACTGAAAGTAATCTGATTGAACTAGTAGATGAACTACC<br>TGAAGAACATGGTCAAGCGCAAGGACCAATCGAGGAAA<br>TTACTGAAAACAATCATCATATTTCTCATTCTGGTTTAGG<br>AACTGAAAATGGTCACGGTAATTATGGCGTGATTGAAGA<br>AATCGAAGAAAATAGCCACGTGGATATTAAGAGTGAATT<br>AGGTTACGAAGGTGGCCAAAATAGCGGTAATCAGTCATT<br>TGAGGAAGACACAGAAGAAGATAAACCGAAATATGAAC<br>AAGGTGGCAATATCGTAGATATCGATTTTCGATAGTGTAC<br>CTCAAATTCATGGTCAAAAATAATGGTAACCAATCATTCTG<br>AAGAAGATACAGAGAAAGACAAACCTAAGTATGAACAA<br>GGTGGTAATATCATTGATATCGACTTCGACAGTGTGCCAC<br>ATATTCACGGATTCAATAAGCACACTGAAATTATTGAAG<br>AAGATACAAATAAAGATAAACCAAATTATCAATTCGGTG<br>GACACAATAGTGTTGACTTTGAAGAAGATACACTTCCAC<br>AAGTAAGTGGTCATAATGAAGGTCAACAAACGATTGAAG<br>AAGATACAACACCTCCAATCGTGCCACCAACGCCACCGA<br>CACCAGAAGTACCAAGCGAGCCGAAACACCAACACCA<br>CCGACACCAGAAGTACCAAGCGAGCCGAAACACCAAC<br>ACCGCCAACGCCAGAGGTACCAACTGAACCTGGTAAACC<br>AATACCACCTGCTAAAGAAGAACCTAAAAAACCTTCTAA<br>ACCAAGTGGAACAAGGTAAAGTAGTAACACCTGTTATTGA<br>AATCAATGAAAAGGTTAAAGCAGTGGTACCAACTAAAAA<br>AGCACAACTAAGAAATCTGAACTACCTGAAACAGGTGG<br>AGAAGAATCAACAAACAACGGCATGTTGTTTCGGCGGATT<br>ATTTAGCATTTTAGGTTTAGCGTTATTACGCAGAAATAAA |
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|    |                                       | AAGAATCACAAAGCATAA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| 12 | Fibronectin-binding protein A (FnBPA) | <p>MKNNLRYGIRKHKLGAA SVFLGTMIVVGMGQDKEAAASE</p> <p>QKTTTVEENGNSATDNKVNETQTTTTNVNTIDETQSYSATA</p> <p>TEQPSNATQVTTEKAPKAVQAPQTAQPANVETVKEEVVKE</p> <p>EANPQVKETTQSQDNSGDQRQVDLTPKKATQNQAAETQVE</p> <p>VAQPRTVSESKPRVTRSADVAEAEKEASDAKVETGTDVTSK</p> <p>VTVESGSIEAPQGNKVEPHAGQRVVLKYKLKFEKGLHKGD</p> <p>YFDFTLSNNVNTYGVSTARKVPEIKNGSVVMATGQLLGNG</p> <p>KIRYTFDYIDYKVNVTADLEINLFIDPKTVQSNGQQTITSTL</p> <p>NDKETKNTLPIEYNPGVSNSYANVNGSIETFDKGNNKFTHV</p> <p>AYIKPQNGHKSDSVSITGTLTQGSKANGNVPTVKVYEV LKD</p> <p>AKELPESVYANISDSTMFKDVTQEMKDKLKENNGSYKLD</p> <p>IEKLEKSYVIHYDGEYLSGSDQVNFRTHMFGYPEQQYKYY</p> <p>YTHLGYQLTWDNGLVLYSNKAKGDGTNGTITESNNMTFDE</p> <p>EYGTGVITGQYDKNLVTTVEEEYDSSTLDIDYHTAIDGEGG</p> <p>YVDGYIETIEETDSSAIDIDYHTAVDSEAGHVGGYTESSEES</p> <p>NPIDFEESTHENS KHHADVVEYEEDTNPGGGQVTTESNLVE</p> <p>FDEESTKGIVTGAVSDHTTIEDTKEYTTESNLIELVDELPEEH</p> <p>GQAQGP IEEITENNHHSGLGTENGHGNYGVIEEIEENSH</p> <p>VDIKSELGYEGGQNSGNQSFEEDTEEDKPKYEQGGNIVDID</p> <p>FDSVPQIHGQNKGDQSFEEDTEKDKPKYEQGGNIIDIDFDSV</p> <p>PQIHGFNKHNEIIEEDTNKDKPNYQFGGHNIVDFEEDTLPKV</p> <p>SGQNEGQQTIEEDTTPPTPPTPEVPSEPETPTPPTPEVPSEPET</p> <p>PTPPTPEVPSEPETPTPPTPEVPSEPETPTPPTPEVPVEPGKPVP</p> <p>PAKEEPKKPSKPVEQ GKVVTPVIEINEKVKAVAPTKQKQSK</p> <p>KSELPETGGEESTNKGMLFGGLFSILGLALLRRNKKNNKA</p> |
| 13 | Fibronectin-binding protein B (FnBPB) | <p>MKSNLRYGIRKHKLGAA SVFLGTMIVVGMGQEKEAAASEQ</p> <p>NNTTVEESGSSATESKASETQTTTNNVNTIDETQSYSATSTE</p> <p>QPSQSTQVTTEEAPKTVQAPKVETSRVDLPSEKVADKETT G</p> <p>TQVDIAQPSNVSEIKPRMKRSTDVTA VAEKEVVEETKATGT</p> <p>DVTNKVEVEEGSEIVGHKQDTNVVNPHNAERVTLKYKWK</p> <p>FGEGIKAGDYFDFTLSDNVETHGISTLRKVPEIKSTDGQVMA</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |

|  |  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|--|--|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  |  | TGEIIGERKVRYTFKEYVQEKKDLTAELSLNLFIDPTTVTQK<br>GNQNVEVKLGETTVSKIFNIQYLGGVRDNWGV TAN GRIDT<br>LNKVDGKF SHFAYMKPNNQSLSSVTVTGQVTKGNKPGVN<br>NPTVKVYKHIGSDDLAE SVYAKLDDVSKFEDVTDNMSLDF<br>DTNGGYSLNFNNLDQSKNYVIK YEGYYDSNASNLEFQTHL<br>FGYYNYYYTSNL TWKNGVAFYSNNAQGDGDKDLKEPIEH<br>STPIELEFKSEPPVEKHELTGTIEESNDSKPIDFEYHTAVEGA<br>EGHAEGTIE TEEDSIHVD FEESTHENS KHHADVVEYEEDTNP<br>GGGQVTTESNLVEFDEDSTKGIVTGAVSDHTTIEDTKEYTTE<br>SNLIELVDELPEEHGQAQGP IEEITENNNHHISHSGLGTENGHG<br>NYGVIEEIEENSHVDIKSELGYEGGQNSGNQSFEEDTEEDKP<br>KYEQGGNIVDIDFDSVPQIHGQNNGNQSFEEDTEKDKPKYE<br>QGGNIIDIDFDSVPHIHGFNKHTEIIEEDTNKDKPNYQFGGH<br>NSVDFEEDTLPQVSGHNEGQQTIEEDTTPPIVPPTPPTPEVPS<br>EPETPTPTPEVPSEPETPTPTPEVPTEPGKPIPPAKEEPKKPS<br>KPVEQGKV VTPVIEINEKVKAVVPTKKAQSKKSELPETGGE<br>ESTNNGMLFGGLFSILGLALLRRNKKNHKA |
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**CLAIMS**

1. A recombinant, probiotic lactic acid bacterium, wherein the bacterium comprises a non-replicating plasmid vector comprising (a) a tumor suppressor gene or anti-inflammatory gene  
5 operably linked to a first promoter that directs expression of the tumor suppressor gene or anti-inflammatory gene in a mammalian cell, and (b) an adhesin gene operably linked to a second promoter that directs expression of the adhesin gene in the bacterium.
2. The recombinant, probiotic lactic acid bacterium of claim 1, wherein the bacterium is a  
10 lactic-acid producing bacterium.
3. The recombinant, probiotic lactic acid bacterium of claim 1 or 2, wherein the bacterium is of the *Lactococcus* genus.
- 15 4. The recombinant, probiotic lactic acid bacterium of any one of claims 1 to 3, wherein the bacterium is *Lactococcus lactis*.
5. The recombinant, probiotic lactic acid bacterium of any one of claims 1 to 4, wherein the adhesin is fibronectin-binding protein or internalin.  
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6. The recombinant, probiotic lactic acid bacterium of any one of claims 1 to 5, wherein the tumor suppressor gene or anti-inflammatory gene has tumor-suppressing or anti-inflammatory effects on cells of the gastrointestinal tract.
- 25 7. The recombinant, probiotic lactic acid bacterium of claim 6, wherein the tumor suppressor gene is selected from the group consisting of *DUSP10*, *TP53*, *APC*, *CD95*, *PTEN*, *TRAIL*, *MDA7*, and *PMAIP1*, and/or the anti-inflammatory gene is selected from the group consisting of *DUSP10*, *IL10*, *IL11*, *IL13*, *IL1-ra*, and *TGFb*.
- 30 8. The recombinant, probiotic lactic acid bacterium of claim 7, wherein the tumor suppressor gene or anti-inflammatory gene is *DUSP10* gene.

9. A method of treating cancer or reducing inflammation in a subject, wherein the method comprises administering a pharmaceutically effective amount of the recombinant, probiotic lactic acid bacterium of any one of claims 1 to 9 into the gastrointestinal tract of the subject, and wherein the cancer or inflammation is a cancer or inflammation of the gastrointestinal tract.
10. A method of treating cancer in a subject, wherein the method comprises administering a pharmaceutically effective amount of a recombinant, probiotic lactic acid bacterium, wherein the bacterium comprises a non-replicating plasmid vector comprising an adhesin gene operably linked to a promoter that directs expression of the adhesin gene in the bacterium.
11. The method of claim 10, wherein the bacterium is a lactic-acid producing bacterium.
12. The method of claim 10 or 11, wherein the bacterium is of the *Lactococcus* genus.
13. The method of any one of claims 10 to 12, wherein the bacterium is *Lactococcus lactis*.
14. The method of any one of claims 10 to 13, wherein the adhesin is fibronectin-binding protein or internalin.
15. The method of claim 9 or 10, wherein the method further comprises administering an pharmaceutically effective amount of immune checkpoint inhibitor, optionally wherein the immune checkpoint inhibitor is anti-programmed death ligand-1 (PDL-1).
16. The method of any one of claims 9 to 15, wherein the cancer is selected from the group consisting of esophageal cancer, gastric cancer, colorectal cancer and anal cancer.
17. The method of claim 16, wherein the cancer is colorectal cancer.
18. The method of claim 9, wherein the inflammation is the inflammation of the esophagus, the stomach, the small intestine, the casum, the large instestine, or the anus.

19. The method of any one of claims 9 to 18, wherein the recombinant, probiotic lactic acid bacterium is administered orally.

20. The method of any one of claims 9 to 19, wherein the subject is a human.

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