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(54) Title: BACTERIAL STRAINS ISOLATED FROM PIGS

(57) Abstract: A first aspect of the invention relates to a porcine lactic acid bacterial strain, wherein said bacterial strain is characterised by one or more of the following characteristics: (i) the ability to exhibit antimicrobial activity against *E. coli*; (ii) the ability to exhibit antimicrobial activity against *S. enteritidis*; (iii) the ability to suppress inflammation in IPEC cells induced by 12-0- tetradecaboylphorbol-13-acetate (PMA); (iv) the ability to block the attachment or invasion of IPEC cells by *S. enteritidis*; (v) the ability to block the attachment or invasion of IPEC cells by *E. coli*; (vi) the absence of antibiotic resistance to one or more antibiotics selected from the following: ampicillin; cefotaxime; chloramphenicol; erythromycin; gentamicin; tetracycline; vancomycin; metronizadole; nalidixic acid; and kanamycin; and (vii) the ability to exhibit heat stability when subjected to three cycles of heating, each cycle comprising heating at a temperature of 70 °C for a period of 15 minutes. Further aspects of the invention relate to compositions comprising said bacterial strains, and therapeutic uses of said bacterial strains.

BACTERIAL STRAINS ISOLATED FROM PIGS

The present invention relates to bacterial strains isolated from pigs. More specifically, the invention relates to the isolation of lactic acid bacteria from organically-reared pigs. The claimed lactic acid bacteria have useful probiotic and therapeutic applications.

BACKGROUND TO THE INVENTION

The composition of the microbial flora of pigs, their gut innate immune function and possible susceptibility to infection is greatly influenced by the environment in which they were reared during early life (Mulder *et al*, 2009). Outdoor-reared pigs generally have a more developed gut immune system, perform better and are healthier than indoor-reared counterparts. The outdoor environment dramatically influences microbial diversity of the gut and is associated with high levels of *Firmicutes*, in particular Lactic Acid Bacteria [LAB].

LAB comprise a clade of gram-positive, low-GC, acid-tolerant, generally non-sporulating, non-respiring bacteria that are associated with certain common metabolic and physiological characteristics. LAB are rod-shaped bacilli or coccus that are characterized by an increased tolerance to a lower pH range. LAB produce lactic acid as the major metabolic end-product of carbohydrate fermentation and are amongst the most important groups of microorganisms used in the food industry.

Lactobacilli are predominant in the gut flora of organically (outdoor) reared pigs. In contrast, the numbers of these bacteria are low in indoor-reared pigs and levels of potentially pathogenic phylotypes are high (Mulder *et al*, 2009). Furthermore, gut immune development and function of indoor-reared pigs is known to deviate from normal. In particular, expression of Type 1 interferon genes, Major Histocompatibility Complex class I and several chemokines are known to be increased (Mulder *et al*, 2009).

Lactic acid bacteria may modify the flora and gut structure and function in several ways (Cotter *et al*, 2005; Ohashi and Ushida, 2009). For example, they may compete with harmful bacteria for key nutrients or attachment sites on the gut, resulting in their exclusion. Alternatively, they can produce bioactive substances that aid or promote colonisation by beneficial bacteria or kill/interfere with the growth of potentially harmful

or pathogenic bacteria. Alternatively, these bioactive factors can be immune-modulators that promote immune development and barrier integrity of the gut. Strains of LAB vary greatly in their biological activity. The present invention seeks to provide LAB strains that have therapeutically useful properties. More specifically, the invention seeks to provide LAB strains that are capable of promoting gut and immune development and health, thereby having considerable therapeutic potential as probiotics.

STATEMENT OF INVENTION

The present applicant has shown that the microbiota of out-door reared pigs contain LAB strains that produce potent and specific anti-microbial or cell- /immune-modulating bioactive factors.

Aspects of the invention, together with preferred embodiments, are set forth in the accompanying claims.

A first aspect of the invention relates to a porcine lactic acid bacterial strain, wherein said bacterial strain is characterised by one or more of the following characteristics:

(i) the ability to exhibit antimicrobial activity against *E. coli*;

(ii) the ability to exhibit antimicrobial activity against *S. enteritidis*;

(iii) the ability to suppress inflammation in IPEC cells induced by 12-O-tetradecaboylphorbol-13-acetate (PMA);

(iv) the ability to block the attachment or invasion of IPEC cells by *S. enteritidis*;

(v) the ability to block the attachment or invasion of IPEC cells by *E. coli*;

(vi) the absence of antibiotic resistance to one or more antibiotics selected from the following: ampicillin; cefotaxime; chloramphenicol; erythromycin; gentamicin; tetracycline; vancomycin; metronizadole; nalidixic acid; and kanamycin; and

(vii) the ability to exhibit heat stability when subjected to three cycles of heating, each cycle comprising heating at a temperature of 70 °C for a period of 15 minutes.

A second aspect relates to a composition comprising one or more lactic acid bacterial strains according to the invention and a pharmaceutically acceptable excipient, carrier or diluent.

A third aspect relates to a probiotic composition comprising one or more lactic acid bacterial strains according to the invention.

A fourth aspect relates to one or more lactic acid bacterial strains according to the invention for use in medicine.

A fifth aspect relates to one or more lactic acid bacterial strains according to the invention for use in treating an intestinal disorder in a subject.

A sixth aspect relates to the use of one or more lactic acid bacterial strains according to the invention in the preparation of a medicament for treating an intestinal disorder in a subject.

A seventh aspect relates to a method of treating an intestinal disorder in a subject, said method comprising administering to the subject a pharmaceutically effective amount of one or more lactic acid bacterial strains or composition according to the invention.

An eighth aspect of the invention relates to one or more lactic acid bacterial strains according to the invention for improving intestinal microbiota.

A ninth aspect of the invention relates to a method of improving intestinal microbiota in a subject, said method comprising administering to the subject one or more lactic acid bacterial strains or composition according to the invention.

A tenth aspect relates to a feedstuff comprising one or more lactic acid bacterial strains according to the invention.

An eleventh aspect relates to a food product comprising one or more lactic acid bacterial strains according to the invention.

A twelfth aspect relates to a dietary supplement comprising one or more lactic acid bacterial strains according to the invention.

A thirteenth aspect relates to a food additive comprising one or more lactic acid bacterial strains according to the invention.

A fourteenth aspect relates to a process for producing a probiotic, said process comprising culturing a lactic acid bacterial strain according to the invention.

A fifteenth aspect of the invention relates to a process for obtaining a porcine lactic acid bacterial strain, said process comprising obtaining faeces from an organically reared pig and extracting one or more porcine lactic acid bacterial strains from said faeces.

A sixteenth aspect of the invention relates to one or more porcine lactic acid bacterial strains obtained by, or obtainable by, the process described above.

DETAILED DESCRIPTION OF THE INVENTION

As mentioned above, the present invention relates to one or more porcine lactic acid bacterial strains. The lactic acid bacterial strain is characterised by one or more of the following characteristics:

(i) the ability to exhibit antimicrobial activity against *E. coli*;

(ii) the ability to exhibit antimicrobial activity against *S. enteritidis*;

(iii) the ability to suppress inflammation in IPEC cells induced by 12-O-tetradecaboylphorbol-13-acetate (PMA);

(iv) the ability to block the attachment or invasion of IPEC cells by *S. enteritidis*;

(v) the ability to block the attachment or invasion of IPEC cells by *E. coli*;

(vi) the absence of antibiotic resistance to one or more antibiotics selected from the following: ampicillin; cefotaxime; chloramphenicol; erythromycin; gentamicin; tetracycline; vancomycin; metronizadole; nalidixic acid; and kanamycin; and

(vii) the ability to exhibit heat stability when subjected to three cycles of heating, each cycle comprising heating at a temperature of 70 °C for a period of 15 minutes.

As used herein, the term "porcine" means "of or pertaining to swine", i.e. of or pertaining to any of several mammals of the family Suidae, especially the domesticated hog, *Sus scrofa domesticus,* or *Sus domesticus* when young or of comparatively small size.

Preferably, the pig is less than 3 months old, preferably, less than 2 months old.

Preferably, the porcine lactic acid bacterial strain is from an organically reared pig. In this regard, preferably, the pigs are reared free range, outside (with exposure to soil) and in the absence of antibiotics, growth promoters and/or growth enhancers.

Preferably, the porcine lactic acid bacterial strain is from an outdoor reared pig. Preferably, the pigs are reared outside for at least 60 % of their lives. More preferably, the animals are reared outside for at least 80 % of their lives, more preferably, at least 90 % of their lives, even more preferably still, 100 % of their lives.

In one preferred embodiment, the lactic acid bacterial strain is selected from *L. johnsonii*, *L. reuteri*, *L. plantarum*, *L. gasseri*, *L. pentosus*, *L. acidophilus*, *L. vaginalis* and *L. mucosae*.

In one preferred embodiment, the lactic acid bacterial strain is selected from *L. johnsonii*, *L. reuteri* and *L. plantarum*.

In another preferred embodiment, the lactic acid bacterial strain is in the form of a live bacterial population, a lyophilized bacterial population, a non-viable bacterial preparation, or the cellular components thereof. Preferably, where the bacterial strain is in the form of a non-viable bacterial preparation, it is selected from heat-killed bacteria, irradiated bacteria and lysed bacteria.

In one preferred embodiment, the lactic acid bacterial strain is in the form of a live bacterium, a dead bacterium, or the cellular components thereof.

In one preferred embodiment, the lactic acid bacterial strain is in isolated form. As used herein, the term “isolated” means isolated from its native environment.

In one preferred embodiment, the lactic acid bacterial strain is in biologically pure form. As used herein the term “biologically pure” refers to a bacterial strain in the form of a laboratory culture that is substantially free from other species of organism. Preferably, the lactic acid bacterial strain is in the form of a culture of a single species of organism.

As used herein, the term “lactic acid bacterial strain” also encompasses mutants of said lactic acid bacterial strain. As used herein, the term "mutant" includes derived bacterial

strains having at least 93% homology, preferably at least 96% homology, more preferably 98% homology to the polynucleotide sequence of a referenced strain, but otherwise comprising mutations in other sequences in the bacterial genome. Mutants are obtainable by genetic engineering techniques inferring alteration of the genetic material of the strains of the invention or inferring a recombination of the genetic material of the strains of the invention with other molecules. Typically, in order to obtain such mutant strains, a person skilled in the art can use standard mutagenesis techniques such as UV radiation or exposure to mutagenic chemical products.

As used herein, the term "mutations" includes natural or induced mutations comprising at least single base alterations including deletions, insertions, transversions, and other modifications known to those skilled in the art, including genetic modification introduced into a parent nucleotide or amino acid sequence whilst maintaining at least 50% homology to the parent sequence. Preferably, the sequence comprising the mutation or mutations has at least 60%, more preferably at least 75%, more preferably still 85% homology with the parental sequence. As used herein, sequence "homology" can be determined using standard techniques known to those skilled in the art. For example, homology may be determined using the on-line homology algorithm "BLAST" program, publicly available at http)://www.ncbi.nlm.nih.gov/BLAST/.

As used herein, the term “lactic acid bacterial strain” also encompasses homologues of the lactic acid bacterial strains. As used herein the term “homologue” refers to a lactic acid bacterial strain having a nucleotide sequence having a degree of sequence identity or sequence homology with the nucleotide sequence of the parent lactic acid bacterial strain (hereinafter referred to as a “homologous sequence(s)”). Here, the term “homologous” means an entity having a certain homology with the subject nucleotide sequence. Here, the term “homology” can be equated with “identity”.

In the present context, a homologous sequence is taken to include a nucleotide sequence which may be at least 50, 60, 70, 75, 80, 85 or 90 % identical, preferably at least 95%, 97%, 98% or 99% identical to the nucleotide sequence of the parent lactic acid bacterial strain (the subject sequence).

Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available

computer programs can calculate % homology between two or more sequences. % homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the the Vector NTI (Invitrogen Corp.). Examples of software that can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel et al 1999 Short Protocols in Molecular Biology, 4th Ed - Chapter 18), BLAST 2 (see FEMS Microbiol Lett 1999 174(2): 247-50; FEMS Microbiol Lett 1999 177(1): 187-8), FASTA (Altschul et al 1990 J. Mol. Biol. 403-410) and AlignX for example. At least BLAST, BLAST 2 and FASTA are available for offline and online searching (see Ausubel et al 1999, pages 7-58 to 7-60).

Preferably, the degree of identity with regard to a nucleotide sequence is determined over at least 20 contiguous nucleotides, preferably over at least 30 contiguous nucleotides, preferably over at least 40 contiguous nucleotides, preferably over at least 50 contiguous nucleotides, preferably over at least 60 contiguous nucleotides, preferably over at least 100 contiguous nucleotides. Preferably, the degree of identity with regard to a nucleotide sequence may be determined over the whole sequence.

The traditional identification of bacteria on the basis of phenotypic characteristics is generally not as accurate as identification based on genotypic methods. Comparison of the bacterial 16S rRNA gene sequence has emerged as a preferred genetic technique and allows for new strains to be identified by comparison of sequences with known bacterial DNA sequences using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The 16S rRNA gene sequence is universal in bacteria, and so relationships can be

measured across many different bacteria. In general, the comparison of the 16S rRNA sequence allows differentiation between organisms at the genus level across all major phyla of bacteria, in addition to classifying strains at multiple levels, including species and sub-species level. The 16S rRNA gene sequence has been determined for a large number of strains. GenBank, the largest databank of nucleotide sequences, has over 20 million deposited sequences, of which over 90,000 are of 16S rRNA genes. This means that there are many previously deposited sequences against which to compare the sequence of an unknown strain.

In one preferred embodiment, the lactic acid bacterial strain has a 16S rRNA gene sequence selected from SEQ ID NOS 1-87, or a homologue or variant thereof. Another embodiment of the invention relates to a lactic acid bacterial strain that comprises a 16S rRNA gene sequence selected from SEQ ID NOS 1-87, or a homologue or variant thereof. Preferred uses/methods apply to this aspect *mutatis mutandis.*

The term "homologue" is as defined hereinabove. As used herein, the term "variant" includes any variation wherein: (a) one or more nucleotides are substituted by another nucleotide or deleted, (b) the order of two or more nucleotides is reversed, (c) both (a) and (b) are present together. Preferably, the variants arise from one of (a), (b) or (c). More preferably, one or two nucleotides are substituted or deleted. Even more preferably, one nucleotide is substituted by another.

In one preferred embodiment of the invention, the lactic acid bacterial strain is characterised by the ability to exhibit antimicrobial activity against *E. coli.* The observed antimicrobial activity is most likely by virtue of anti-microbial substances produced by the lactic acid bacterial strains of the invention, although nature of these anti-microbial substances has not been determined.

In the context of the present invention, the ability to exhibit antimicrobial activity against *E. coli* can be determined by measuring inhibition of the growth of *E. coli* in an *in vitro* well diffusion assay. Further details of the well diffusion assay are set forth in the accompanying examples. The assay is carried out using *Escherichia coli* K88 on MacConkey No 3 agar, incubating the plates for 16 hours at 37°C. More specifically, *Escherichia coli* K88 is added to the agar (1ml of a 1:1000 dilution of an overnight

culture of *Escherichia coli* K88 in 200 ml agar to give the equivalent of 106 CFU/ml). The agar is poured into petri dishes and allowed to set. The plates are marked off into quadrants and an approximately 5 mm well cut out in each quadrant. An aliquot (60 µl) of conditioned media or MRS broth is added to the wells. The plates are covered and incubated for 16 hours at 37°C. They are photographed using a digital camera. Images are transferred to Photoshop, and the diameter of the well and zone of inhibition were determined using the measure tool.

In the context of killing *E. coli* in the above well diffusion assay, preferably the lactic acid bacterial strain of the invention exhibits <20000 units of inhibition, more preferably 20000-40000 units, even more preferably 40000-60000 units, more preferably 60000-80000 units, more preferably 80000-100000 units of inhibition, even more preferably still >100000 units of inhibition.

In one preferred embodiment of the invention, the lactic acid bacterial strain is characterised by the ability to exhibit antimicrobial activity against *S. enteritidis*. Again, the observed antimicrobial activity is most likely by virtue of anti-microbial substances produced by the lactic acid bacterial strains of the invention, although nature of these anti-microbial substances has not been determined.

In the context of the present invention, the ability to exhibit antimicrobial activity against *S. enteritidis* can be determined by measuring the ability to inhibit the growth of *S. enteritidis* in an *in vitro* well diffusion assay. Further details of the well diffusion assay are set forth in the accompanying examples. The assay is carried out using *Salmonella enteritidis* S1400 on XLD agar, incubating the plates for 16 hours at 37°C. XLD agar is prepared as per manufacturer's instructions and cooled to 45°C. *Salmonella enteritidis* S1400 is added to the XLD agar (1ml of a 1:1000 dilution of an overnight culture of *Salmonella enteritidis* S1400 in 200 ml agar to give the equivalent of 106 CFU/ml). The XLD agar is poured into petri dishes and allowed to set. The plates are marked off into quadrants and an approximately 5 mm well cut out in each quadrant. An aliquot (60 µl) of conditioned media or MRS broth is added to the wells. The plates are covered and incubated for 16 hours at 37°C and the data analysed as described above for the *E. coli* assay.

In the context of killing *Salmonella enteritidis* in the above well diffusion assay, preferably the lactic acid bacterial strain of the invention exhibits <20000 units of inhibition, more preferably 20000-40000 units, even more preferably 40000-60000 units, more preferably 60000-80000 units, more preferably 80000-100000 units of inhibition, even more preferably still >100000 units of inhibition.

In an alternative embodiment, the ability to exhibit antimicrobial activity against *S. enteritidis* can be determined by measuring the ability to inhibit *S. enteritidis in vivo* in C3H/HeN or C57Bl/6 mice. Further details of appropriate *in vivo* assays are set forth in the accompanying examples.

Specifically, C3H/HeN and C57Bl/6 mice are treated with a lactic acid bacterial strain according to the invention prior to and post-challenge with *Salmonella enteritidis*. The mice are euthanased and dissected 6 (C57Bl/6) or 10 (C3H/HeN) days post-infection and viable salmonella are detected in systemic tissues (e.g. the mesenteric lymph node, liver and spleen), in the intestine (e.g. caecum, colon) and in the faeces as compared to appropriate controls. The *in vivo* activity of the lactic acid bacterial strain of the invention can also be measured by determining the level of myeloperoxidase [MPO], a marker for neutrophils, in the intestine of C3H/HeN mice treated with salmonella or salmonella plus LAB. MPO in the intestine is greatly increased by salmonella infection, due to recruitment of neutrophils to the intestine part of the host response to infection. Co-treatment with a lactic acid bacterial strain according to the invention reduces MPO activity in the intestine of salmonella-infected mice, indicating that the intestinal inflammatory responses to infection are lowered in these animals, relative to control experiments.

In one preferred embodiment of the invention, the lactic acid bacterial strain is characterised by the ability to suppress inflammation in IPEC cells induced by 12-O-tetradecaboylphorbol-13-acetate (PMA). In the context of the present invention, this refers to the ability of the lactic acid bacterial strain to block interleukin-8 (IL-8) gene expression triggered by PMA. More specifically, it can be determined by measuring the suppression of inflammation in IPEC-J2 cells induced by PMA when incubated for 2 hours at 37°C, 5% CO_2, 95% humidity. Following RNA and reverse transcription, real time PCR is carried out on a 7500 Fast Real-time PCR system operating with 7500 Fast System v 1.4.0 Sequence Detection Software version 1.4 (Applied Biosystem),

using primers for porcine IL-8 and TNF-α (prepared by Sigma Aldrich). The reaction mix is: 10 µl Power Sybergreen Master mix, 2.5 µl of forward primer, 2.5 µl of reverse primer and 5 µl of cDNA, The Real Time PCR is then run according to the Standard 7500 protocol (95 °C, 10 min, 1 cycle. 95 °C, 15 sec, 40 cycles. 60 °C, 1 min, 40 cycles. 95 °C, 15 sec, 1 cycle. 60 °C, 1 min, 1 cycle. 95 °C, 15 sec, 1 cycle. 60 °C, 15 sec, 1 cycle). Expression of IL-8 and TNF-α genes are analysed and compared to that of the 'house-keeping' gene β-actin. For comparison, values are given as the ratio of IL-8 and TNF-α per β-actin or fold-change. Further details of this assay are set forth in the accompanying examples.

In one preferred embodiment of the invention, the lactic acid bacterial strain is characterised by the ability to block the attachment or invasion of IPEC cells by *S. enteritidis*. This can be measured by the assay set forth in the accompanying examples. Specifically, monolayers of IPEC-J2 cells are grown to 3 days post-confluence in 24-well plates and synchronised by the addition of DTS media 24 hrs prior to use. Overnight cultures of pig LAB (10 ml) are centrifuged and the bacteria re-suspended in phosphate buffered saline [PBS]. An aliquot (50 µl) of LAB is added to the wells. The plates are incubated for 2 hours at 37°C, 5% CO_2, 95% humidity. An overnight culture of *Salmonella enterica* serovar *Enteritidis* S1400 [*S. enteritidis* S1400] is sub-cultured (0.5ml in 10 ml) into Luria Bertani (LB) media and incubated aerobically for 2-3 hours at 37°C until it reaches an optical density (560nm) of 0.8 (a concentration equivalent to 1 x 108 CFU/ml). The culture is centrifuged and the bacteria re-suspended in PBS. An aliquot (50 µl) is added to the wells of IPEC-J2 cells. The plates are incubated for a further 2 hours at 37°C, 5% CO_2, 95% humidity. The IPEC-J2 cell monolayers are washed with HBSS. A solution (0.5 ml) of PBS containing Triton-X100 (10 ml/litre) is added to each well, the monolayer scraped off and dispersed. Viable salmonella are estimated on XLD agar plates (incubated for 24 hours at 37°C) by the Miles and Misra method. Lactic acid bacteria are determined by the same procedure (incubated anaerobically for 48 hours at 37°C).

Preferably, in the context of the adherence/invasion of IPEC cells by *S. enteritidis* the lactic acid bacterial strain of the invention exhibits 0-20% inhibition of adherence/invasion, more preferably 20-40%, even more preferably 40-60%, more preferably still, 60-80%, even more preferably still, 80-100% inhibition of adherence/invasion as measured by the above assay.

In one preferred embodiment of the invention, the lactic acid bacterial strain is characterised by the ability to block the attachment or invasion of IPEC cells by *E. coli*. This can be measured by a similar assay to that described above for *S. enteritidis,* and as set forth in the accompanying examples.

Preferably, in the context of the adherence/invasion of IPEC cells by *E. coli* K88 the lactic acid bacterial strain of the invention exhibits 0-20% inhibition of adherence/invasion, more preferably 20-40%, even more preferably 40-60%, more preferably still, 60-80%, even more preferably still, 80-100% inhibition of adherence/invasion as measured by the above assay.

In one preferred embodiment of the invention, the lactic acid bacterial strain is characterised by the absence of antibiotic resistance to one or more antibiotics selected from the following: ampicillin; cefotaxime; chloramphenicol; erythromycin; gentamicin; tetracycline; vancomycin; metronizadole; nalidixic acid; and kanamycin. In the context of the present invention, antibiotic resistance can be determined by measuring the effect of various antibiotic-containing discs on an MRS agar plate culture of the lactic acid bacterial strain, when placed in an anaerobic jar and incubated for 24 hours at 37°C. Further details of the assay are set forth in the accompanying examples. More specifically, pig LAB [0.5ml of a 1:100 dilution of an overnight culture] is spread onto the surface of an MRS agar plate and dried off. The plates are marked off into 4 quadrants and in each quadrant is placed an antibiotic-containing disc [Ampicillin, 10 µg. Cefotaxime, 30 µg. Chloramphenicol, 10 µg. Erythromycin, 15 µg. Gentamicin, 10 µg. Kanamycin, 30 µg. Metronizadole, 50 µg. Nalidixic acid, 30 µg. Tetracycline, 30 µg. Vancomycin, 30 µg]. The plates are covered, placed in an anaerobic jar and incubated for 24 hours at 37°C. The plates are photographed using a digital camera. Images are transferred to Photoshop, and the diameter of the zone of inhibition is determined using the measure tool. For each antibiotic, the exclusion area for the test strain is taken and divided with the maximum area of exclusion obtained for that antibiotic.

Preferably, the LAB of the invention is characterised by the absence of resistance to the antibiotics ampicillin, cefotaxime, chloramphenicol, erythromycin, gentamicin, tetracycline, vancomycin, metronizadole, nalidixic acid and kanamycin. More preferably, the LAB of the invention is characterised by the absence of resistance to

the antibiotics ampicillin, cefotaxime, chloramphenicol, erythromycin, gentamicin, tetracycline and vancomycin.

In one preferred embodiment of the invention, the lactic acid bacterial strain is characterised by the ability to exhibit heat stability when subjected to three cycles of heating, each cycle comprising heating at a temperature of 70 ºC for a period of 15 minutes. Further details of heat stability studies are set forth in the accompanying examples. More specifically, in the context of the present invention, heat stability is measured by centrifuging an overnight culture (10ml) of isolated pig LAB and resuspending the pellet in fresh MRS broth (10ml). An aliquot (1 ml) is heated at 70°C for 15 min and then plated out (0.5 ml) out on MRS agar and incubated in an anaerobic jar for 48 hours at 37°C. A small number of colonies are detected, picked off, seeded into Hungate tubes containing MRS broth and incubated for 48 hours at 37°C. This culture is centrifuged, re-suspended in MRS broth, heated again at 70°C for 15 min, plated out on MRS agar, incubated in an anaerobic jar for 48 hours at 37°C, picked off, seeded into Hungate tubes containing MRS broth and incubated for 48 hours at 37°C. This culture is centrifuged, re-suspended in MRS broth, re-heated at 70°C for 15 min, plated out (0.5 ml) out on MRS agar, incubated in an anaerobic jar for 48 hours at 37°C, picked off, seeded into Hungate tubes containing MRS broth and incubated for 48 hours at 37°C.

In one preferred embodiment, the lactic acid bacterial strain has any two of the characterising features selected from the group consisting of (i), (ii), (iii), (iv), (v), (vi) and (vii) set forth above.

In one preferred embodiment, the lactic acid bacterial strain has any three of the characterising features selected from the group consisting of (i), (ii), (iii), (iv), (v), (vi) and (vii) set forth above.

In one preferred embodiment, the lactic acid bacterial strain has any four of the characterising features selected from the group consisting of (i), (ii), (iii), (iv), (v), (vi) and (vii) set forth above.

In one preferred embodiment, the lactic acid bacterial strain has any five of the characterising features selected from the group consisting of (i), (ii), (iii), (iv), (v), (vi) and (vii) set forth above.

In one preferred embodiment, the lactic acid bacterial strain has any six of the characterising features selected from the group consisting of (i), (ii), (iii), (iv), (v), (vi) and (vii) set forth above.

In one preferred embodiment, the lactic acid bacterial strain has all seven of the characterising features (i), (ii), (iii), (iv), (v), (vi) and (vii) set forth above.

In one particularly preferred embodiment, (A), the lactic acid bacterial strain is characterised by features (i) and (ii) above.

In one particularly preferred embodiment, (B), the lactic acid bacterial strain is characterised by features (iv) and (v) above.

In one particularly preferred embodiment, (C), the lactic acid bacterial strain is characterised by features (iv) and (v) above.

In one particularly preferred embodiment, the lactic acid bacterial strain is characterised by features denoted (D) to (G) as follows:

(D) (i) and (iv); or

(E) (i) and (v); or

(F) (ii) and (iv); or

(G) (ii) and (v);

More preferably, the lactic acid bacterial strain is further characterised by feature (vi) in addition to those features recited in any one of embodiments (A) to (G) above.

Even more preferably, the lactic acid bacterial strain is further characterised by feature (iii) in addition to those features recited in any one of embodiments (A) to (G) above.

Even more preferably still, the lactic acid bacterial strain is further characterised by feature (vii) in addition to those features recited in any one of embodiments (A) to (G) above.

Biological Deposits

One embodiment of the invention relates to a lactic acid bacterial strain isolated from the faeces of organically reared pigs and selected from the group consisting of strains deposited on 27 June 2011 under the terms of the Budapest Treaty at National Collections of Industrial, Food and Marine Bacteria (NCIMB) at NCIMB Ltd, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, UK, AB21 9YA, under the following accession numbers:

NCIMB 41846: *Lactobacillus reuteri* GGDK31;
NCIMB 41847: *Lactobacillus plantarum/pentosus/paraplantarum* GGDK161;
NCIMB 41848: *Lactobacillus johnsonii/taiwanensis/acidophilus/gasseri* GGDK255;
NCIMB 41849: *Lactobacillus plantarum/pentosus/helveticus/paraplantarum* GGDK258;
NCIMB 41850: *Lactobacillus johnsonii* GGDK266.

The above deposits NCIMB 41846, NCIMB 41847, NCIMB 41848, NCIMB 41849 and NCIMB 41850, were made by Dr George Grant of the Rowett Institute of Nutrition and Health, University of Aberdeen, Greenburn Road, Aberdeen, AB21 9SB on behalf of the Applicant, GT Biologics Limited.

Subsequent studies by the Applicant revealed that the strain deposited as NCIMB 41847 was a mixture of *Lactobacillus paraplantarum* and *Lactobacillus reuteri.* Subsequent studies by the Applicant revealed that the strain deposited as NCIMB 41850 was a mixture of *Lactobacillus johnsonii* and *Lactobacillus reuteri.* Subsequent studies by the Applicant revealed that the strain deposited as NCIMB 41848 was *Lactobacillus reuteri.* Isolated strains for the respective components of strains NCIMB 41847 and NCIMB 41850 were subsequently deposited (see below).

Another embodiment of the invention relates to a lactic acid bacterial strain isolated from the faeces of organically reared pigs and selected from the group consisting of strains deposited on 12 July 2012 under the terms of the Budapest Treaty at National Collections of Industrial, Food and Marine Bacteria (NCIMB) at NCIMB Ltd, Ferguson

Building, Craibstone Estate, Bucksburn, Aberdeen, UK, AB21 9YA, under the following accession numbers:

NCIMB 42008 *Lactobacillus johnsonii*;
NCIMB 42009 *Lactobacillus reuteri*;
NCIMB 42010 *Lactobacillus plantarum*;
NCIMB 42011 *Lactobacillus reuteri*;
NCIMB 42012 *Lactobacillus reuteri*

The above deposits NCIMB 42008, NCIMB 42009, NCIMB 42010 and NCIMB 42011 and NCIMB 42012, were made by Professor Denise Kelly of GT Biologics Limited, c/o Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, Aberdeensshire, AB25 2ZD, UK, on behalf of the Applicant, GT Biologics Limited.

The invention also encompasses mutant strains, which can be obtained from said strains, and strains exhibiting a DNA-DNA homology of at least 70 % and/or a 16S RNA identity of at least 99.5 % with a strain selected from those deposited under the above accession numbers.

As used herein the term "16S rRNA identity" refers to the percentage identity with a known bacterial strain. In one preferred embodiment, the lactic acid bacterial strain has a 16S rRNA identity of at least 85% or at least 90%, or at least 95, 96, 97, 98 or 99 % with a strain selected from those deposited under the above accession numbers. In one highly preferred embodiment, the lactic acid bacterial strain has a 16S rRNA identity of at least 99.5% with a strain selected from those deposited under the above accession numbers.

In the context of the present invention, the term "DNA-DNA homology" refers to how closely related two or more separate strands of DNA are to each other, based on their nucleotide sequence. Typically, this is measured in terms of their % identity. In one preferred embodiment, the lactic acid bacterial strain has a DNA-DNA homology of at least 70% with a strain selected from those deposited under the above accession numbers, more preferably, at least 80%, or at least 85%, more preferably still, at least 90, 95, 97, 98 or 99 % homology with a strain selected from those deposited under the above accession numbers.

In one highly preferred embodiment, the lactic acid bacterial strain has a DNA-DNA homology of at least 70% and a 16S rRNA identity of at least 99.5% with a strain selected from those deposited under the above accession numbers.

Compositions

Another aspect of the invention relates to a composition comprising one or more lactic acid bacterial strains as described above and a pharmaceutically acceptable excipient, carrier or diluent. Suitable excipients, diluents, carriers are described below.

The composition may be any composition, but is preferably a composition to be administered orally, enterally or rectally. For example, the composition may be an edible composition. "Edible" means a material that is approved for human or animal consumption.

Another aspect of the invention relates to a probiotic composition comprising a lactic acid bacterial strain as described above.

Another aspect of the invention relates to combinations of two more lactic acid bacterial strains as described herein. In a particularly preferred embodiment, such combinations exhibit a synergistic functionality, for example, the combination is synergistic, i.e. the resultant effect is greater than the simple additive effects attributable to the individual lactic acid bacterial components in the combination.

One preferred embodiment of the invention relates to a combination of two, three, four or five different lactic acid bacteria, more preferably, two, three or four different lactic acid bacteria, more preferably, two or three different lactic acid bacteria. Where the invention relates to a combination of more than one lactic acid bacterial strain, the individual components of the combination may be present in any ratio.

More preferably still, the invention relates to a combination of two different lactic acid bacteria. Preferably, the two different lactic acid bacteria are present in a ratio of from 1/99.9 to 99.9/1 by weight, for example, 1/99 to 99/1 or 10/90 to 90/10, or 20/80 to 80/20, or 30/70 to 70/30 and the like.

In one highly preferred embodiment, the combination is a mixture of *Lactobacillus johnsonii* and *Lactobacillus reuteri*. Even more preferably, the combination is NCIMB 41850: *Lactobacillus johnsonii* and *Lactobacillus reuteri* GGDK266 as described above. Surprisingly, this particular combination of lactic acid bacteria unexpectedly gives rise to beneficial *in vivo* responses in early weaned pigs (see Examples).

In another highly preferred embodiment, the combination is a mixture of *Lactobacillus plantarum* and *Lactobacillus reuteri*. Even more preferably, the combination is NCIMB 41847: *Lactobacillus plantarum/pentosus/paraplantarum* and *Lactobacillus reuteri* GGDK161 as described above.

As used herein, the term "probiotic" means microbial cell preparations or components of microbial cells with a beneficial effect on the health or well-being of the host. (Salminen S, Ouwehand A. Benno Y. et al "Probiotics: how should they be defined" Trends Food Sci. Technol. 1999:10 107-10).

Preferably, the probiotic composition is an orally administrable composition of metabolically active, i.e., live and/or or lyophilized, or non-viable heat-killed, irradiated or lysed probiotic bacteria. The probiotic composition may contain other ingredients. The probiotic composition of the invention can be administered orally, i.e., in the form of a tablet, capsule or powder. Alternatively, the probiotic composition of the invention may be administered orally as a food or nutritional product, such as milk or whey based fermented dairy product, or as a pharmaceutical product.

A suitable daily dose of the probiotic bacteria is from about 1×10^3 to about 1×10^{11} colony forming units (CFU), more preferably from about 1×10^7 to about 1×10^{10} CFU, more preferably, about 1×10^6 to about 1×10^{10} CFU.
In one preferred embodiment, the composition contains bacterial strains and/or their cellular components, as active ingredients, in an amount of from about 1×10^6 to about 1×10^{12} CFU/g, respect to the weight of the composition, preferably from about 1×10^8 to about 1×10^{10} CFU/g. The dose may be of 1 g, 3 g, 5 g, and 10 g, by way of example.

Typically, a probiotic is optionally combined with at least one suitable prebiotic compound. A prebiotic is usually a non-digestible carbohydrate such as an oligo- or

polysaccharide, or a sugar alcohol which is not degraded or absorbed in the upper digestive tract. Known prebiotics include commercial products such as inulin and transgalacto-oligosaccharides.

Preferably, the composition of the present invention includes a prebiotic in an amount of from about 1 to about 30% by weight, respect to the total weight composition, preferably from 5 to 20% by weight. Preferred carbohydrates are selected from: fructo-oligosaccharides (or FOS), short-chain fructo-oligosaccharides, inulin, isomalt-oligosaccharides, pectins, xylo-oligosaccharides (or XOS), chitosan-oligosaccharides (or COS), beta-glucans, arable gum modified and resistant starches, polydextrose, D-tagatose, acacia fibers, carob, oats, and citrus fibers. Particularly preferred prebiotics are the short-chain fructo-oligosaccharides (for simplicity shown hereinbelow as FOSs-c.c); said FOSs-c.c. are not digestable glucides, generally obtained by the conversion of the beet sugar and including a saccharose molecule to which three glucose molecules are bonded.

Preparation of lactic acid bacteria

A further aspect of the invention relates to a process for producing a probiotic, said process comprising culturing a lactic acid bacterial strain according to the invention. The skilled person in the art will be familiar with standard techniques and conditions suitable for culturing a bacterial strain according to the invention.

A further aspect of the invention relates to a method of preparing one or more bacterial strains according to the invention, said method comprising the steps of:

(i) obtaining faeces from an organically reared pig;

(ii) freezing the faeces and dispersing in a suitable diluent;

(iii) applying the dispersed faeces obtained in step (ii) to a suitable agar, optionally in the presence of supplemental pig colostrum carbohydrates, and incubating under an anaerobic conditions;

(v) selecting off distinct colonies of bacteria formed during step (iv) and seeding into a suitable broth, optionally in the presence of supplemental pig colostrum carbohydrates;

(vi) incubating the seeded colonies obtained in step (v).

Suitable agars include, for example, MRS or LAMVAB agar plates. However, other suitable agars can also be used, and would be familiar to the skilled person.

Suitable broths include, for example, MRS broth. However, other suitable broths can also be used, and would be familiar to the skilled person.

Preferably, step (iii) involves incubating the agar for at least 72 hours at a temperature of about 37 °C.

Preferably, step (vi) involves incubating the seeded colonies for at least 48 hours at a temperature of about 37 °C.

A further aspect of the invention relates to a process for obtaining a porcine lactic acid bacterial strain, said process comprising obtaining faeces from an organically reared pig and extracting one or more porcine lactic acid bacterial strains from said faeces.

Preferably, the process comprises the steps of:

(i) obtaining faeces from an organically reared pig;

(ii) freezing the faeces and dispersing in a suitable diluent;

(iii) applying the dispersed faeces obtained in step (ii) to a suitable agar, optionally in the presence of supplemental pig colostrum carbohydrates, and incubating under an anaerobic conditions;

(v) selecting off distinct colonies of bacteria formed during step (iv) and seeding into a suitable broth, optionally in the presence of supplemental pig colostrum carbohydrates;

(vi) incubating the seeded colonies obtained in step (v).

Another aspect of the invention relates to a porcine lactic acid bacterial strain obtained by, or obtainable by, the process described above.

Therapeutic Applications

Another aspect of the invention relates to one or more lactic acid bacterial strains as defined above for use in medicine.

Another aspect of the invention relates to one or more lactic acid bacterial strains as defined above for use in treating an intestinal disorder.

Another aspect of the invention relates to the use of one or more lactic acid bacterial strains or a composition as defined above in the preparation of a medicament for treating an intestinal disorder.

The term "medicament" as used herein encompasses medicaments for both human and animal usage in human and veterinary medicine. In addition, the term "medicament" as used herein means any substance which provides a therapeutic and/or beneficial effect. The term "medicament" as used herein is not necessarily limited to substances which need Marketing Approval, but may include substances which can be used in cosmetics, nutraceuticals, food (including feeds and beverages for example), probiotic cultures, and natural remedies. In addition, the term "medicament" as used herein encompasses a product designed for incorporation in animal feed, for example livestock feed and/or pet food.

Another aspect of the invention relates to a method of treating an intestinal disorder in a subject, said method comprising administering to the subject a pharmaceutically effective amount of one or more lactic acid bacterial strains or a pharmaceutical composition or a probiotic composition as described above.

Preferably, the intestinal disorder is selected from irritable bowel syndrome (IBS), inflammatory bowel disorder (IBD), functional dyspepsia, functional constipation, functional diarrhoea (including antibiotic associated diarrhoea, traveller's diarrhoea and pediatric diarrhoea), functional abdominal pain, functional bloating, Epigastric Pain Syndrome, Postprandial Distress Syndrome, Crohn's disease, ulcerative colitis, gastrointestinal reflux disease (GERD), allergies, atopic diseases e.g. atopic dermatitis, necrotising enterocolitis, other infections, and combinations thereof.

In one preferred embodiment, the intestinal disorder is IBS. The precise pathophysiology of IBS remains to be elucidated. Recent studies have described mucosal inflammation and alterations in intestinal microbiota in IBS patients and a disease correlation with intestinal infections.

In one highly preferred embodiment, the disorder is salmonellosis. Salmonellosis is a disease caused by various strains of salmonella that is characterized by fever and intestinal disorders.

Another aspect of the invention relates to one or more lactic acid bacterial strains as defined above for improving intestinal microbiota.

Another aspect of the invention relates to a method of improving intestinal microbiota in a subject, said method comprising administering to the subject a composition comprising one or more lactic acid bacterial strains or a pharmaceutical composition or a probiotic composition according to the invention.

The lactic acid bacterial strains according to the invention may also be used in prophylactic applications. In prophylactic applications, compositions according to the invention are administered to a patient susceptible to, or otherwise at risk of, a particular disease in an amount that is sufficient to at least partially reduce the risk of developing a disease. Such an amount is defined to be "a prophylactic effective dose". The precise amounts depend on a number of patient specific factors such as the patient's state of health and weight.

The lactic acid bacterial strains and probiotic compositions according to the invention may also be used in animal nutrition (e.g. in pig nutrition), particularly in the early-weaned period and growing fattening period. The probiotics are expected to enhance immune function reduce and prevent infectious diseases, beneficially alter the microbiota composition, and improve growth and performance of animals, for example, through increased feed conversion efficiency. The term “animal” includes all animals including humans. Examples of animals are non-ruminants and ruminants. Ruminant animals include for example, sheep, goat, and cattle eg. cow as beef cattle and dairy cows. In a particular embodiment, the animal is a non-ruminant animal. Non-ruminant animals include pet animals, eg horses, cats, and dogs; monogastric eg pigs or swine (including but not limited to, piglets growing pigs and sows); poultry such as turkeys, ducks, and chickens (including but not limited to broiler chicks, layers); fish (including but not limited to salmon, trout, tilapia, catfish and carp); and crustaceans (including but not limited to shrimp and prawn).

Feedstuffs/products

A further aspect of the invention relates to food products, dietary supplements, nutraceuticals, nutritional formulae, drinks and medicaments containing one or more bacterial strains according to the invention.

In one preferred embodiment, the composition comprises additionally at least one other kind of other food grade bacterium, wherein the food grade bacterium is preferably selected from the group consisting of lactic acid bacteria, bifidobacteria, propionibacteria or mixtures thereof.

One aspect of the invention relates to a food product comprising one or more lactic acid bacterial strains according to the invention. The term "food product" is intended to cover all consumable products that can be solid, jellied or liquid. Suitable food products may include, for example, functional food products, food compositions, pet food, livestock feed, health foods, feedstuffs and the like. In one preferred embodiment, the food product is a health food.

As used herein, the term "functional food product" means food that is capable of providing not only a nutritional effect, but is also capable of delivering a further beneficial effect to the consumer. Accordingly, functional foods are ordinary foods that have components or ingredients (such as those described herein) incorporated into them that impart to the food a specific functional - e.g. medical or physiological benefit - other than a purely nutritional effect.

Examples of specific food products that are applicable to the present invention include milk-based products, ready to eat desserts, powders for re-constitution with, e.g., milk or water, chocolate milk drinks, malt drinks, ready-to-eat dishes, instant dishes or drinks for humans or food compositions representing a complete or a partial diet intended for pets or livestock.

In one preferred embodiment the composition according to the present invention is a food product intended for humans, pets or livestock. The composition may be intended for animals selected from the group consisting of dogs, cats, pigs, cattle, horses, goats, sheep or poultry. In a preferred embodiment, the composition is a food product intended for adult species, in particular human adults.

In the present invention, "milk-based product" means any liquid or semi-solid milk or whey based product having a varying fat content. The milk-based product can be, e.g., cow's milk, goat's milk, sheep's milk, skimmed milk, whole milk, milk recombined from powdered milk and whey without any processing, or a processed product, such as yoghurt, curdled milk, curd, sour milk, sour whole milk, butter milk and other sour milk products. Another important group includes milk beverages, such as whey beverages, fermented milks, condensed milks, infant or baby milks; flavoured milks, ice cream; milk-containing food such as sweets.

One aspect of the invention relates to a feedstuff or animal feed comprising one or more bacterial strains according to the invention.

Feedstuff can be a food additive, a feed premixor an animal feed. Particular examples of feedstuffs according to the invention include the following: animal feed additive comprising (a) porcine lactic acid bacteria according to the present invention (b) at least one fat soluble vitamin (c) at least one water soluble vitamin (d) at least one trace mineral and/or at least one macro mineral; an animal feed composition comprising a porcine lactic acid bacteria according to the present invention and a crude protein content of 50-88g/kg feed. The so-called premixes are examples of animal feed additives of the invention. A premix designates a preferably uniform mixture of one or more micro-ingredients with diluent and/or carrier. Premixes are used to facilitate uniform dispersion of micro-ingredients in a larger mix.

Further, optional, feed-additive ingredients are coloring agents, e.g. carotenoids such as beta-carotene, astaxanthin, and lutein; aroma compounds; stabilisers; antimicrobial peptides; polyunsaturated fatty acids; reactive oxygen generating species; and/or at least one enzyme selected from amongst phytase (EC 3.1.3.8 or 3.1.3.26); xylanase (EC 3.2.1.8); galactanase (EC 3.2.1.89); alpha-galactosidase (EC 3.2.1.22); protease (EC 3.4.), phospholipase A1 (EC 3.1.1.32); phospholipase A2 (EC 3.1.1.4); lysophospholipase (EC 3.1.1.5); phospholipase C (EC 3.1.4.3); phospholipase D (EC 3.1.4.4); amylase such as, for example, alpha-amylase (EC 3.2.1.1); and/or beta-glucanase (EC 3.2.1.4 or EC 3.2.1.6).

Examples of polyunsaturated fatty acids are C18, C20 and C22 polyunsaturated fatty acids, such as arachidonic acid, docosohexaenoic acid, eicosapentaenoic acid and gamma-linoleic acid.

Examples of reactive oxygen generating species are chemicals such as perborate, persulphate, or percarbonate; and enzymes such as an oxidase, an oxygenase or a syntethase.

Usually fat- and water-soluble vitamins, as well as trace minerals form part of a so-called premix intended for addition to the feed, whereas macro minerals are usually separately added to the feed. Either of these composition types, when enriched with a porcine lactic acid bacteria according to the present invention, is an animal feed additive within the scope of the invention.

The following are non-exclusive lists of examples of these components:Examples of fat-soluble vitamins are vitamin A, vitamin D3, vitamin E, and vitamin K, e.g. vitamin K3. Examples of water-soluble vitamins are vitamin B12, biotin and choline, vitamin B1, vitamin B2, vitamin B6, niacin, folic acid and panthothenate, e.g. Ca-D-panthothenate. Examples of trace minerals are manganese, zinc, iron, copper, iodine, selenium, and cobalt. Examples of macro minerals are calcium, phosphorus and sodium.

The nutritional requirements of these components (exemplified with poultry and piglets/pigs) are listed in Table A of WO 01/58275. Nutritional requirement means that these components should be provided in the diet in the concentrations indicated. In the alternative, the animal feed additive of the invention comprises at least one of the individual components specified in Table A of WO 01/58275. At least one means either of, one or more of, one, or two, or three, or four and so forth up to all thirteen, or up to all fifteen individual components. More specifically, this at least one individual component is included in the additive of the invention in such an amount as to provide an in-feed-concentration within the range indicated in column four, or column five, or column six of Table A of WO 01/58275.

Animal feed compositions or diets typically have a relatively high content of protein. Poultry and pig diets can be characterized as indicated in Table B of WO 01/58275, columns 2-3. Fish diets can be characterized as indicated in column 4 of this Table B.

Furthermore such fish diets usually have a crude fat content of 200-310 g/kg. WO 01/58275 corresponds to US 09/779334 which is hereby incorporated by reference.

An animal feed composition according to the invention typically has a crude protein content of 50-800 g/kg, and furthermore comprises a porcine lactic acid bacteria according to the present invention thereof as described and/or claimed herein.

Furthermore, or in the alternative (to the crude protein content indicated above), the animal feed composition of the invention may have a content of metabolisable energy of 10-30 MJ/kg; and/or a content of calcium of 0.1-200 g/kg; and/or a content of available phosphorus of 0.1-200 g/kg; and/or a content of methionine of 0.1-100 g/kg; and/or a content of methionine plus cysteine of 0.1-150 g/kg; and/or a content of lysine of 0.5-50 g/kg.

In certain preferred embodiments, the content of metabolisable energy, crude protein, calcium, phosphorus, methionine, methionine plus cysteine, and/or lysine is within any one of ranges 2, 3, 4 or 5 in Table B of WO 01/58275 (R. 2-5). Crude protein is calculated as nitrogen (N) multiplied by a factor 6.25, i.e. Crude protein (g/kg)= N (g/kg) x 6.25. The nitrogen content is determined by the Kjeldahl method (A.O.A.C., 1984, Official Methods of Analysis 14th ed., Association of Official Analytical Chemists, Washington DC). Metabolisable energy can be calculated on the basis of the NRC publication Nutrient requirements in swine, ninth revised edition 1988, subcommittee on swine nutrition, committee on animal nutrition, board of agriculture, national research council. National Academy Press, Washington, D.C., pp. 2-6, and the European Table of Energy Values for Poultry Feed-stuffs, Spelderholt centre for poultry research and extension, 7361 DA Beekbergen, The Netherlands. Grafisch bedrijf Ponsen & looijen bv, Wageningen. ISBN 90-71463-12-5.

The dietary content of calcium, available phosphorus and amino acids in complete animal diets is calculated on the basis of feed tables such as Veevoedertabel 1997, gegevens over chemische samenstelling, verteerbaarheid en voederwaarde van voedermiddelen, Central Veevoederbureau, Runderweg 6, 8219 pk Lelystad. ISBN 90-72839-13-7.

In one preferred embodiment, the animal feed composition of the invention contains at least one vegetable protein or protein source. It may also contain animal protein, such as Meat and Bone Meal, and/or Fish Meal, typically in an amount of 0-25%. The term vegetable proteins as used herein refers to any compound, composition, preparation or mixture that includes at least one protein derived from or originating from a vegetable, including modified proteins and protein-derivatives. In certain particularly preferred embodiments, the protein content of the vegetable proteins is at least 10, 20, 30, 40, 50, or 60% (w/w).

Vegetable proteins may be derived from vegetable protein sources, such as legumes and cereals, for example materials from plants of the families Fabaceae (Leguminosae), Cruciferaceae, Chenopodiaceae, and Poaceae, such as soy bean meal, lupin meal and rapeseed meal. In a particular embodiment, the vegetable protein source is material from one or more plants of the family Fabaceae, e.g. soybean, lupine, pea, or bean. Other examples of vegetable protein sources are rapeseed, sunflower seed, cotton seed, and cabbage. Other examples of vegetable protein sources are cereals such as barley, wheat, rye, oat, maize (corn), rice, triticale, and sorghum.

Animal diets can e.g. be manufactured as mash feed (non pelleted) or pelleted feed. Typically, the milled feed-stuffs are mixed and sufficient amounts of essential vitamins and minerals are added according to the specifications for the species in question. A porcine lactic acid bacteria according to the present invention thereof can be added as solid or liquid formulations.

The compositions of the present invention may be - or may be added to - food supplements, also referred to herein as dietary supplements or food additives. Thus, another aspect of the invention relates to a dietary supplement or food additive comprising one or more bacterial strains according to the invention.

Another embodiment of the invention relates to the use of a feedstuff as described above for improving animal growth performance as measured by daily weight gain and/or feed conversion ratio.

In a preferred embodiment, the invention relates to methods for using a feedstuff comprising one or more porcine lactic acid bacteria according to the present invention

in animal feed for improving daily weight gain, improving the Feed Conversion Ratio (FCR) and/or for modulation of the gut microflora.

In alternative preferred embodiments, the feedstuff comprising one or more porcine lactic acid bacteria according to the present invention improves animal feed digestibility, and/or maintains animal health by aiding in proper digestion and/or supporting immune system function.

The FCR may be determined on the basis of a piglet growth trial comprising a first treatment in which the feedstuff comprising a porcine lactic acid bacteria according to the present invention is added to the animal feed in a suitable concentration per kg feed, and a second treatment (control) with no addition of a porcine lactic acid bacteria according to the present invention to the animal feed. In the present context, the term Feed Conversion Ratio, or FCR, is used synonymously with the term feed conversion. The FCR is calculated as the feed intake in g/animal relative to the weight gain in g/animal. As it is generally known, an improved FCR is lower than the control FCR. In particular embodiments, the FCR is improved (i.e., reduced) as compared to the control by at least 1.0%, preferably at least 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, 2.0%, 2.1%, 2.2%, 2.3%, 2.4%, or at least 2.5%.

The term "gut" as used herein designates the gastrointestinal or digestive tract (also referred to as the alimentary canal) and it refers to the system of organs within multicellular animals which takes in food, digests it to extract energy and nutrients, and expels the remaining waste.

The term gut "microflora" as used herein refers to the natural microbial cultures residing in the gut and maintaining health by aiding in proper digestion and/or supporting immune system function.

The term "modulate" as used herein in connection with the gut microflora generally means to change, manipulate, alter, or adjust the function or status thereof in a healthy and normally functioning animal, i.e. a non-therapeutic use.

Diluents, excipients and carriers

As mentioned above, the invention also relates to compositions, more preferably pharmaceutical compositions, comprising a lactic acid bacterial strain according to the invention. The lactic acid bacterial strains of the present invention are generally administered in admixture with a pharmaceutical carrier, excipient or diluent, particularly for human therapy. The pharmaceutical compositions may be for human or animal usage in human and veterinary medicine.

Examples of such suitable excipients for the various different forms of pharmaceutical compositions described herein may be found in the "Handbook of Pharmaceutical Excipients, 2nd Edition, (1994), Edited by A Wade and PJ Weller.

Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985).

Examples of suitable carriers include lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol and the like. Examples of suitable diluents include ethanol, glycerol and water.

The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, or in addition to, the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s).

Examples of suitable binders include starch, gelatin, natural sugars such as glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweeteners, natural and synthetic gums, such as acacia, tragacanth or sodium alginate, carboxymethyl cellulose and polyethylene glycol.

Examples of suitable lubricants include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like.

Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate,

sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

Administration

The compositions of the present invention may be adapted for oral, rectal, vaginal, parenteral, intramuscular, intraperitoneal, intraarterial, intrathecal, intrabronchial, subcutaneous, intradermal, intravenous, nasal, buccal or sublingual routes of administration. Preferably, the compositions of the present invention are adapted for oral, rectal, vaginal, parenteral, nasal, buccal or sublingual routes of administration.

For oral administration, particular use is made of compressed tablets, pills, tablets, gellules, drops, and capsules.

Other forms of administration comprise solutions or emulsions which may be injected intravenously, intraarterially, intrathecally, subcutaneously, intradermally, intraperitoneally or intramuscularly, and which are prepared from sterile or sterilisable solutions. The pharmaceutical compositions of the present invention may also be in form of suppositories, pessaries, suspensions, emulsions, lotions, ointments, creams, gels, sprays, solutions or dusting powders.

An alternative means of transdermal administration is by use of a skin patch. For example, the active ingredient can be incorporated into a cream consisting of an aqueous emulsion of polyethylene glycols or liquid paraffin. The lactic acid bacterial strain can also be incorporated into an ointment consisting of a white wax or white soft paraffin base together with such stabilisers and preservatives as may be required.

Compositions may be formulated in unit dosage form, i.e., in the form of discrete portions containing a unit dose, or a multiple or sub-unit of a unit dose.

Dosage

A person of ordinary skill in the art can easily determine an appropriate dose of one of the instant compositions to administer to a subject without undue experimentation. Typically, a physician will determine the actual dosage which will be most suitable for an individual patient and it will depend on a variety of factors including the activity of the specific bacterial strain employed, the metabolic stability and length of action of that

strain, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the individual undergoing therapy. The dosages disclosed herein are exemplary of the average case. There can of course be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

The usual effective daily dose in humans or in animals is from about 1 x 10^3 to about 1x 10^{11}, more preferably, from about 1 x 10^7 to about 1x 10^{11}, even more preferably, from about 1 x 10^6 to about 1x 10^{10} CFU.

Combinations

In one preferred embodiment, the compositions of the invention are administered in any combination, for example, two or more of the lactic acid bacteria may be administered in any combination or ratio.

In another particularly preferred embodiment, the compositions of the invention are administered in combination with one or more other active agents. In such cases, the compositions of the invention may be administered consecutively, simultaneously or sequentially with the one or more other active agents.

Isolation and characterisation of bacterial strains

The LAB strains isolated (total of 436 individual colony picks) from faeces of organically-reared pigs were predominantly *L. reuteri*, *L. johnsonii*, *L. gasseri*, *L. pentosus*, strains with a small number of *L. plantarum*, *L. acidophilus*, *L. vaginalis*, a single *L. mucosae* and several uncultured strains.

Most of the LAB produced substances that could inhibit the growth of *S. enteritidis* and / or *E. coli K88 in vitro*. The potency of these anti-pathogen effects varied greatly between the individual bacterial strains.

Certain strains were selected on the basis of anti-microbial potency as determined *in vitro*. These bacteria were further screened for their ability to block adherence/invasion of intestinal pig epithelial cells (IPEC) by pathogens *in vitro* and their susceptibility to antibiotics.

Certain strains were assayed for substrate range and specificity and their capacity to suppress inflammation in IPEC cells *in vitro*. From these, fourteen LAB (5 *L. johnsonii*, 6 *L. reuteri* and 3 *L. Plantarum*) with favourable properties were identified. Two of these strains [GGDK266 and GGDK31] were prepared in bulk for *in vivo* evaluation in newly-weaned piglets. Other potentially important candidates were present amongst this set of 14 LAB.

Small losses in viability were evident on freeze drying and storage of LAB dried in skimmed milk powder. A combination of skimmed milk powder and simple sugars was slightly more effective, but difficult to maintain. Bulk preparations of GGDK266 and GGDK31 were freeze-dried and stored in this medium.

Five heat-conditioned cultures of LAB were obtained. However, the biological properties *in vitro* and probiotic potential of three strains were adversely affected by heat-treatment. Nonetheless, two of the bacteria retained the biological properties of the native non-heat-treated forms.

Oral treatment of mice with pig LAB (*L. reuteri* or *L. mucosae*) greatly reduced the pathogenicity of *S. enteritidis* in acute (C57Bl/6 mouse) and chronic (C3H/HeN mouse) forms of salmonellosis.

The data indicate that LAB from organically-reared pigs have considerable potential as a source of novel and potent probiotics.

Studies carried out by the applicant involved isolating large numbers of LAB from organically-reared pigs and screening for potent probiotic LAB strains by assessing their biological potency and mode of action both *in vitro* and *in vivo*.

More specifically, experiments were undertaken to establish cultures of LAB derived from faeces of organically-reared pigs. The LAB strains were screened for anti-microbial activity against a number of pathogens in *vitro*. Experiments were undertaken to determine whether the LAB strains could block the attachment of pathogens to pig epithelial cells *in vitro*. Studies were also undertaken to evaluate the capacity of LAB to block inflammatory responses in pig epithelial cells *in vitro*. Strains demonstrating a

good bioactive profile *in vitro* were selected and cultured in bulk for a large-scale study *in vivo*.

Further details on the experimental techniques are described in the accompanying examples section. In brief, LAB strains were isolated and cultured from pig faeces using selective microbiological media. Individual bacterial colonies were isolated and 16S rRNA gene sequences were analysed to enable genotypic identification of bacterial strains. Phenotypic characteristic of potential probiotics was further determined following measurement of adherence, anti-bacterial and anti-inflammatory activities, antibiotic susceptibility and finally heat stability. Anti-bacterial activity of conditioned media derived from LAB was evaluated using well-diffusion assays to determine killing activity against the enteric pathogens *Salmonella enteritidis* and *E. coli* K88. The ability of LAB strains to block or interfere with *S. enteritidis* and *E. coli* K88 adherence / invasion of pig epithelial (IPEC) was also evaluated, as was their capacity to suppress inflammation in IPEC cells induced by 12-O-Tetradecaboylphorbol-13-acetate [PMA]. In addition, the metabolic properties of LAB strains (API CH 50 kit) and their susceptibility to antibiotics was further determined. A ranking system, based on scoring the biological properties of LAB was established and used for the selection of candidate LAB strains for probiotic evaluation in vivo.

Further details on the results of the above experiments are described in the accompanying examples.

The LAB (436 individual colony picks) isolated from faeces of organically-reared pigs were predominantly *L. johnsonii* or *L. johnsonii*-related and *L. reuteri* or *L. reuteri*-related with small numbers of L. *plantarum-related* and uncultured strains. This represented a much narrower range of porcine-associated LAB than reported by others (Martin *et al*, 2009; Yun *et al,* 2009; Lähteinen *et al*, 2010; Yao *et al*, 2011). However, in comparison to conventionally/intensively-reared pigs, out-door organically-reared pigs had high levels of LAB and more developed intestinal immune function (Mulder *et al*, 2009). The present bacterial data indicate that *L. johnsonii* and *L. reuteri* strains are of particular importance in proper development of the gut and immune system in young pigs. In addition, the inclusion of other lactic acid bacteria derived from the gut or faeces of organically-reared pigs, in particular, *Lactobacillus delbrueckii* and

Lactobacillus amylovorous may enhance the immune homeostatic properties of *Lactobacillus reuteri* , *Lactobacillus plantarum* and *Lactobacillus johnsonii.*

All of the isolated pig LAB produced substances that could kill or interfere with the growth of *S. enteritidis* in a well-diffusion assay and the majority killed or suppressed growth of *E. coli* K88. The potency of the anti-microbial activities varied greatly between individual colonies, irrespective of whether they were *L. reuteri*, *L. johnsonii* or *L. plantarum*. There was no general correlation between the anti-salmonella and anti-E.coli K88 potency of each of the LAB. LAB are known to produce a range of active factors, including organic acids, small anti-microbial compounds and anti-bacterial peptides (Cintas *et al*, 2001). The nature of these anti-microbial substances produced by LAB from organically-reared pigs has not been established.

Thirty three pig LAB strains, selected on the basis of anti-pathogen activity, were tested for the ability to block attachment/invasion of IPEC cells by *S. enteritidis* and *E. coli* K88. They were all able to dramatically reduce attachment / invasion of IPEC cells by salmonella. The majority could also block *E. coli* K88. As with pathogen killing, there was no general correlation between the abilities of the LAB to block salmonella and E.coli K88. Without wishing to be bound by theory, it is believe that the LAB may limit the access of pathogens to the epithelial layer by occupying binding-sites on the cell monolayer or by production of factors that interfere with attachment of the pathogen to the epithelial cells, such as blocking binding sites of surface adhesins (Ljungh and Wadstrom, 2006; Blandino *et al*, 2008; Williams, 2010).

Pig LAB may also block or suppress inflammatory gene (Interleukin-8, IL-8)-expression triggered in IPEC cells by PMA. Individual cultures varied greatly in their ability to affect inflammation, but five strains (RINH vial 29, 30, 31 86 and 266) had potent anti-inflammatory properties. Certain LAB strains are known to have immuno-modulatory or anti-inflammatory properties (Cotter *et al*, 2005; Blandino *et al*, 2008; Ohashi and Ushida, 2009; Elmadfa *et al*, 2010; Liu *et al*, 2010). The mechanisms involved remain unclear, but are likely to involve modulation of molecular signalling systems by bioactive factors produced by the LAB.

Antibiotic resistance is an increasing problem and can spread between bacteria by gene transfer (Korhonen *et al*, 2007; Gousia *et al*, 2011; Nicolau, 2011). Ideally, candidate probiotics should have little or no resistance to antibiotics to minimise the

risk of transfer of resistance genes to the host flora. Pig LAB (33 strains) were screened for resistance to 10 individual antibiotics. One strain (RINH vial 266) was susceptible to all the tested antibiotics. Most were susceptible to ampicillin, cefotaxime, chloramphenicol, erythromycin, gentamicin, tetracycline and vancomycin. However, most exhibited resistance to metronizadole, nalidixic acid and to a lesser extent kanamycin. This relatively low incidence of antibiotic resistance amongst these LAB isolates may be linked to the environment in which the source piglets were reared [organic out-door reared] (Mulder *et al*, 2009).

L. johnsonii, *L. reuteri* and *L. plantarum*, as expected, exhibited strain-specific general substrate reaction profiles, when assayed using an API CH 50 kit. However, most genotype strains exhibited fine differences in their substrate reactivity. This indicated that they were unique individual strains of the genotype.

On the basis of their biological activities *in vitro*, fourteen LAB [4 *L. plantarum*-related, 3 *L. johnsonii*-related and 1 *L. reuteri*] were identified as having potential for testing *in vivo*. Two of these LAB strains [GGDK266 and GGDK31] were prepared in bulk. Interestingly, 7 of the fourteen LAB (RINH vials 85, 86, 131, 230, 255, 266) had been isolated from LAB-selective agars supplemented with carbohydrate fractions from pig colostrum. The growth and bioactivity profile of LAB is, in part, dependent on the carbohydrate substrate in which it is grown (Gopal *et al*, 2001; Tzortzis *et al*, 2004), The present data may indicate that some of the LAB are host-adapted and require certain pig-associated carbohydrates for optimal growth or bioactivity.

It is advantageous if the LAB can withstand being freeze dried to allow them to be handled and processed as probiotics. However, their viability can be greatly reduced during freezing and drying (Tomas et al, 2009; Strasser et al, 2009; Reddy et al, 2009). Skimmed milk powder, alone or in combination with simple sugars, is often used as a cryo-protectant to preserve the viability of the bacteria (Tomas *et al*, 2009; Strasser *et al*, 2009). In the present study, small losses in viability were evident on drying and storage of pig LAB in skimmed milk powder alone. Sucrose or lactose in combination with skimmed milk powder was slightly more protective. However, the product was hygroscopic and difficult to store or handle. It was therefore decided to dry and store pig LAB in skimmed milk powder.

Supplemental feeds for animal are often given as pellets, production of which involves high temperatures (De Angelis *et al*, 2006). LAB to be added to animal feeds should therefore have a significant degree of heat-stability to minimise loss of viability during processing. In the present study, five LAB were subject to heating three times for 15 minutes at 70°C. All of the bacteria that were recovered after the third heat-treatment were viable and in most cases grew at rates similar to the native forms of the bacteria. Two of the bacteria retained the biological properties of the native non-heat-treated forms. However, one of the heat-treated strains had lost the ability to block attachment of pathogen to epithelial cells *in vitro* and another had greatly reduced blocking activity. A further strain was unable to block PMA-induced inflammation in epithelial cells *in vitro*, although the native form was a potent suppressor of inflammation. Heat-treatment can thus differentially affect the biological properties of individual LAB. This needs to be taken into account when considering inclusion of LAB in pelleted animal feeds.

Experiments demonstrated that the pathogenicity of *S. enteritidis* was attenuated if mice were co-treated with LAB derived from organically-reared pigs. RINH vial 323 (*L. mucosae*) greatly reduced the ability of *S. enteritidis* to invade, spread to and proliferate in systemic tissues in acute (C57Bl/6 mouse) and chronic (C3H/Hen mouse) salmonellosis. Furthermore, RINH vial 31 [GGDK31], RINH vial 32, RINH vial 46 or RINH vial 47 (all *L. reuteri*) reduced colonisation of the large intestine, invasion and systemic spread and proliferation in C3H/HeN mice by *S. enteritidis*. Overall, RINH vial 31 [GGDK31] and RINH vial 32 were the most effective in this chronic model of salmonellosis. These LAB have potential as novel probiotics to promote gut health or increase resistance to infection *in vivo*.

Infection by salmonella is a multi-factorial process (Naughton and Grant, 2005). *S. enteritidis* colonises the whole gastro-intestinal tract, moves through the mucus layer and attaches to the mucosa. The large intestine acts as a reservoir for the pathogen but invasion is primarily via M cells, present on the Peyer's patches of the ileum. Most invaded salmonella spread to the mesenteric lymph nodes and then out to the liver and spleen (Naughton and Grant, 2005). Without wishing to be bound by theory, it is believed that LAB could be blocking salmonella at various stages of the infection (Cintas *et al*, 2001; Cotter *et al*, 2005; Ohashi and Ushida, 2009). By competing for nutrients, killing of pathogen or blocking of attachment sites, LAB could limit the numbers of salmonella in the large intestine reservoir. LAB may also prevent

attachment to ileal mucosal cells, in a manner similar to that observed here with IPEC-J2 cells and with Caco-2 cells (Neeser *et al*, 2000) and thereby limit invasion. Alternatively, LAB may directly modulate host responses to the infection, in particular suppression of inflammation. By limiting gut damage and preserving barrier integrity (Smith et al, 2008; Schreiber et al, 2009), the ability of salmonella to invade and spread would be greatly reduced.

The present invention is further described by way of non-limiting example, and with reference to the following non-limiting figures, wherein:

Figure 1 shows an assay of antibacterial activity of conditioned media from Lactic Acid Bacteria.

Figures 2a & b show inhibitory activity against *S. enteritidis* S1400 (expressed as area of inhibition in a well diffusion assay) of conditioned media of all individual LAB cultured from faeces of organically-reared pigs.

Figures 3a & b show inhibitory activity against *E. coli* K88 (expressed as area of inhibition in a well diffusion assay) of conditioned media of all individual LAB cultured from faeces of organically-reared pigs.

Figures 3c & d show inhibitory activity (expressed as area of inhibition in a well diffusion assay) of conditioned media of all individual LAB cultured from faeces of organically-reared pigs.

Figures 4a, b, c shown inhibition of adherence by (a) *S. enteritidis* S1400; and (b) *E. coli* K88 to IPEC cells in culture by LAB cultured from faeces of organically-reared pigs; (c) comparison between inhibition of *S. enteritidis* S1400 and (b) *E. coli* K88.

Figure 5 shows an assay of the antibiotic susceptibility of Lactic Acid Bacteria using discs impregnated with a defined amount of antibiotic.

Figure 6 shows an evaluation of substrate profile of LAB using an API CH 50 kit [49 substrates, pale colour indicated positive reaction, except 25 where positive reaction is black, dark colour indicates no reaction].

Figure 7 shows the ΔCt (a), ratio (b) and fold-change (c) for IL-8 gene expression in IPEC cells treated with PMA and pig LAB.

Figure 8 shows the stability of pig LAB after freeze-drying in skimmed milk powder (SKP, (100g/l), SKP + lactose (both 100g/l), SKP + sucrose (both 100g/l) or SKP (200g/l).

Figure 9 shows the stability of isolated LAB to heat-treatment (a), the ratio (b) and fold-change (c) for IL-8 gene expression in IPEC cells treated with PMA and naive or heat-treated pig LAB; (d) Antibiotic susceptibility of native and heat-treated RINH vial 31.

Figure 10 shows a protocol for the C3H/HeN mouse study to evaluate efficacy of vial 323 (*L. mucosae*) to counteract salmonella infection *in vivo*.

Figures 11a-c show the distribution of *S. enteritidis* S1400 in tissues at 10 days post-infection in C3H/HeN mice that had or had not been co-treated with 323 (*L. mucosae*, LM).

Figures 12a-b show spleen weight (mg/100g BW) and intestinal (ileal) myeloperoxidase (μg) at 10 days post-infection in C3H/HeN mice that had or had not been co-treated with vial 323 (*L. mucosae*).

Figure 13 shows a protocol for the C57 Bl/6 mouse study to evaluate efficacy of vial 323 (*L. mucosae*) to counteract acute salmonella infection *in vivo*.

Figures14a-c shows the distribution of *S. enteritidis* S1400 in tissues at 6 days post-infection in C57Bl/6 mice that had or had not been co-treated with RINH vial 323.
Figure 15 shows spleen weight (mg/100g BW) at 6 days post-infection in C57Bl/6 mice that had or had not been co-treated with vial 323 (*L. mucosae*).

Figure 16 shows a protocol for the C3H/HeN mouse study to evaluate efficacy of selected LAB from faeces of organically reared pigs to counteract salmonella infection *in vivo*.

Figures 17a & b show excretion of *S. enteritidis* in faeces at 7-8 days post-infection by C3H/HeN mice that had or had not been co-treated with selected LAB.

Figures 18a-b show the distribution of *S. enteritidis* (Log10 CFU/g) in ileum (a), caecum (b) and colon (c) at 10 days post-infection of C3H/HeN mice that had or had not been co-treated with selected LAB.

Figures 19a-c show the distribution of *S. enteritidis* (Log10 CFU/g) in mesenteric lymph node (a), liver (b) and spleen (c) at 10 days post-infection of C3H/HeN mice that had or had not been co-treated with selected LAB.

Figure 20 shows the performance of pigs fed GGDK266 and GGDK31 versus a control (daily weight gain, DWG, in g/day) for days 0-7, 7-14 and 0-14.

Figure 21 shows microbial diversity analysis using denaturing gel gradient electrophoresis (DGGE; Trial 1). DGGE using universal primers revealed no differences in overall microbial diversity between the treatments and placebo. Bands on the gel are visualised by silver staining.

Figure 22 shows microbial diversity analysis using DGGE. DGGE using lactic acid bacteria (LAB) specific primers revealed significant differences in LAB diversity between treatment with GGDK266 and placebo in both caecal and ileal samples. Bands on the gel are visualised by silver staining.

Figure 23 shows microbial diversity analysis using DGGE. DGGE using lactic acid bacteria (LAB) specific primers revealed significant differences in LAB diversity between treatment with GGDK266 and placebo in ileal samples. Bands on the gel are visualised by silver staining.

Figure 24 shows microbial diversity analysis using DGGE. DGGE using lactic acid bacteria (LAB) specific primers revealed significant differences in LAB diversity between treatment with GGDK266 and placebo in caecal samples. Bands on the gel are visualised by silver staining.

Figure 25 shows the gene ontology biological processes significantly down-regulated by oral administration of GGDK266.

Figure 26 shows changes in immune response and response to stimuli in animals treated with GGDK266 versus animals treated with placebo (percent of genes versus a range of different GO annotations).

Figure 27 shows the gene ontology biological processes significantly enriched by oral administration of GGDK266.

EXAMPLES

Materials and Methods

Materials: Pig faeces samples collected during the course of the study of outdoor- and indoor-reared pigs (Mulder *et al*, 2009) were used in these studies. The culture collection was based primarily on LAB collected from frozen samples 411, 412 and 416, which were from outdoor-reared pigs with particularly high levels of LAB in their faeces. MRS broth premix, agar and vancomycin, anaerobe gas packs and indicator and antibiotic discs were purchased from Oxoid, anaerobe catalyst from Fisher Scientific and cysteine-HCL, bromocresol green and skimmed milk powder from Sigma-Aldrich. Pig colostrum carbohydrate fractions were prepared as part of the SMART 163 programme of D. Kelly. DNA extraction kits were purchased from MP Biomedicals and PCR reagents and clean-up kits from Promega. API CH 50 kits were purchased from Biomerieux UK Ltd.

Standard media: MRS broth and MRS agar were prepared according to the manufacturer's instructions. LAMVAB agar was prepared according to the method of Jackson *et al.* (2002). Agar plates were prepared immediately before use. MRS broth was decanted (10 ml per tube) into sterile Hungate tubes under anaerobic conditions and stored at room temperature.

Carbohydrate-supplemented media: SMART 163 ammonium sulphate precipitate of pig colostrum: precipitated at 0, 20, 25, 30, 35, 45, 50, 55 or 65% saturation or soluble at 65% saturation were weighed out in proportion to the amounts recovered from 15 ml or 50 ml of colostrum. Carbohydrate fractions were each dispersed in 15 ml of MRS or

LAMVAB agar, held at 45°C, and then individual plates were poured for each fraction. They were also dispersed in MRS broth (50ml) and the supplemented broth decanted to eight (6 ml/ tube) sterile Hungate tubes under anaerobic conditions.

Animals: Female C3H/HeN and C57Bl/6 mice (5-6 weeks old) were purchased from Harlan UK. They were housed as groups or pairs in standard caging within HEPA-filtered flexifilm isolators situated in a class 2 containment facility. They had free access to a high quality rodent chow and sterile deionised water at all times and were allowed to acclimatise for 7 to 10 days prior to commencement of experiments. The Rowett Institute of Nutrition and Health (RINH) is licensed under the UK Animals (Scientific Procedures) Act 1986. Studies herein were carried out under the auspices of an approved Home Office Project Licence by staff holding the requisite Home Office Personal Licence (as defined and set out in the UK Animals (Scientific Procedures) Act 1986), and were reviewed and approved by the RINH Ethical Review Committee.

Methods

Culture of LAB: In initial studies, a small amount of frozen faeces (100 mg) was dispersed in 1ml of maximum recovery diluent (MRD). Two further sequential ten-fold dilutions were made. All three suspensions were streaked out on MRS or LAMVAB agar plates. In later studies, the faeces sample was dispersed in 5ml of MRD, further diluted (1:40) in MRD and 0.5ml of this dilution spread over the surface of MRS or LAMVAB agar plates with or without supplemental pig colostrum carbohydrates. In all cases, the plates were incubated in an anaerobic jar for 72 hours at 37°C. Distinct colonies (at least 8 per plate) were picked off the agar plates and seeded into Hungate tubes containing MRS broth or where appropriate MRS broth containing pig colostral carbohydrates. The tubes were incubated for 48 hours at 37°C.

Frozen stock: An aliquot (0.7 ml) of each culture was drawn off with a sterile syringe and needle and dispensed into a plastic tube that was flushed with CO_2 and contained 0.3 ml glycerol and 2 mg L-cysteine. The tube was sealed with a plastic stopper, labelled, the contents mixed, frozen and stored at -80°C.

Conditioned medium: The remaining culture was transferred to a Corning 15 ml centrifuge tube, centrifuged at 1000g x 5 min at room temperature, the supernatant

decanted, aliquoted and frozen. The pellets were either extracted immediately for 16S rRNA gene analysis or frozen.

16S rRNA gene analysis (Clarridge, 2004): Bacterial DNA was extracted using a FastDNA® Spin kit for Soil in conjunction with a Fastprep 120 bead beater system, according to the protocol supplied with the kit. PCR was carried out (reaction mix: buffer, 10 µl. dNTPs (2mM), 5 µl. 27F Primer (20pmol/ µl), 2 µl. 1492R Primer (20pmol/ µl). 2 µl Go Taq Flexi Polymerase, 0.5 µl. MgCl2, 5 µl. H2O, 23.5 µl and 2 µl of extracted DNA) using MJ Research PTC-200 Peltier Thermal Cycler run through 35 cycles of 95°C for 3 minutes, 95°C for 30 seconds, 57°C for 30 seconds and 72°C for two minutes. Primer: 27F (F01) AGAGTTTGATCCTGGCTCAG; 1492R (RP2) ACGGCTACCTTGTTACGACTT. PCR product cleanup was done with a Wizard® SV Gel and PCR Clean-up kit (Promega), used according to the manufacturer's instructions. 16S PCR products were sequenced using fully automated genetic analysers based on capillary electrophoresis technology (Genomics Section, RINH, UoA) using the reverse and forward primers 519R and 926F. Bacterial strains were identified by comparison of sequences with known bacterial DNA sequences using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Antibacterial activity: XLD agar was prepared as per manufacturer's instructions and cooled to 45°C. *Salmonella enteritidis* S1400 was added to the XLD agar [1ml of a 1:1000 dilution of an overnight culture of salmonella in 200 ml XLD agar to give the equivalent of 106 CFU/ml]. The agar was poured into petri dishes and allowed to set. The plates were marked off into 4 quadrants and an approximately 5 mm well cut out in each quadrant. An aliquot (60 µl) of conditioned media or MRS broth was added to the wells. The plates were covered and incubated for 16 hours at 37°C. They were photographed using a digital camera. Images transferred to Photoshop, and the diameter of the well and zone of inhibition were determined using the measure tool. Values were calculated and stored on an Excel spreadsheet. The same procedure was used with *Escherichia coli* K88, except that MacConkey No 3 agar was used.

Antibiotic susceptibility: Pig LAB [0.5ml of a 1:100 dilution of an overnight culture] was spread onto the surface of an MRS agar [90mm] plate and dried off. The plates were marked off into 4 quadrants and in each quadrant was placed an antibiotic-containing disc [Ampicillin, 10 µg. Cefotaxime, 30 µg. Chloramphenicol, 10 µg.

Erythromycin, 15 µg. Gentamicin, 10 µg. Kanamycin, 30 µg. Metronizadole, 50 µg. Nalidixic acid, 30 µg. Tetracycline, 30 µg. Vancomycin, 30 µg]. The plates were covered, placed in an anaerobic jar and incubated for 24 hours at 37°C. They were photographed using a digital camera. Images transferred to Photoshop, and the diameter of the zone of inhibition was determined using the measure tool. Values were calculated and stored on an Excel spreadsheet.

Prevention of adherence / invasion by salmonella *in vitro:* Monolayers of IPEC-J2 cells were grown to 3 days post-confluence in 24-well plates and synchronised by the addition of DTS media 24 hrs prior to use. Overnight cultures of pig LAB (10 ml) were centrifuged [1000g x 5 min at room temperature] and the bacteria re-suspended in 1 ml of phosphate buffered saline [PBS]. An aliquot (50 µl) of LAB was added to the wells. The plates were incubated for 2 hours at 37°C, 5% CO_2, 95% humidity. An overnight culture of *Salmonella enterica* serovar *Enteritidis* S1400 [*S. enteritidis* S1400] was sub-cultured (0.5ml in 10 ml) into Luria Bertani (LB) media and incubated aerobically for 2-3 hours at 37°C until it reached an optical density (560nm) of 0.8. This gave a concentration equivalent to 1x108 CFU/ml. The culture was centrifuged [1000g x 5 min at room temperature], the bacteria re-suspended in 10 ml of PBS. An aliquot (50 µl) was added to the wells of IPEC-J2 cells. Wells treated with PBS were used as controls. The plates were incubated for a further 2 hours at 37°C, 5% CO_2, 95% humidity. The IPEC-J2 cell monolayers were washed 5 times with HBSS. A solution (0.5 ml) of PBS containing Triton-X100 (10 ml / litre) was added to each well, the monolayer scraped off and dispersed. Viable salmonella were estimated on XLD agar plates [incubated for 24 hours at 37°C] by the Miles and Misra method [Robertson *et al*, 2003]. LAB were determined by the same procedure [incubated anaerobically for 48 hours at 37°C].

Inhibition of inflammatory responses: Monolayers of IPEC-J2 cells were grown to 3 days post-confluence in 24-well plates and synchronised by the addition of DTS media 24 hrs prior to use. Overnight cultures of pig LAB (10 ml) were centrifuged [1000g x 5 min at room temperature] and the bacteria re-suspended in 1 ml of PBS. An aliquot (50 µl) of LAB was added to each well [3 wells for each sample] along with 220 ng 12-O-Tetradecaboylphorbol-13-acetate [PMA] per well. PMA or PBS alone served as controls. The plates were incubated for 2 hours at 37°C, 5% CO_2, 95% humidity. Culture media was removed from the dishes and the cells washed twice with PBS. RLT buffer (0.5 ml) containing mercaptoethanol was added to each well, the cells scraped

off and transferred to an eppendorf tube [for each sample scrapings from 3 wells were combined]. RNA extraction was done using RNeasy® Mini kit in accordance with the manufacturer's protocols and reverse transcription with a high capacity cDNA Reverse Transcription Kit (Applied Biosystems). Real Time PCR was done on a 7500 Fast Real-time PCR system operating with 7500 Fast System v 1.4.0 Sequence Detection Software version 1.4 (Applied Biosystem). Primers for porcine IL-8 and TNF-α [IPEC-J2, SY100604186-096 IL-8-2 Reverse, SY100604186-090 TNF1a Reverse, SY100604186-095 IL-8 2 Forward, SY100604186-089 TNFa1 Forward, and SY100604186-093] were prepared by Sigma Aldrich. The reaction mix was: 10 µl Power Sybergreen Master mix, 2.5 µl of forward primer, 2.5 µl of reverse primer and 5 µl of cDNA, The Real Time PCR was then run according to the Standard 7500 protocol [95 °C, 10 min, 1 cycle. 95 °C, 15 sec, 40 cycles. 60 °C, 1 min, 40 cycles. 95 °C, 15 sec, 1 cycle. 60 °C, 1 min, 1 cycle. 95 °C, 15 sec, 1 cycle. 60 °C, 15 sec, 1 cycle]. Expression of IL-8 and TNF-α genes were analysed and compared to that of the 'house-keeping' gene β-actin. For comparison, values were given as the ratio of IL-8 and TNF-α per β-actin or fold-change.

For example:

a. Calculate ΔCt (2h) for IL-8 [Ct IL-8 minus Ct β-actin]
b. Calculate ΔCt (2h) for PMA [Ct PMA minus Ct β-actin]
c. Divide ΔCt (IL-8) with ΔCt (PMA)
d. Round up value to whole number

Substrate reactivity: The carbohydrate reactivity of individual LAB was determined using an API CH 50 kit (Biomerieux UK Ltd). Assays were done according to the manufacturer's instructions and reactions were recorded after incubation for 24 and 48 hours at 37°C. There are 50 capules on an API CH 50 plate. These contain various potential substrates and negative controls. The range of substrates is as follows: Monosaccharides 16,, Monosaccharides/alcohols 4, Disaccharides 8, Trisaccharides 2, Polysaccharides 3, Alcohols 6, Others 7. For each substrate group the number of positive reactions is counted. This is divided by the maximum possible to give the rank for that substrate group. The sum of all the substrate scores gives the overall ranking for the bacterium. High Ranking indicates broad spectrum of substrate reactivity

Heat-treatment of LAB: A small amount of frozen faeces (100 mg) was dispersed in 5ml of maximum recovery diluent (MRD). Sediment was allowed to settle out and the

upper layer was decanted into eppendorf tubes (1ml / tube). The tubes were heated at 50°C, 60°C or 70°C for 10 min. An aliquot (0.4 ml) of each was plated out on MRS agar and incubated in an anaerobic jar for 72 hours at 37°C. A small number of colonies were detected after heating at 70°C. Distinct colonies were picked off, seeded into Hungate tubes containing MRS broth and incubated for 48 hours at 37°C.

In a second study, a small amount of frozen faeces (100 mg) was dispersed in 5ml of maximum recovery diluent (MRD). Sediment was allowed to settle out and the upper layer was decanted into eppendorf tubes (1ml / tube). The tubes were heated at 50°C for 20 min, 50°C for 20 min plus 60°C for 20 min or 50°C for 20 min plus 60°C for 20 min plus 70°C for 20 min. An aliquot (0.5 ml) of each was plated out on MRS agar and incubated in an anaerobic jar for 48 hours at 37°C. A small number of colonies were detected, picked off, seeded into Hungate tubes containing MRS broth and incubated for 48 hours at 37°C.

In the third study, an overnight culture (10ml) of isolated pig LAB was centrifuged (1000g x 5 min at room temperature), the pellet re-suspended in fresh MRS broth (10ml). An aliquot (1 ml) was heated at 70°C for 15 min and then plated out (0.5 ml) out on MRS agar and incubated in an anaerobic jar for 48 hours at 37°C. A small number of colonies were detected, picked off, seeded into Hungate tubes containing MRS broth and incubated for 48 hours at 37°C. This culture was centrifuged, re-suspended in MRS broth, heated again at 70°C for 15 min, plated out on MRS agar, incubated in an anaerobic jar for 48 hours at 37°C, picked off, seeded into Hungate tubes containing MRS broth and incubated for 48 hours at 37°C. As before, this culture was centrifuged, re-suspended in MRS broth, re-heated at 70°C for 15 min, plated out (0.5 ml) out on MRS agar, incubated in an anaerobic jar for 48 hours at 37°C, picked off, seeded into Hungate tubes containing MRS broth and incubated for 48 hours at 37°C.

Stability of freeze dried bacteria: Overnight cultures of LAB were centrifuged (1000 g x 5 min at room temperature. Pellets were re-suspended in 2 ml sterile PBS and re-centrifuged. The subsequent pellets were then re-suspended in 5ml of freezing solution [defatted skimmed milk powder (SKP), 100g/l; SKP + lactose, both 100g/l; SKP + sucrose, both 100g/l; or SKP, 200g/l]. The samples were frozen at -20°C (2-3 hours) and then stored at -80°C overnight. They were freeze-dried for 48 hours and dried material stored at room temperature. Viable bacteria in the samples were determined

at 0 and approximately 40 and 80 days after completion of freeze drying. They were plated out on MRS agar and incubated anaerobically for 48 hours at 37°C.

Bulk preparation of GGDK31 and GGDK266: Two 500 ml batches of MRS broth were prepared in 500ml glass screw-top bottles, autclaved and allowed to cool to room temperature (in proximity to gas flame) whilst being flushed with CO_2. Four ml of a 24 hour culture of GGDK31 or GGDK266 was added to each bottles of MRS and the lids lightly closed. The bottles were placed in an anaerobic jar and incubated at 37°C for 24 hours. The culture was centrifuged [1000g x 5 min at room temperature] in 6 sterile 50 ml centrifuge tubes. The supernatant was discarded, tubes refilled with culture and re-centrifuged until all the bacteria had been recovered. Each of the 6 tubes contained almost equal amounts of bacteria. The bacteria in each tube were re-suspended in 40ml of sterile PBS, re-centrifuged and the supernatant discarded. he bacteria in each tube was re-suspended in 20 ml of SKM (100g/l), frozen at -20°C (2-3 hours) and then overnight at -80°C, freeze-dried for 48-72 hours and stored at 4°C. To evaluate viable bacteria in the sample, one tube of freeze dried material was re-suspended in 20 ml of MRS broth, incubated at room temperature for 2 hours, diluted, plated out on MRS agar and incubated anaerobically for 48 hours at 37°C.

***L. mucosae in vivo* Study 1:** Sixteen (6 week) old female C3H/HeN mice were dosed with an overnight culture of vial 323 (*L. mucosae*; 50 µl; >109 CFU) at day -7, -4, -2 and 0 and daily thereafter up to day +9. A further 16 mice (control) were given media. On day 0, eight mice *(L. mucosae*-treated) and eight control mice were given, by gavage, a single dose of *Salmonella enteritidis* S1400 (50 µl; ≥108 CFU). In addition, eight mice *(L. mucosae*-treated) and eight control mice were given a single dose of culture medium. Body weight and health score were monitored twice daily post-salmonella infection. The mice were euthanased (isoflurane overdose and exsanguination) and dissected at 10 days post-salmonella infection. Stomach, representative portions of jejunum and ileum, caecum plus contents, colon plus contents, spleen and liver and one kidney and the mesenteric lymph node were collected under near aseptic conditions for microbiology. Representative portions of upper jejunum, mid jejunum, ileum, caecum and ascending and descending colon were placed in neutral buffered formalin or RNA-later and stored for future analysis.

***L. mucosae in vivo* Study 2:** Five (6 week) old female C57Bl/6 mice were dosed with an overnight culture of vial 323 (*L. mucosae*; 50 µl; >109 CFU) at day -7, -4, -2 and 0 and daily thereafter up to day +5. A further 5 mice were given media. On day 0, all ten mice were given, by gavage, a single dose of *Salmonella enteritidis* S1400 (50 µl; ≥107CFU). The mice were euthanased and dissected on day 6, according to the procedure for study 1.

Novel pig LAB *in vivo*: Four (6 week) old female C3H/HeN mice were dosed with an overnight culture of RINH vial 31 (L. reuteri; 50 µl; >109 CFU), four with RINH vial 32 (L. reuteri). Four with vial 323 (L. mucosae), four with RINH vial 46 (L. reuteri), four with RINH vial 47 (L. reuteri) and eight with MRS. This was done at day -6, -4, -2 and 0 and daily thereafter up to day +9. On day 0, all lactobacilli-treated mice and four control mice were given, by gavage, a single dose of *Salmonella enteritidis* S1400 (50 µl; ≥108 CFU). In addition, the remaining four control mice were given a single dose of culture medium. The mice were euthanased and dissected on day 10, according to the procedure for study 1.

Microbiology: Tissues were homogenised [1:100 w/v] in MRD using a Janke-Kunkel Ultra-Turrax T25 tissue homogeniser at 20,000 rpm for 30 seconds, as were jejunal and ileal contents. Up to eight sequential dilutions (1:10 v/v) of the primary homogenates were made, plated out onto XLD agar and MacConkey No. 3 agar and incubated overnight at 37°C. Viable counts were estimated as before [Robertson et al, 2003].

Statistical analysis: Where appropriate data were initially assessed by one-way analysis of variance (ANOVA) regarding treatment outcome. If ANOVA indicated that there were significant differences ($p<0.05$) amongst all groups, the data was then analysed by the Tukey-Kramer Multiple Comparisons Test or the Kruskal-Wallis Multiple Comparisons Test as appropriate. This was done using the Instat Statistical Package (GraphPad Software Inc., San Diego, USA).

Based on the outputs from the multiple comparison tests, means in tables or graphs were marked with superscript letters. Means that differed significantly from each other ($p<0.05$) were allocated distinct superscript letters. Means that did not differ significantly from each other were allocated common superscript letters.

Results

1. Isolation of LAB

Faeces from organically-reared piglets were plated out on selective agars and were incubated under anaerobic conditions. From all studies, a total of 436 individual colonies of Lactic Acid Bacteria [LAB] were picked off, seeded into MRS broth and incubated under anaerobic conditions. Each culture was given a unique RINH vial number and an aliquot was frozen down in MRS media containing 30% glycerol and L-cysteine (~2 mg/ml) and stored at -80°C. 16S rRNA gene analysis was done and bacterial strains were identified by comparison of sequences with known bacterial DNA sequences (Table 1).

The majority of the cultured LAB colonies were *L. johnsonii and L. johnsonii*-related strains [*L. johnsonii, L. johnsonii / gasseri, L. johnsonii / taiwanensis*] (240 / 436) and *L. reuteri* or *L. reuteri*-related [*L. reuteri, L. reuteri / pontis, L. reuteri / vaginalis, L. reuteri / acidophilus* (169 / 436)]. There were 7 *L. plantarum / pentosus* colonies, 19 other species and 5 uncultured strains.

2. Anti-salmonella activity *in vitro*

Conditioned media from isolated LAB were screened for anti-bacterial activity against *Salmonella enteritidis* S1400 using a well-diffusion assay (Figure 1).
Conditioned media from individual colonies of LAB varied greatly in their activity against *S. enteritidis* (Figures 2a). This was not strain dependent. The range of anti-salmonella activities amongst *L. johnsonii* was similar to that amongst *L. reuteri.* On an arbitrary basis, the cultures were separated into groupings on the basis of their capacity to inhibit salmonella *in vitro* (Figure 2b). Group 1 had <20000 units of inhibition, Group 2 20000-40000 units of inhibition, Group 3 40000-60000 units of inhibition, Group 4 60000-80000 units of inhibition, Group 5 80000-100000 units of inhibition and Group 6 >>100000 units of inhibition (Figure 2b). Group 1 comprised of 14 strains (3.4% of total), Group 2 of 95 strains (22.8%), Group 3 of 99 strains (23.7%), Group 4 of 99 strains (23.7%), Group 5 of 86 strains (20.6%) and Group 6 of 24 strains (5.8%). The latter group comprised of seventeen *L. johnsonii and L. johnsonii*-related, six *L. reuteri* or *L. reuteri*-related strains and one uncultured strain.

3. Anti-E. coli K88 activity *in vitro*

Conditioned media from LAB were also screened for anti- *Escherichia coli* K88 activity by the well diffusion assay. Activity against *E. coli* K88, as with salmonella, varied greatly between individual colonies of LAB (Figure 3a). The range and variation in the activity was similar amongst the *L. johnsonii* and *L. reuteri* strains. In general, there was no direct correlation between the anti-salmonella and anti *E. coli* K88 activities for any individual LAB (Figure 3c, 3d). However of the ten strains in *E. coli* K88 group 5 (Figure 3b), seven had relatively high activities against both pathogens, two had high activity against E. coli K88 but moderate activity against salmonella and one was active primarily against *E. coli* K88 .

4. Initial selection of candidate LAB

Thirty-three strains were identified for further testing *in vitro* (Table 2).

These comprised 18 *L. johnsonii* and *L. johnsonii*-related strains, 11 *L. reuteri* or *L. reuteri*-related and 4 *L. plantarum* and *L. plantarum*-related strains (Table 2a).

5. Attachment / invasion of Pig Intestinal Epithelial [IPEC-J2] Cells

The capacity of LAB to block adhesion / invasion of IPEC cells by *S. enteritidis* and *E. coli* K88 was evaluated (Figure 4a, 4b, 4c). The candidate LAB all greatly reduced attachment and invasion of IPEC cells by salmonella. Most of them were also very effective against E. coli K88. However, 3 of the strains had only limited effects on adhesion / invasion of IPEC cells by E. coli K88.

6. Susceptibility of LAB to antibiotics.

Thee susceptibility of the candidate LAB to a range of antibiotics was evaluated (Table 4, Figure 5). All but one strain (RINH vial 266) exhibited some degree of resistance to individual antibiotics. All were susceptible to ampicillin (10 µg), cefotaxime (30 µg) and chloramphenicol (10 µg). The majority were susceptible to erythromycin (15 µg), gentamicin (10 µg), tetracycline (30 µg) and vancomycin (30 µg). Most strains were resistant to metronizadole (50 µg) and nalidixic acid (30 µg) and to a lesser extent kanamycin (30 µg). 23

7. Refined selection of candidate LAB

Twenty-three high ranking strains were identified for further testing *in vitro*.

8. Substrate specificity of LAB

The candidate LAB were screened for substrate reactivity using an API CH 50 kit (Table 5, 6, Figure 6). *L. johnsonii*, *L. reuteri* and *L. plantarum* each exhibited strain-specific general substrate reaction profiles. In addition, most strains of each genotype exhibited fine differences in their substrate reactivity, indicative that they were unique individual strains.

9. Suppression of inflammation in Pig Intestinal Epithelial [IPEC-J2] Cells

The ability of candidate LAB to block or suppress inflammatory responses triggered in IPEC cells by 12-O-Tetradecaboylphorbol-13-acetate [PMA] was tested (Figure 7; Table 7). The candidate strains varied greatly in their capacity to block interleukin-8 (IL-8) gene-expression triggered by PMA. Five strains (RINH vial 29, 30, 31 86 and 266) had potent anti-inflammatory effects.

10. Final selection of candidate LAB

Fourteen strains were identified having killing and blocking activities against salmonella and *E. coli* K88, susceptibility to antibiotics carbohydrate reactivity and capacity to suppress inflammation *in vitro*. Seven of these were particularly preferred. The latter set comprised 4 *L. plantarum*-related, 3 *L. johnsonii*-related and one *L. reuteri*. Two of these LAB strains [GGDK266 and GGDK31] were prepared in bulk for evaluation in a trial with newly-weaned piglets (Table 8).

11. Freeze drying and storage of LAB

The survival and viability of LAB after freeze drying in skimmed milk powder [SKP], SKP plus lactose or SKP plus sucrose was evaluated (Figure 8). Small losses in viability were evident on storage for 42 and 84 days at room temperature of samples dried in SKP. This was less marked when skimmed milk powder and sugars were used in combination. However, the 24 latter preparations tended to be hygroscopic and difficult to maintain. Bulk preparations of GGDK266 and GGDK31 were therefore prepared by drying the bacteria in skimmed milk powder [100g/l] (Table 8).

12. Heat-treatment studies

Suspensions of faeces from organically reared pigs were heat treated for varying periods of time at 50-70°C, plated out on MRS agar, colonies picked off and cultured in MRS broth [RINH vial 417-506]. The strain types recovered were variable and

clostridium species formed a high proportion, the isolated strains remained sensitive to heat.

Isolated cultures of LAB were subject to heating three times for 15 minutes at 70°C Figure 9). Viable bacteria decreased by 3-4 log orders after heat-treatment for the first time. However, the surviving bacteria had a degree of heat-resistance. With one exception, losses of viable bacteria were low when the bacteria were re-cultured and re-heated a further two times.

Heat-treatment three times at 70°C altered the biological activities of the strains Figure 9. RINH vial 521 (vial 255 heat-treated) was not able to block attachment of pathogens to IPEC cells and the capacity of RINH vial 520 (vial 230 heat-treated) to prevent attachment was reduced. The ability of RINH vial 517 (vial 31 heat-treated) to abolish inflammatory responses triggered in IPEC cells was abolished. In contrast, the biological properties of RINH vial 518 (vial 85 heat-treated) and RINH vial 519 (vial 86 heat-treated) were similar to those of the native strains.

13. Mouse infection studies

13.1 *L. mucosae* (RINH vial 323)

C3H/HeN mice develop a persistent but non-lethal, intestinal and systemic infection, which has many characteristics of the major form of human salmonellosis, when challenged with high levels of *Salmonella enteritidis* S1400. In contrast, C57Bl/6 mice develop a severe primarily systemic, infection, reminiscent of acute infection in humans, when challenged with the same pathogen. To evaluate the capacity of *L. mucosae* (vial 323) to ameliorate salmonellosis, C3H/HeN and C57Bl/6 mice were treated with L. mucosae prior to and post-challenge with *Salmonella enteritidis* (Figures 10, 13). The mice were euthanased and dissected 6 (C57Bl/6) or 10 (C3H/HeN) days post-infection.

Systemic tissues: Oral treatment with *L. mucosae* limited the capacity of *S. enteritidis* to cause systemic infection both in C3H/HeN and C57Bl/6 mice (Figure 11a-c; 14a-c). High numbers of viable salmonella were detected in the mesenteric lymph node, liver and spleen of mice. In contrast, the numbers present in these tissues were greatly reduced if the mice had been co-treated with RINH vial 323 (*L. mucosae*). Salmonella infection caused enlargement of the spleen (Figure 12a; 15). This tissue response was

significantly reduced in mice treated with both RINH vial 323 (*L. mucosae*) and salmonella.

Intestine: Intestinal myeloperoxidase [MPO], a marker for neutrophils, was determined in C3H/HeN mice treated with salmonella or salmonella plus RINH vial 323 (*L. mucosae*). MPO in the intestine was greatly increased by salmonella infection, due to recruitment of neutrophils to the intestine part of the host response to infection (Figure 12b), Co-treatment with RINH vial 323 (*L. mucosae*) reduced MPO activity in the intestine of salmonella-infected mice, indicating that the intestinal inflammatory responses to infection were lowered in these animals.

13.2 Novel pig LAB

Four LAB were selected: RINH vial 31, RINH vial 32, RINH vial 46 and RINH vial 47 (All *L. reuteri*; LR31, LR 32, LR 36 and LR47 respectively). To assess their efficacy to ameliorate a pathogen infection, C3H/HeN mice were treated with these LAB or RINH vial 323 (*L. mucosae*, LM] prior to and post-challenge with *Salmonella enteritidis* (Figure 16). The mice were euthanased and dissected 10 days post-infection. Faecal excretion of *S. enteritidis* was reduced, if the mice had been co-treated with LAB (Figure 17a, b). LR31 and LR32 tended to have the greatest effects on faecal salmonella outputs.

Intestine: Treatment with LR31, LR32, LM, LR46 or LR47 significantly reduced the numbers of salmonella in the caecum (Figure 18a). Furthermore, LR31, LR32, LR46 and LR47 but not LM lowered salmonella numbers in the colon (Figure 18 b). The reductions tended to be greater with LR31 and LR32. In contrast to the large intestine, the LAB had no significant effects on numbers of salmonella in the small intestine.

Systemic tissues: Treatment with LR31, LR32, LM, LR46 or LR47 greatly reduced the numbers of salmonella detected in the spleen and liver (Figures 19a-c). The reductions were more marked with LR31 and LR32 than with LM, LR46, or LR47. Salmonella numbers in the mesenteric lymph node were lowered following treatment with LR31, LR32 and LR46 but not with LM or LR47.

Discussion

The LAB strains isolated (total of 436 individual colony picks) from faeces of organically-reared pigs were predominantly *L. reuteri*, *L. johnsonii*, *L. gasseri, L. pentosus,* strains with a small number of *L. plantarum*, *L. acidophilus*, *L. vaginalis*, a single *L. mucosae* and several uncultured strains. Most of the LAB produced substances that could inhibit the growth of *S. enteritidis* and / or *E. coli K88 in vitro.* The potency of these anti-pathogen effects varied greatly between the individual bacterial strains. A proportion of LAB had high activity against *S. enteritidis* but low activity against *E. coli* K88 and vice-versa, but the majority had similar activities against both pathogens.

Thirty-three strains were selected on the basis of anti-microbial potency as determined *in vitro.* These bacteria were further screened for their ability to block adherence/invasion of intestinal pig epithelial cells (IPEC) by pathogens *in vitro* and their susceptibility to antibiotics.

Twenty-three strains were assayed for substrate range and specificity and their capacity to suppress inflammation in IPEC cells *in vitro.* From these, fourteen LAB (5 *L. johnsonii*, 6 *L. reuteri* and 3 *L. Plantarum*) with particularly favourable properties were identified.

Two LAB strains [GGDK266 and GGDK31] were prepared in bulk for *in vivo* evaluation in newly-weaned piglets. Other potentially important candidate strains were present in this set of 14 LAB.

The survival and viability of LAB after freeze drying in various solutions was also evaluated. Small losses in viability were evident on prolonged storage of samples dried with skimmed milk powder. This was less marked when skimmed milk powder and sugars were used. However, the latter preparations were hygroscopic and were difficult to maintain. It was therefore decided to use a skimmed milk powder suspension for freeze drying and storage of LAB. The bulk preparations of GGDK266 and GGDK31 were freeze-dried in this medium.

Heat stability is a useful feature for LAB to be used in pelleted animal foods. Five heat-conditioned viable strains of isolated pig LAB were obtained. However, the biological properties *in vitro* and probiotic potential of three of the strains were adversely affected

by heat-treatment. Nonetheless, two of the bacteria retained the biological properties of their native non-heat-treated forms.

Five pig LAB (*L. reuteri* [4] or *L. mucosae* [1]) were tested for ability to ameliorate salmonellosis *in vivo*. Treatment of mice with these LAB greatly reduced the pathogenicity of *S. enteritidis*.

14. Evaluation of Oral Administration of Organic Lactobacilli Probiotic Strains on the modulation of the Gut Microbiota and Performance of early Weaned Pigs

In vivo trials were carried out on early weaned piglets to test the effect of two probiotic strains according to the invention, Lactobacilli strains GGDK266 and GGDK31.

Trial design

Animals:

- 24 Large – White x Redon piglets
- Early weaned (21 days old, ≈ 7 - 8 kg), born in a local farm
- Weighted then distributed equally between the different group
- 3 experimental treatments (n=8):

  A – Basal diet + Placebo

  B – Basal diet + probiotic GDDK 266 - dose 10x 10^{12}

  C – Basal diet + probiotic GDDK 31- dose 10x 10^{12}
- Observation period: 14 days

Diet:

Diets based on barley, wheat & soybean meal

- Feed composition (%):

Barley	36.5
Wheat	21
SBM 48	19

Corn	10
Soy oil	4
Sugar	4
Potato protein	2
Premix	3.5

- feed *ad libitum* in pelleted form

Tissue Sampling and Measurements

Sampling: Day 0 Slaughter of 6 "naïve" piglets for collection of the caecum
Individual collection of faeces (if possible)

Day 7 Individual collection of faeces during weight measurement

Day 14 Slaughter of 24 piglets for collection of:

Content (5g):	Tissus (10cm):
- Gastric	- Jejunum
- Jejunum	- Ileum
- Ileum	- Caecum
- Caecum	- Lymphatic nodes (distal ileum level)

Storage: All samples were weighed, frozen in liquid nitrogen and stored at -80°C.

Performance (1st step): Daily Weight gain (DWG), Feed Intake (FI) and Feed Conversion Ratio (FCR)

Analysis: (2nd step)

- Determination of the microbiota profile in the different gut content samples by the molecular microbiology technique Denaturing gradient gel electrophoresis (DGGE).
- Molecular analysis of gene expression data using pig affymetrix gene expression arrays to determine gene modulation patterns.
- Determination of immunity markers in intestinal tissues

Microbial analysis using Denaturing Gel Gradient Electrophoresis DGGE (Trial 1)

DGGE methodology

DNA is extracted from faecal or tissue samples utilizing the MP Bio FastDNA™ spin kit for soil sample - 116560000. The DNA is then amplified using Muyzer primers, as it is essential to use primers with a GC Clamp to be run on the gel. For samples of lactobacillus, specialised lactobacillus primers with a GC clamp were used.

Target Group	Primer	Primer Sequence (5'-3')	Amplicon Size (bp)	Annealing temperature (°C)	DGGE gradient (%)
All Bacteria	MF MR-GC[a]	ATTACCGCGGCTGCTGG GC-clamp-CCTACGGGAGGCAGCAG	233	55	35-70
LABs	Lac1 Lac2-GC[a]	AGCAGTAGGGAATCTTCCA GC-Clamp-ATTYCACCGCTACACATG[c]	327	55	30-50

Annotations:

[a] The GC clamp is as follows:

CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG

[c] Y = C or T

PCR Program:

Time	Temperature	Cycles
5 minutes	94oC	1
30 seconds	94oC	35
30 seconds	55oC	
2 minutes	72oC	
10 minutes	72oC	1

DGGE is a genetic analysis technique in which amplified PCR products are separated by the denaturants formamide and urea within the gel, based on the genetic sequence by as little as a single base difference. DGGE can be utilised to visualise the differences in microbial diversity between samples. DNA obtained from a range of samples can be used in DGGE e.g. tissue and faecal samples. Bands on the gel were visualised using silver staining.

Molecular analysis and gene expression profiles of pig tissues

RNA extraction and Affymetrix Microarray analysis

RNA was isolated from both animal tissue and cultured cells for use on Affymetrix GeneChips. For animal tissue, approximately 200 mg tissue sample was removed from RNAlater (Ambion) and lyzed in Trizol (Invitrogen) using a polytron homogenizer. The tissue was further homogenized by passing the lysate through a syringe fitted with a 19G needle 3-5 times. The samples were incubated for 5 min at RT to permit the complete dissociation of nucleoprotein complexes. Then, chloroform, isopropanol and ethanol steps were performed according to the manufacturer's instructions. Briefly, 0.2 mL of chloroform was added per 1 mL of Trizol, vortexed and incubated at RT for 5 min. The samples were centrifuged at 12,000 x g for 15 min at 4°C. The resultant aqueous phase was transferred to a fresh tube, and the RNA was precipitated by the addition of 0.5 mL of isopropanol per 1 mL of Trizol. The tubes were shaken vigorously by hand for 10s, incubated at 4°C for 10 min and centrifuged at 12,000 x g for 10 min at 4°C.

The RNA precipitate was washed with ice-cold 75% ethanol, adding at least 1 mL of 75% ethanol per 1 mL of Trizol. The samples were vortexed and centrifuged at 7,400 x g for 5 min at 4 °C. After air-drying the resultant RNA pellet, the RNA was resuspended in up to 100 µL RNase-free water. Total RNA was further extracted with the RNeasy kit (Qiagen) according to the manufacturer's instructions, including an RNase-free DNase I (Qiagen) digestion step.

Cultured cells were homogenized by adding 350 µL Buffer RLT + 1% β-mercaptoethanol. The cells were scraped off culture dishes with a filter tip and further homogenized by passing the lysate through a syringe fitted with a 19G needle 3-5 times. The cell lysate was then further processed using the RNeasy kit (Qiagen) according to the manufacturer's instructions, including an RNase-free DNase I (Qiagen) digestion step.

RNA concentration and integrity was ascertained using a Nanodrop instrument and/or Agilent Bioanalyzer, and purified RNA was stored at -70°C.

250 ng RNA was processed for Affymetrix GeneChips using the GeneChip 3' IVT Express Kit (Affymetrix) according to the manufacturer's instructions. aRNA quality was determined by Agilent 2100 Bioanalyzer. Hybridization to the GeneChip Mouse Genome 430 2.0 and GeneChip Human Genome U133 Plus 2.0 (Affymetrix) on a GeneChip Fluidics Station 450 (Affymetrix) was performed at the Institute of Medical Sciences Microarray Core Facility (University of Aberdeen, UK). Chips were scanned with an Affymetrix GeneChip Scanner 3000 (Affymetrix). Image quality analysis was performed using Gene Chip Operating Software (GCOS) (Affymetrix). Further quality analysis, normalization (gcRMA), statistical analysis and heatmap generation was performed with the freely available software packages R (http://www.r-project.org) and Bioconductor (http://www.bioconductor.org). Microarray data were submitted to the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo).

Results

Performance of pigs fed probiotics GGDK266 and GGDK31

The results for pigs fed probiotics GGDK266 and GGDK31 are shown in Figure 20.

DWG (Daily weight gain), FI (food intake) and FCR (feed conversion ratio) are shown below:

GGDK266	DWG	FI	FCR
d0-d7	+++ (*)	+	+
d7-d14	=	+	+
d0-d14	+	+	+

Piglets fed GGDK266 exhibited significantly improved daily weight gain (DWG) during the first week post-weaning relative to GGDK31 and placebo fed piglets.

Microbial diversity analysis using DGGE (Trial 1)

DGGE using universal primers revealed no differences in overall microbial diversity between the treatments and placebo (see Figure 21).

DGGE using lactic acid bacteria (LAB) specific primers revealed significant differences in LAB diversity between treatment with GGDK 266 and placebo in both caecal and ileal samples (see Figure 22).

DGGE using LAB specific primers revealed significant differences in LAB diversity between the treatment with GGDK266 and placebo in ileal samples (see Figure 23).

DGGE using LAB specific primers revealed significant differences in LAB diversity between the treatment with 266 and placebo in caecal samples (see Figure 24).

Overall the microbial diversity analysis revealed significant clustering of the LAB population in piglets fed GGDK266 indicating that the populations in individual animals on this treatment has a similar and stable microbiota.

Molecular analysis of ileal tissue samples: Affymetrix pig arrays

Downregulated in GDK266 versus placebo

Gene ontology analysis of differentially expressed gene revealed that a significant reduction in immune system processes and pro-inflammatory activation in response to feeding young piglets probiotic GGDK266 relative to placebo (see Figure 25).

Results reveal that GGDK266 had a very specific and targeted effect on the immune system and the functional groups associated with response to stimuli (see Figure 26).

Upregulated in GGDK266 versus Placebo

In contrast to the effects on the immune system, GGDK266 promoted metabolic processes particularly in relation to nitrogen (see Figure 27). Without wishing to be bound by theory, it is believed that these effects may explain the improved DWG in animals fed GGDK266.

Top differentially expressed genes between GGDK266 and Placebo

affy.id	Gene Name	Product	FC	p-value
Ssc.645.1.S1_at	CSTA	Cystatin A	44.06	0.00000
Ssc.11608.1.A1_at	TIP_HUMAN	T-cell immunomodulatory protein precursor	28.92	0.00030
Ssc.10837.1.A1_at	ROBO1	Roundabout homolog 1 precursor	13.35	0.00178
Ssc.8960.1.A1_at	BPI	Bactericidal permeability-increasing protein precursor	11.65	0.00476
Ssc.16234.1.S1_at	TCN1	Transcobalamin I precursor	11.48	0.00023
Ssc.1411.1.S1_at	THBS4	Thrombospondin 4 precursor	8.92	0.00198
Ssc.837.1.A1_at	BPI	Bactericidal permeability-increasing protein precursor	4.55	0.00573
Ssc.30008.1.A1_at	ESR1	Estrogen receptor	4.48	0.00053
Ssc.13539.1.A1_at	PLAGL1	Zinc finger protein PLAGL1	4.42	0.00881
Ssc.26324.1.S1_at	NP_981932	Iodotyrosine dehalogenase 1 protein	4.26	0.00200
Ssc.29413.1.A1_at	B3GALT2	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 2	4.00	0.00046
Ssc.27410.1.S1_at	MYCN	N-myc proto-oncogene protein	3.80	0.00261
Ssc.25176.1.A1_at	GOLPH4	Golgi phosphoprotein 4	3.80	0.00009
Ssc.15890.1.S1_at	VNN1	Pantetheinase precursor	3.61	0.00271
Ssc.23427.1.A1_at	CYB561	Cytochrome b561	3.29	0.01512
Ssc.16186.1.S1_at	CD3E	T-cell surface glycoprotein CD3 epsilon chain precursor	-2.62	0.00764
Ssc.22676.1.S1_at	CXCR6	C-X-C chemokine receptor type 6	-2.63	0.01652
Ssc.15565.1.S1_at	LCP2	Lymphocyte cytosolic protein 2	-2.76	0.00024
Ssc.18652.1.S1_at	IL16	Interleukin-16 precursor	-2.97	0.01132
Ssc.181.1.S1_at	TRGV9	T-cell receptor gamma chain V region PT-gamma-1/2 precursor	-3.04	0.01615
Ssc.23489.1.S1_at	CD8A	T-cell surface glycoprotein CD8 alpha chain precursor	-3.08	0.00071
Ssc.428.6.S1_a_at	TCA_HUMAN	T-cell receptor alpha chain C region	-3.15	0.00027
Ssc.10357.1.A1_at	FMN2	Formin 2	-3.46	0.00308
Ssc.27354.1.S1_at	STXBP5	Tomosyn	-3.88	0.02438
Ssc.28909.3.A1_at	TPH2	Tryptophan 5-hydroxylase 2	-4.36	0.00717
Ssc.25976.1.S1_at	GZMH	Granzyme H precursor	-5.46	0.00179
Ssc.11070.1.S1_at	IGHM	Ig alpha-1 chain C region	-9.07	0.00115
Ssc.16566.1.S1_at	LCT	Lactase-phlorizin hydrolase precursor	-11.31	0.00328
Ssc.13273.1.A1_at	GCNT3	glucosaminyl (N-acetyl) transferase 3, mucin type	-19.75	0.00016
Ssc.11098.1.S1_at	IFITM3	Interferon-induced transmembrane protein 3	-51.36	0.00044

Gene expression data revealed that a number of genes were significantly increased including antimicrobial peptides (eg. CSTA, BP1) and immune-regulatory genes (TIP). In contrast GGDK266 reduced the expression of a diverse panel of genes involved in pro-inflammatory immunity (IFITM3, IL-16).

Conclusions

- Cellular and metabolic processes, particularly in relation to nitrogen, are increased in animals treated with GGDK266 relative to placebo.
- Immune system processes are downregulated in animals treated with GGDK266 relative to placebo. Examples include T-cell markers CD3 and CD8, T cell receptor chains, chemokines/cytokines and IFN-related genes.
- Animals administered with GGDK266 exhibited a stable population of lactic acid bacteria revealed by clustering of the bacterial profile of the individual induced by the adminiatration of probiotic GGDK266.
- FCR and performance were significantly improved during the first weeks of post-weaning life.
- This improvement in growth performance correlated with the reduction in inflammatory immune responses and the increase in specific metabolic processing.

Various modifications and variations of the described aspects of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes of carrying out the invention which are obvious to those skilled in the relevant fields are intended to be within the scope of the following claims.

Table 1: Summary of bacteria colonies selected from cultures of faeces from organically-reared pigs.

Total number of cultured colony picks	443
Media:	
LAMVAB agar	55
LAMVAB agar + pig colostral carbohydrate	88
MRS agar	29
MRS agar + pig colostrum carbohydrate	176
Glucose-free MRS agar + carbohydrate	57
MRS agar after heat-treatment at up to 70°C	38
Main strains identified:	
Lactobacillus reuteri	
Lactobacillus johnsonii	
Lactobacillus plantarum	

Five isolated LAB were heated once, twice or three times at 70°C for 15 min. Surviving bacteria were re-grown.

In stock

5 LAB heated once at 70°C

5 LAB heated twice at 70°C

5 LAB heated three times at 70°C

Table 2: Candidate LAB strains for further study selected on the basis of killing activity in well diffusion assays (note 266 and 161 contain LR)

RINH Vial no.		Pathogen killing (units) Well diffusion assay	
		anti-SE	anti-KSS
85	LR	129886	60168
255	LJ	101477	64390
266	LJ	101335	60168
436	LJ	81656	85010
161	LP	77894	103346
12	LJ	162709	42977
16	LJ	117621	41365
29	LR	174471	45720
31	LR	116867	46907
86	LR	98520	75147
230	LJ	95705	64340
256	LJ	94012	77459
314	LJ	103497	48936
361	LJ	100770	40254
17	LJ	144765	23072
30	LR	125463	36050
32	LR	168892	32572
258	LP	70724	68612
260	LP	78197	68562
320	LJ	66350	78044
364	LJ	99137	55123
433	LJ	95083	51461
15	LP	77459	58669
218	LJ	62329	50416
220	LJ	68612	53834
356	LJ	72986	55302
363	LJ	79125	45555
131	LR	42223	44108
434	LR	10000	81656
166	LJ	17064	79621
431	LR	48657	31674
47	LR	20722	34633
46	LR	19867	34633

LJ. L. johnsonii. LR. L. reuteri. LP. L. Plantarum

Table 2a: Identification of candidate LAB strains (by 16S rRNA gene sequence) selected on the basis of killing activity in well diffusion assays (note 266 and 161 contain LR)

RINH Vial no.	forward sequence	reverse sequence
85	Lactobacillus reuteri	Lactobacillus reuteri
255	Lactobacillus johnsonii, taiwanensis, acidophilus	Lactobacillus johnsonii, gasseri
266	Lactobacillus johnsonii	Lactobacillus johnsonii
436	lactobacillus johnsonii str. 466	lactobacillus johnsonii F19785
161	Lactobacillus plantarum, pentosus, paraplantarum	Lactobacillus plantarum, pentosus
12	Lactobacillus johnsonii, gasseri, taiwanensis	Lactobacillus johnsonii, gasseri
16	Lactobacillus johnsonii, gasseri, taiwanensis	Lactobacillus johnsonii
29	Lactobacillus reuteri, pontis, vaginalis, frumenti	Lactobacillus reuteri
31	Lactobacillus reuteri	Lactobacillus reuteri
86	Lactobacillus reuteri	Lactobacillus reuteri
230	Lactobacillus johnsonii, taiwanensis, acidophilus	Lactobacillus johnsonii
256	Lactobacillus johnsonii, taiwanensis, acidophilus	Lactobacillus johnsonii
314	lactobacillus johnsonii BR0315	uncultured bacterium
361	lactobacillus johnsonii str. NCC2822	lactobacillus johnsonii F19785
17	Lactobacillus johnsonii, gasseri, taiwanensis	Lactobacillus johnsonii
30	Lactobacillus reuteri, pontis	Lactobacillus reuteri
32	Lactobacillus reuteri	Lactobacillus reuteri
258	Lactobacillus plantarum, pentosus, helveticus	Lactobacillus plantarum, pentosus, paraplantarum
260	Lactobacillus plantarum, pentosus, paraplantarum	Lactobacillus pentosus, plantarum, paraplantarum
320	lactobacillus johnsonii NCC2822	Lactobacillus johnsonii F19785
364	lactobacillus johnsonii 466	lactobacillus johnsonii F10785
433	lactobacillus johnsonii str. CECT 289	lactobacillus johnsonii F19785
15	Lactobacillus plantarum, pentosus	Lactobacillus plantarum, pentosus
218	Lactobacillus johnsonii, taiwanensis	uncultured Firmicutes, Lactobacillus johnsonii
220	Lactobacillus johnsonii, taiwanensis	uncultured Firmicutes, Lactobacillus johnsonii
356	lactobacillus johnsonii NCC2822	lactobacillus johnsonii F19785
363	lactobacillus johnsonii 466	lactobacillus johnsonii F10785
131	Lactobacillus reuteri	Lactobacillus reuteri
434	Lactobacillus reuteri NM99-1	lactobacillus reuteri
166	Lactobacillus johnsonii, taiwanensis, acidophilus	Lactobacillus johnsonii
431	lactobacillus reuteri str.Probio-16	lactobacillus reuteri JCM 1112
47	Lactobacillus reuteri	Lactobacillus reuteri
46	Lactobacillus reuteri	Lactobacillus reuteri

Table 3: Candidate LAB strains for further study selected on the basis of killing activity in well diffusion assays and capacity to block adherence of pathogen to IPEC cells

RINH Vial no.	Inhibition of adherence (%)	
	SE	KSS
85	88.31	87.93
255	82.37	99.93
266	88.03	98.09
161	98.32	96.94
12	96.89	99.92
29	93.7	99.91
31	98.64	99.75
86	81	99.98
256	82.47	99.92
361	85.07	99.44
17	84.56	99.66
30	96.44	99.91
32	87.74	99.86
230	78.89	82.45
258	96.37	86.5
260	90.22	88.79
314	79.68	94.2
433	99.99	96.23
16	87.68	45.38
218	91.53	86.49
363	85.61	99.93
364	82.13	78.12
15	79.19	99.52
131	95.5	96.03
220	91.04	78.6
320	92.7	44.17
356	82.15	78.4
434	94.78	98.85
436	99.97	1
166	91.45	95.97
431	96.35	86.47
47	90.47	99.47
46	83.51	99.7

Table 4: Area of inhibition of LAB by defined amounts of antibiotic (arbitrary units)

	ampicillin	cefotaxime	chloramphenicol	erythromycin	gentamicin	kanamycin	metronizadole	nal. acid	tetracycline	vancomycin
12	244011	340402	186699	13151	0	0	0	0	37668	22581
15	277117	311725	204282	214008	0	0	0	0	0	0
16	266033	294166	187805	64681	17000	7157	0	0	0	105209
17	387224	400570	235430	277145	9193	0	0	0	50328	117741
29	410335	444193	190293	114511	0	0	0	0	252497	11483
30	292728	335927	77133	208117	31261	0	0	0	187805	31402
31	334789	410966	165904	262226	38221	0	0	0	214037	24901
32	404496	402291	247436	350238	71608	23786	0	0	261979	10691
46	359232	402588	210421	251461	29550	0	0	0	21382	25069
47	328283	410579	185515	270105	30342	0	0	0	211556	22231
85	356114	369916	204992	309439	0	0	0	0	276800	3971
86	250812	381270	183399	250805	41858	0	31264	0	16643	13355
131	349955	473065	248521	123562	82466	14932	0	0	19354	7479
161	338497	412977	258724	261133	51991	4536	29126	0	20435	5542
166	268783	417393	185508	251607	61136	17671	0	0	24606	0
218	209117	271547	148617	0	0	0	0	0	88668	122870
220	209371	319970	165815	34230	58814	32572	0	0	34636	111666
230	254614	335143	164405	51078	65717	45705	0	0	36644	41991
255	330364	392169	217758	59224	56563	8486	0	0	29872	0
256	456892	502325	228531	71258	93058	0	0	0	20955	42203
258	401257	271932	195909	233326	28608	0	0	0	223143	0
260	286400	364573	203796	33393	78821	78364	0	0	21757	62792
266	287070	322869	198614	247085	54008	3079	6437	2737	48286	107882
314	297057	332853	154830	44115	0	0	0	0	24194	90259
356	291920	339895	203692	62656	10472	5890	0	0	0	8202
361	320695	323713	201886	234140	0	0	0	0	28212	91863
363	275304	308159	193271	44491	86683	0	0	0	18322	18146
364	288514	341651	194320	143978	45880	0	0	0	0	103995
431	339016	380459	226484	311725	74991	0	0	0	0	26302
433	241710	203588	174124	63381	19139	0	0	0	19965	79034
434	198112	261065	172223	68052	6049	0	0	0	60344	45863
436	290458	287331	185812	142842	0	0	0	0	52279	61810

Nal. Acid, naladixie acid.

Table 5: Substrates in capsules of API CH 50 Kit

	Substrates in capules of API CH 50 kit	
1	glycerol	polyol
2	erythritol	polyol
3	D-arabinose	monosaccharide
4	L-arabinose	monosaccharide
5	D-ribose	monosaccharide
6	D-xylose	monosaccharide
7	L-xylose	monosaccharide
8	D-adonotol	alcohol
9	Methyl-βD-Xylopyranoside	cyclic
10	D-galactose	monosaccharide
11	D-glucose	monosaccharide
12	D-fructose	monosaccharide
13	D-mamose	monosaccharide
14	L-sorbose	monosaccharide
15	L-rhamose	monosaccharide
16	dulcitol	monosaccharide/alcohol
17	inositol	polyol
18	D-mamitol	polyol
19	D-sorbitol	sugar/alcohol
20	Methyl-αD-Mannopyranoside	cyclic
21	Methyl-αD-Glucopyranoside	cyclic
22	N-acetylglucosamine	monosaccharide
23	amygdalin	glycoside
24	arbutin	glycoside
25	esculin ferric citrate	
26	salicin	glycoside
27	D-cellobiose	disaccharide
28	D-maltose	disaccharide
29	D-lactose (bovine)	disaccharide
30	D-Melibiose	disaccharide
31	D-saccharose	disaccharide
32	D-trehalose	disaccharide
33	inulin	polysaccharide
34	D-melezitose	trisaccharide
35	D-rafinose	trisaccharide
36	amidon (starch)	polysaccharide
37	glycogen	polysaccharide
38	xylitol	monosaccharide/alcohol
39	gentiobiose	disaccharide
40	D-turanose	disaccharide
41	D-lyxose	monosaccharide
42	D-tagatose	monosaccharide
43	D-fucose	monosaccharide
44	L-fucose	monosaccharide
45	D-arabitol	monosaccharide/alcohol
46	L-arabitol	monosaccharide/alcohol
47	potassium gluconate	sequestrant
48	potassium 2-ketogluconate	sequestrant
49	potassium 5-ketogluconate	sequestrant

Table 6: Substrate profile of LAB using an API CH 50 kit

	monosaccharides	alcohol/ monosaccharides	disaccharides	trisaccharides	polysaccharides	alcohols	others
17	0.4	0.0	0.8	0.5	0.3	0.0	0.4
30	0.2	0.0	0.0	0.0	0.0	0.0	0.3
31	0.3	0.0	0.6	0.5	0.0	0.0	0.6
32	0.3	0.0	0.5	0.5	0.0	0.0	0.3
46	0.2	0.0	0.5	0.5	0.0	0.0	0.4
47	0.2	0.3	0.5	0.5	0.0	0.0	0.4
85	0.1	0.0	0.3	0.0	0.0	0.0	0.4
86	0.3	0.0	0.5	0.5	0.0	0.0	0.4
131	0.4	0.0	1.0	1.0	0.3	0.3	0.9
161	0.7	0.3	0.9	1.0	0.0	0.3	0.9
166	0.4	0.0	0.6	0.0	0.7	0.0	0.3
220	0.1	0.0	0.5	0.5	0.0	0.0	0.4
230	0.3	0.0	0.8	0.5	0.0	0.0	0.4
255	0.1	0.0	0.6	1.0	0.3	0.0	0.4
256	0.2	0.0	0.5	1.0	0.0	0.2	0.6
258	0.6	0.3	1.0	1.0	0.7	0.7	0.9
260	0.4	0.3	0.9	1.0	1.0	0.0	0.6
266	0.3	0.0	0.9	0.5	0.3	0.0	0.4
320	0.3	0.0	0.5	0.5	0.3	0.0	0.3
363	0.4	0.0	0.8	1.0	0.3	0.0	0.4
364	0.4	0.0	0.8	0.5	0.3	0.0	0.4
433	0.2	0.0	0.5	0.0	0.0	0.2	0.3

Table 7: Candidate LAB strains selected on the basis of killing activity, capacity to block adherence of pathogen to IPEC cells, antibiotic susceptibility, substrate reactivity and ability to suppress inflammation (note 266 and 161 contain LR)

RINH Vial no.	forward sequence	reverse sequence
266	Lactobacillus johnsonii	Lactobacillus johnsonii
31	Lactobacillus reuteri	Lactobacillus reuteri
258	Lactobacillus plantarum, pentosus, helveticus	Lactobacillus plantarum, pentosus, paraplantarum
260	Lactobacillus plantarum, pentosus, paraplantarum	Lactobacillus pentosus, plantarum, paraplantarum
255	Lactobacillus johnsonii, taiwanensis, acidophilus	Lactobacillus johnsonii, gasseri
161	Lactobacillus plantarum, pentosus, paraplantarum	Lactobacillus plantarum, pentosus
256	Lactobacillus johnsonii, taiwanensis, acidophilus	Lactobacillus johnsonii
86	Lactobacillus reuteri	Lactobacillus reuteri
85	Lactobacillus reuteri	Lactobacillus reuteri
32	Lactobacillus reuteri	Lactobacillus reuteri
230	Lactobacillus johnsonii, taiwanensis, acidophilus	Lactobacillus johnsonii
131	Lactobacillus reuteri	Lactobacillus reuteri
30	Lactobacillus reuteri, pontis	Lactobacillus reuteri
364	lactobacillus johnsonii 466	lactobacillus johnsonii F10785

Table 8: Identity for pig LAB strains selected for bulk preparation (note 266 and 161 contain LR)

GGDK266

RINH vial no	Seq code primer 926F	Bacteria identified by BLAST	Seq code primer 519R	Bacteria identified by BLAST
266	S10CM218	Lactobacillus johnsonii	S10CM171	Lactobacillus johnsonii

GGDK31

RINH vial no	Seq code primer 926F	Bacteria identified by BLAST	Seq code primer 519R	Bacteria identified by BLAST
31	S10BL123	Lactobacillus reuteri	S10BL141	Lactobacillus reuteri

SEQ ID NO: 1

31 S10BL123 with 926F

GGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAG

GCGTTCCCTTCGGGGACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC

AACGAGCGCAACCCTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAG

GTGGGGACGACGTCAGATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGAGTCGCAA

GCTCGCGAGAGTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGTCGGAAT

CGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTT

TGTAACGCCCAAAGTCGGTGGCCTAACCATTATGGAGGGAGCCGCCTAAGTGCGGGACAGATGACTGGGGTGAAGTCGTA

ACAAGGTAGCCTGTATTTTCTTGCGGTTGTTCCCCCCCCNGGCGGGACTGCCTTACTCCTTTCACCNCCCGCGCCCCTGG

AGGGGGCCGGAACCCCCCTCCCAACCCCCCTAACCCACCTCCTTCCTTTTAACCNGCT

SEQ ID NO: 2

31 S10BL141 with 519R

GACTTTCTAGGTTGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTTCTTCTCCAACAACAGAGCTTTACGAGC

CGAAACCCTTCTTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGT

AGGAGTATGGACCGTGTCTCAGTTCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCG

TTACCTTACCAACTAGCTAATGCACCGCAGGTCCATCCCAGAGTGATAGCCAAAGCCATCTTTCAAACAAAAGCCATGTG

GACTTTCTTGTTATGCGGTATTAGCATCTGTTTCCAAATGTTATCCCCCGCTCCGGGGCAGGTTACCTACGTGTTACTCA

CCCGTCCGCCACTCACTGGTGATCCATCGTCAATCAGGTGCAAGCACCATCAATCAGTTGGGCCAGTGCGTACGACTTGC

ATGTATTAGGCACACCGCCGGCGTTCATCCTGAGCCATGATCAAACTCTANGCGTCAGTTTTACGGTCTCGGCTCGTTTC

TCTGTTNTCTGACATCAACGTGCGTTACATTTGCGGTTTACGCATTGATTGTACTCCCTCCACATAGGTGGCGGCATACC

CTTCGTGCTCCTCTACTCATCTCGTTCATTACAACTCGCTTTGTTACCTTCCCGGTGGGGTTCTCTACCTCCTTCGTTTT

CTCTCACCTCATTCTCTCTCCCATCCTCTCNCTTTCCTCTTGCTC

SEQ ID NO: 3

161 S10BL282 with 926F

GGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAG
ACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTAGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTGTTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAG
GTGGGGATGACGTCAAATCATCATGCCCCTTGATGACCTGGGCTAGACACGTGCTACAATGGATGGTACAACGAGTTGCG
AACTCGCGAGAGTAAGCTAATCTCTTAAAGCCATTCTCAGTTACGGATGTGTAGGCTGCAACTCGCCATACATGAAGTCG
GAATCGCTAGTAATCGCGGATACAGCATGCCGCGGTGAATACTGTTCCCGGGCCTATGTGACACACCGCCCGTCACACCA
TGAGCAGTTTGTAATCACCCACACAGTCGGTGGGGTAACCTTTATAGGAACCAGCCGCCTACAGTGCGGGACCGATGATT
ATGGGTGCACTCGTATCACTGTAACTTAAACCCTTGCGGCCGTACTCCCCAGGCGGAATGCTTAATACGTTACCTGCAAC
CCTGAAGGGCGGAATCCCTCCAACGATTATCAAT

SEQ ID NO: 4

161 S10BL300 with 519R

GTGGCTTTCTGGTTAAATACCGTCAATACCTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACGAG
CCGAAACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCG
TAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCCATGGTGAGCC
GTTACCCCACCATCTAGCTAATACGCCGCGGGACCATCCAAAAGTGATAGCCGAAGCCATCTTTCAAGCTCGGACCATGC
GGTCCAAGTTGTTATGCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGGCAGGTTTCCCACGTGTTACTC
ACCAGTTCGCCACTCACTCAAATGTAAATCATGATGAAGCACCAATCAATACCAAGTTCGTTCGACTTGCATGTATTA
GGCACGCCGCCAGCGTTCGTCGCTGAGCCATGATCAAACTACTAAAGGCCCCCNATGCCTCCCACCCGCTTTGTTGCCGG
GGCCCCCCGTTCCCATACCCCTTTTGGACGTTTTCCAGCCCCTTGGCGGGCCCTGTACCTCCCCCCAGGGCGGGGAATGC
CTTAATTGCGTTNACCTTGCACCCCCTGAAGGGGCGGAATCCCTCCAACGATTACCT

SEQ ID NO: 5

255 S10BL504 with 926F

GGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCAGTCGCATAACCTAAGAGATT
AGGTGTTCCCTTCGGGGACGCTGAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTCACATGTTGGGTTAAGTCCC
GCAACGAGCGCAACCCTTGTCATTAGTTGCCATCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGA
AGGTGGGGATGACGTCAAGATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTAGAATGGACGGTACAACGAGATA
GCGAACCTGCGAAGAGCTAAGCGGATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGC
TTGGAATCGCTAGTAATCGCGGATCAGCACTGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCA
TGAGAGTCTGTAACTCCCAAAGTCGGTGGGATAACCTTCTATAGCGAGTGAGTCCGTTCGATGGGTAGGGACAAGATGAA
TGAGCGGTGAAAGGTCGTTAAACCAAGGGTAGCAAGTAAGGATCCCTTTGGGGGTTTTATCTCCACGGGGGGGGTGTTTC
TTTTCTGTCTTTA

SEQ ID NO: 6

255 S10BL530 with 519R

ACTTTCTAGAGTTAGATGATACCGTTCAACATGACAGATGGCCACGTTTACTTACTCTCACTGACTACTGTTCTTTCATC
TCACACAACAGAGCTTTACGAGCCGAAACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGAGCTTTGCGTCCCATTGTG
GAACATTCCCTACTGCTGCCTCCCGTAGGAGTATGGGCCGTGTCTCAGTCCCATTGTGGCCGATCAGTCTCTCAACTCGG
CTATGCATCATCGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATGCACCGCAGGTCCATCCAAGAGTGATAGCCGAA
CCATCTTTCACAACTCTAAACATGCTTGTAGTGTTGTTATTCCGGTATTAACATTCTGTTTCCAGGTTGTTATTCCCAGC
TGATCTCGGGGCAGGGTTTACCCCAACGTTGGTTTACCTTCACCCCCGGTTNCGGCCCGGCTTCGNCCTTGGGTTAGTAC
TNACGATTCTGCTATTATATACGATGGGCTAGACGACCAGCCTAACACAATTTCAATTTCGTNAAGTGTCGAGAGGNCCT
ACGGTCGTCCCGTTAACGTGTAGNCNATTTGGCTTATTTGTTAAGTTGTCCANCGGGCCACCGACCCCCAGGGCCCGGTT
GGTCCGGGTTTCCCCCATTGCAACGTCGCCAAAGTGCGGAAATTTCGAAAATACCCTTAACCAATGAAAAAAACATA

SEQ ID NO: 7

258 S10BL414 with 926F

GGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAG

ACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGGTTAAGTCCCT

CAACGAGCGCAACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAA

GGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCG

AACTCGCGAGAGTAAGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAA

TCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGT

TTGTAACACCCAAAGTCGGTGGGGGTAACCTTTTTAGGAAACCAGCCCGCCCTAAAGGGTGGGGAACAAGAATGAATTAA

GGGGGTTGAAAAGTTCCGTTAAACCAAAAGGGGTTAGCCCCNGNTNNGANNNNNNNNNGAC

SEQ ID NO: 8

258 S10BL438 with 519R

GCTTTCTGGTTAAATACCGTCAATACCTGAACAGTTACTCTCAGATATGTGTCTTCTTTAACAACAGAGTTTTACGAGCC

GAAACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTA

GGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCCATGGTGAGCCGT

TACCCCACCATCTAGCTAATACGCCGCGGGACCATCCAAAAGTGATAGCCGAAGCCATCTTTCAAGCTCGGACCATGCGG

TCCAAGTTGTTATGCGGTATTAGCATCTGTTTCCAGGGTGTTATTCCCCCGCTTCGTGGGCAGGGTTTCCCACGTGTTAC

TCACCAGTTCGCCACTCACTCAAATGTAAATCATGATGCAAGCACCAATCAATACCAGAGTTCGTTCGACTTGCATGTAT

TAGGCACGCCGCCAGCGTTCGTCCTGAGCCATGATCAAACTCNGA

NCIMB 41846 GGDK31 – Lactobacillus reuteri

SEQ ID NO: 10

S12KG200 GGDK 31-1 27F

TGCCTAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACCTGATTGACGATGGATCACCAGTGA
GTGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGATGCTAATACCGCAT
AACAACAAAAGCCACATGGCTTTTGTTTGAAAGATGGCTTTGGCTATCACTCTGGGATGGACCTGCGGTGCATTAGCTAG
TTGGTAAGGTAACGGCTTACCAAGGCGATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACAATGGAACTGAGACACG
GTCCATACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGCAAGCCTGATGGAGCAACACCGCGTGAGTGA
AGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTGGAGAAGAACGTGCGTGAGAGTAACTGTTCACGCAGTGACGGTATCCA
ACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGT
AAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCGGAAACCGGGCGAC
TTGAGTGCAGAAGAGGACAGTGGAACTC

SEQ ID NO: 11

S12KG201 GGDK 31-1 519F

TCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGC
ATCGGAAACCGGGCGACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAG
AACACCAGTGGCGAAGGCGGCTGTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATA
CCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGAGCTAACGCATTAA
GCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG
GTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCGTTCCCTTCG
GGGACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC
CCTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACG
TCAGATCATCATGCCCCTTATGACCTGGGCTA

SEQ ID NO: 12

S12KG202 GGDK 31-1 926F

GAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCGT
TCCCTTCGGGGACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGG
GGACGACGTCAGATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGAGTCGCAAGCTC
GCGAGAGTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGTCGGAATCGCT
AGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTTGTA
ACGCCCAAAGTCGGTGGCCTAACCATTATGGAGGGAGCCGCCTAAGGCGGGACAGATGACTGGGGTGAAGTCGTAACAAG
GTAGCCGTA

SEQ ID NO: 13

S12KG203 GGDK 31-1 926R

CTCCCCAGGCGGAGTGCTTAATGCGTTAGCTCCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCACTCATCGTTTAC
GGCATGGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACCAGACAGCCG
CCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAA

GTCGCCCGGTTTCCGATGCACTTCTTCGGTTAAGCCGAAGGCTTTCACATCAGACCTAAGCAACCGCCTGCGCTCGCTTT
ACGCCCAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGACTTTCTGGT
TGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTTCTTCTCCAACAACAGAGCTTTACGAGCCGAAACCCTTCT
TCACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGAC
CGTGTCTCAGTTCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCGTTACCTTACCAA
CTAGCTAATGCACCGCAGGTCCATCCCAGAGTGATAGCCAAAGCCATCTTTCAAACAAAAGCCATGTGGCTTTTGTTGT
TATGC

SEQ ID NO: 14

S12KG204 GGDK 31-1 519R

TTTCTGGTTGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTTCTTCTCCAACAACAGAGCTTTACGAGCCGAA
ACCCTTCTTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGA
GTATGGACCGTGTCTCAGTTCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCGTTAC
CTTACCAACTAGCTAATGCACCGCAGGTCCATCCCAGAGTGATAGCCAAAGCCATCTTTCAAACAAAAGCCATGTGGCTT
TTGTTGTTATGCGGTATTAGCATCTGTTTCCAAATGTTATCCCCCGCTCCGGGGCAGGTTACCTACGTGTTACTCACCCG
TCCGCCACTCACTGGTGATCCATCGTCAATCAGGTGCAAGCACCATCAATCAGTTGGGCCAGTGCGTACGACTTGCATGT
ATTAGGCACACCGCCGGCGTTCATCCTGAGCCATGATCAAAC

SEQ ID NO: 15

S12KG205 GGDK 31-1 RP2

CCGCCTTAGGCGGCTCCCTCCATAATGGTTAGGCCACCGACTTTGGGCGTTACAAACTCCCATGGTGTGACGGGCGGTGT
GTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGC
AGCCTACAGTCCGAACTGAGAACGGCTTTAAGAGATTAGCTTACTCTCGCGAGCTTGCGACTCGTTGTACCGTCCATTGT
AGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATCTGACGTCGTCCCCACCTTCCTCCGGTTTGTCACCGGCAGTC
TCACTAGAGTGCCCAACTTAATGCTGGCAACTAGTAACAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGA
CACGAGCTGACGACGACCATGCACCACCTGTCATTGCGTCCCCGAAGGGAACGCCTTATCTCTAAGGTTAGCGCAAGATG
TCAAGACCTGGTAAGGTTCTTCGCGTAGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTT
GAGTTTCCACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTCCGGCACTGAAGGGCGGAAACCCT
CCAACACCTAGCACTCATCGTTTACGGCATGGACTACCAGGG

NCIMB 41847 GGDK161 – contains both Lactobacillus plantarum and Lactobacillus reuteri

Lactobacillus plantarum

SEQ ID NO: 16

S12KG218 GGDK 161-1 27F

GTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGCGAA
CTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCGCATAACAACTT
GGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGAG
GTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAAC
TCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGT
TTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAA
AGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGA

GCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAGCTTGAGTG
CAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGT

SEQ ID NO: 17

S12KG219 GGDK 161-1 519F

CGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGC
ATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAG
AACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATA
CCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAA
GCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG
GTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGG
GGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC
CTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGA

SEQ ID NO: 18

S12KG220 GGDK 161-1 926F

TGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGAC
GTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGT
GGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAAC
TCGCGAGAGTAAGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCG
CTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTG
TAACACCCAAAGTCGGTGGGGTAACCTTTTAGGAACCAGCCGCCTAAGGTGGGACAGATGATTAGGGTGAAGTCGTAAC
AAGGTAGCCCGTA

SEQ ID NO: 19

S12KG221 GGDK 161-1 926R

ACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCATTCATCGTTTA
CGGTATGGACTACCAGGGTATCTAATCCTGTTTGCTACCCATACTTTCGAGCCTCAGCGTCAGTTACAGACCAGACAGCC
GCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCA
AGTTTCCCAGTTTCCGATGCACTTCTTCGGTTGAGCCGAAGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTT
TACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGG
TTAAATACCGTCAATACCTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACGAGCCGAAACCCTTC
TTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGG
CCGTGTCTCAGTCCCAATGTGGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCCATGGTGAGCCGTTACCCCACCA
TCTAGCTAATACGCCGCGGGACCATCCAAAAGTGATAGCCGAAGCCATCTTTCAAACTCGGACCATGCGGTCCAAGTTG
T

SEQ ID NO: 20

S12KG222 GGDK 161-1 519R

GCTTTCTGGTTAAATACCGTCAATACCTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACGAGCCG
AAACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAG

GAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCCATGGTGAGCCGTT
ACCCCACCATCTAGCTAATACGCCGCGGGACCATCCAAAAGTGATAGCCGAAGCCATCTTTCAAACTCGGACCATGCGGT
CCAAGTTGTTATGCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGGCAGGTTTCCCACGTGTTACTCAC
CAGTTCGCCACTCACTCAAATGTAAATCATGATGCAAGCACCAATCAATACCAAAGTTCGTTCGACTTGCATGTATTAG
GCACGCCGCCAGCGTTCGTCCTGAGCCAGATCAAACTCTAA

SEQ ID NO: 21

S12KG223 GGDK 161-1 RP2

CCACCTTAGGCGGCTGGTTCCTAAAAGGTTACCCCACCGACTTTGGGTGTTACAAACTCTCATGGTGTGACGGGCGGTGT
GTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGACTTCATGTAGGCGAGTTGC
AGCCTACAATCCGAACTGAGAATGGCTTTAAGAGATTAGCTTACTCTCGCGAGTTCGCAACTCGTTGTACCATCCATTGT
AGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTC
TCACCAGAGTGCCCAACTTAATGCTGGCAACTGATAATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGA
CACGAGCTGACGACAACCATGCACCACCTGTATCCATGTCCCCGAAGGGAACGTCTAATCTCTTAGATTTGCATAGTATG
TCAAGACCTGGTAAGGTTCTTCGCGTAGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCT
TTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTC
CAACACTTAGCATTCATCGTTTACGGTATGGACTACCAGGGTATCTA

NCIMB 41847 GGDK161 – contains both Lactobacillus plantarum and Lactobacillus reuteri

Lactobacillus reuteri

SEQ ID NO: 22

S12KG309 cGGDK 161-1 27F

ATGCTAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACCTGATTGACGAT
GGATCACCAGTGAGTGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGAT
GCTAATACCGCATAACAACAAAAGCCACATGGCTTTTGTTTGAAAGATGGCTTTGGCTATCACTCTGGGATGGACCTGCG
GTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACAATG
GAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGCAAGCCTGATGGAGCAAC
ACCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTGGAGAAGAACGTGCGTGAGAGTAACTGTTCACGCA
GTGACGGTATCCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGG
ATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCG
GAAACCGGGCGACTTGAGTGCAGAAGAGGACAGTGGAAC

SEQ ID NO: 23

S12KG310 cGGDK 161-1 519F

TCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTG
CATCGGAAACCGGGCGACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAA
GAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGAT
ACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGAGCTAACGCATTA
AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGT
GGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCGTTCCCTTCG
GGGACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC
CCTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACG

TCAGATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGAGTCGCAAGCTCGCGAGAG

SEQ ID NO: 24

S12KG311 cGGDK 161-1 926F

GGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCG
TTCCCTTCGGGGACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTG
GGGACGACGTCAGATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGAGTCGCAAGCT
CGCGAGAGTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGTCGGAATCGC
TAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTTGT
AACGCCCAAAGTCGGTGGCCTAACCTTTATGGAGGGAGCCGCCTAAGGCGGGACAGATGACTGGGGTGAAGTCGTAACAA
GGTAGCCGTA

SEQ ID NO: 25

S12KG312 cGGDK 161-1 926R

TCCCCAGGCGGAGTGCTTAATGCGTTAGCTCCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCACTCATCGTTTACG
GCATGGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACCAGACAGCCGC
CTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAG
TCGCCCGGTTTCCGATGCACTTCTTCGGTTAAGCCGAAGGCTTTCACATCAGACCTAAGCAACCGCCTGCGCTCGCTTTA
CGCCCAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGACTTTCTGGTT
GGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTTCTTCTCCAACAACAGAGCTTTACGAGCCGAAACCCTTCTT
CACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGACC
GTGTCTCAGTTCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCGTTACCTTACCAAC
TAGCTAATGCACCGCAGGTCCATCCCAGAGTGATAGCCAAAGCCATCTTTCAAACAAAAGCCATGTGGCTTTT

SEQ ID NO: 26

S12KG313 cGGDK 161-1 519R

TTTCTGGTTGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTTCTTCTCCAACAACAGAGCTTTACGAGCCGAA
ACCCTTCTTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGA
GTATGGACCGTGTCTCAGTTCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCGTTAC
CTTACCAACTAGCTAATGCACCGCAGGTCCATCCCAGAGTGATAGCCAAAGCCATCTTTCAAACAAAAGCCATGTGGCTT
TTGTTGTTATGCGGTATTAGCATCTGTTTCCAAATGTTATCCCCCGCTCCGGGGCAGGTTACCTACGTGTTACTCACCCG
TCCGCCACTCACTGGTAATCCATCGTCAATCAGGTGCAAGCACCATCAATCAGTTGGGCCAGTGCGTACGACTTGCATG
TATTAGGCACACCGCCGGCGTTCATCCTGAGCCATGATCAAAC

SEQ ID NO: 27

S12KG314 cGGDK 161-1 RP2

GCGGCTCCCTCCATAAAGGTTAGCGCCACCGACTTTGGGCGTTACAAACTCCCATGGTGTGACGGGCG
GTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAG
TTGCAGCCTACAGTCCGAACTGAGAACGGCTTTAAGAGATTAGCTTACTCTCGCGAGCTTGCGACTCGTTGTACCGTCCA
TTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATCTGACGTCGTCCCCACCTTCCTCCGGTTTGTCACCGGC
AGTCTCACTAGAGTGCCCAACTTAATGCTGGCAACTAGTAACAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTC

ACGACACGAGCTGACGACGACCATGCACCACCTGTCATTGCGTCCCCGAAGGGAACGCCTTATCTCTAAGGTTAGCGCAA
GATGTCAAGACCTGGTAAGGTTCTTCGCGTAGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCA
ATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTCCGGCACTGAAGGGCGGAA
ACCCTCCAACACCTAGCACTCATCGTTTACGGCAT

NCIMB 41848 GGDK255 - Lactobacillus reuteri

SEQ ID NO: 28

S12KG237 GGDK 255-1 27F

GTGTGCCTAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACCTGATTGACGATGGATCACCAG
TGAGTGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGATGCTAATACCG
CATAACAACAAAAGCCACATGGCTTTTGTTTGAAAGATGGCTTTGGCTATCACTCTGGGATGGACCTGCGGTGCATTAGC
TAGTTGGTAAGGTAACGGCTTACCAAGGCGATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACAATGGAACTGAGAC
ACGGTCCATACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGCAAGCCTGATGGAGCAACACCGCGTGAG
TGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTGGAGAAGAACGTGCGTGAGAGTAACTGTTCACGCAGTGACGGTAT
CCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGG
CGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCGGAAACCGGGC
GACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTG

SEQ ID NO: 29

S12KG238 GGDK 255-1 519F

TCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGC
ATCGGAAACCGGGCGACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAG
AACACCAGTGGCGAAGGCGGCTGTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATA
CCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGAGCTAACGCATTAA
GCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG
GTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCGTTCCCTTCGG
GGACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC
CTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACG
TCAGATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTAC

SEQ ID NO: 30

S12KG239 GGDK 255-1 926F

TGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGC
GTTCCCTTCGGGGACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGT
GGGGACGACGTCAGATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGAGTCGCAAGC
TCGCGAGAGTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGTCGGAATCG
CTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTTG
TAACGCCCAAAGTCGGTGGCCTAACCTTTATGGAGGGAGCCGCCTAAGGCGGGACAGATGACTGGGGTGAAGTCGTAACA
AGGTAGCCGTA

SEQ ID NO: 31

S12KG240 GGDK 255-1 926R

TACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTCCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCACTCATCGTTT
ACGGCATGGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACCAGACAGC
CGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTC
AAGTCGCCCGGTTTCCGATGCACTTCTTCGGTTAAGCCGAAGGCTTTCACATCAGACCTAAGCAACCGCCTGCGCTCGCT
TTACGCCCAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGACTTTCTG
GTTGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTTCTTCTCCAACAACAGAGCTTTACGAGCCGAAACCCTT
CTTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGG
ACCGTGTCTCAGTTCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCGTTACCTTACC
AACTAGCTAATGCACCGCAGGTCCATCCCAGAGTGATAGCCAAAGCCATCTTTCAAACAAAAGCCATGTGGCTTTTG

SEQ ID NO: 32

S12KG241 GGDK 255-1 519R

TTTCTGGTTGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTTCTTCTCCAACAACAGAGCTTTACGAGCCGAA
ACCCTTCTTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGA
GTATGGACCGTGTCTCAGTTCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCGTTAC
CTTACCAACTAGCTAATGCACCGCAGGTCCATCCCAGAGTGATAGCCAAAGCCATCTTTCAAACAAAAGCCATGTGGCT
TTTGTTGTTATGCGGTATTAGCATCTGTTTCCAAATGTTATCCCCCGCTCCGGGGCAGGTTACCTACGTGTTACTCACC
CGTCCGCCACTCACTGGTGATCCATCGTCAATCAGGTGCAAGCACCATCAATCAGTTGGGCCAGTGCGTACGACTTGCAT
GTATTAGGCACACCGCCGGCGTCCATCCTGAGCCATGATCAAAC

SEQ ID NO: 33

S12KG242 GGDK 255-1 RP2

CCGCCTTAGGCGGCTCCCTCCATAAAGGTTAGGCCACCGACTTTGGGCGTTACAAACTCCCATGGTGTGACGGGCGGTGT
GTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGC
AGCCTACAGTCCGAACTGAGAACGGCTTTAAGAGATTAGCTTACTCTCGCGAGCTTGCGACTCGTTGTACCGTCCATTGT
AGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATCTGACGTCGTCCCCACCTTCCTCCGGTTTGTCACCGGCAGTC
TCACTAGAGTGCCCAACTTAATGCTGGCAACTAGTAACAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGA
CACGAGCTGACGACGACCATGCACCACCTGTCATTGCGTCCCCGAAGGGAACGCCTTATCTCTAAGGTTAGCGCAAGATG
TCAAGACCTGGTAAGGTTCTTCGCGTAGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCT
TTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTCCGGCACTGAAGGGCGGAAACCCTCCAA
CACCTAGCACTCATCGTT

NCIMB 41849 GGDK 258 - Lactobacillus plantarum

SEQ ID NO: 34

S12KG267 GGDK 258-3 27F

GTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGCGAA
CTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCGCATAACAACTT
GGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGAG
GTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAAC

TCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGT
TTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAA
GCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAG
CGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGC
AGAAGAGGACAGTGGAACTC

SEQ ID NO: 35

S12KG268 GGDK 258-3 519F

GGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCAT
CGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAA
CACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACC
CTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGC
ATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
TTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGG
ACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCT
TATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCA
AATCATCATGCCCCTTATGACCTGGGCTAC

SEQ ID NO: 36

S12KG269 GGDK 258-3 926F

GTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGA
CGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA
ACGAGCGCAACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGG
TGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAA
CTCGCGAGAGTAAGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATC
GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTT
GTAACACCCAAAGTCGGTGGGGTAACCTTTTAGGAACCAGCCGCCTAAGGTGGGACAGATGATTAGGGTGAAGTCGTAA
CAAGGTAGCCCGTA

SEQ ID NO: 37

S12KG270 GGDK 258-3 926R

ACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCATTCATCGTTTA
CGGTATGGACTACCAGGGTATCTAATCCTGTTTGCTACCCATACTTTCGAGCCTCAGCGTCAGTTACAGACCAGACAGCC
GCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCA
AGTTTCCCAGTTTCCGATGCACTTCTTCGGTTGAGCCGAAGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTT
TACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGG
TTAAATACCGTCAATACCTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACGAGCCGAAACCCTTC
TTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGG
CCGTGTCTCAGTCCCAATGTGGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCCATGGTGAGCCGTTACCTCACCA
TCTAGCTAATACGCCGCGGGACCATCCAAAAGTGATAGCCGAAGCCATCTTTCAAACTCGGACCATGCGGTCCAAGTTG
TTATGCGGTATTAGCATCTGTTTC

SEQ ID NO: 38

S12KG271 GGDK 258-3 519R

TTTCTGGTTAAATACCGTCAATACCTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACGAGCCGAA
ACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGA
GTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCCATGGTGAGCCGTTAC
CTCACCATCTAGCTAATACGCCGCGGGACCATCCAAAAGTGATAGCCGAAGCCATCTTTCAAACTCGGACCATGCGGTCC
AAGTTGTTATGCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGGCAGGTTTCCCACGTGTTACTCACCAG
TTCGCCACTCACTCAAATGTAAATCATGATGCAAGCACCAATCAATACCAGAGTTCGTTCGACTTGCATGTATTAGGCAC
GCCGCCAGCGTTCGTCCTGAGCCATGATCAAAC

SEQ ID NO: 39

S12KG272 GGDK 258-3 RP2

CCACCTTAGGCGGCTGGTTCCTAAAAGGTTACCCCACCGACTTTGGGTGTTACAAACTCTCATGGTGTGACGGGCGGTGT
GTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGACTTCATGTAGGCGAGTTGC
AGCCTACAATCCGAACTGAGAATGGCTTTAAGAGATTAGCTTACTCTCGCGAGTTCGCAACTCGTTGTACCATCCATTGT
AGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTC
TCACCAGAGTGCCCAACTTAATGCTGGCAACTGATAATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGA
CACGAGCTGACGACAACCATGCACCACCTGTATCCATGTCCCCGAAGGGAACGTCTAATCTCTTAGATTTGCATAGTATG
TCAAGACCTGGTAAGGTTCTTCGCGTAGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCT
TTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTC
CAACACTTAGCATTCATCGTTTACGGTATGGACTACCAGGGTATCTAATCCTGTTTGCTACCCATACTTTCGAGCCTCAGC
GTCAGTTACAGACCAGACAGCCGCCT

NCIMB 41850 GGDK 266 – contains both Lactobacillus johnsonii and Lactobacillus reuteri

Lactobacillus johnsonii

SEQ ID NO: 40

S12KG273 GGDK 266-1 27F – repeat

GTGCCTAATACATGCAAGTCGAGCGAGCTTGCCTAGATGATTTTAGTGCTTGCACTAAATGAAACTAGATACAAGCGAGC
GGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCAAGAGACTGGGATAACACCTGGAAACAGATGCTAATACCGGATAA
CAACACTAGACGCATGTCTAGAGTTTGAAAGATGGTTCTGCTATCACTCTTGGATGGACCTGCGGTGCATTAGCTAGTTG
GTAAGGTAACGGCTTACCAAGGCAATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCC
CAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGA
AGGGTTTCGGCTCGTAAAGCTCTGTTGGTAGTGAAGAAAGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATTACTT
AGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA
AGCGAGTGCAGGCGGTTCAATAAGTCTGATGTGAAAGCCTTCGGCTCAACCGGAGAAT

SEQ ID NO: 41

S12KG274 GGDK 266-1 519F

TCCGGATTTATTGGGCGTAAAGCGAGTGCAGGCGGTTCAATAAGTCTGATGTGAAAGCCTTCGGCTCAACCGGAGAATTG
CATCAGAAACTGTTGAACTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAA
GAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGAT

ACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTA
AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGT
GGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCAGTGCAAACCTAAGAGATTAGGTGTTCCCTTC
GGGGACGCTGAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGT

SEQ ID NO: 42

S12KG275 GGDK 266-1 926F – repeat

GGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCAGTGCAAACCTAAGAGATTAGGTG
TGTCCCTTCGGGGACGCTGAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTTGTCATTAGTTGCCATCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGT
GGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGAGAAGCGAAC
CTGCGAAGGCAAGCGGATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGCTGGAATCG
CTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAG
TCTGTA

SEQ ID NO: 43

S12KG276 GGDK 266-1 926R

ACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAGAGGCGGAAACCTCCCAACACTTAGCACTCATCGTTTA
CGGCATGGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACCAGAGAGCC
GCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACACATGGAGTTCCACTCTCCTCTTCTGCACTCA
AGTTCAACAGTTTCTGATGCAATTCTCCGGTTGAGCCGAAGGCTTTCACATCAGACTTATTGAACCGCCTGCACTCGCTT
TACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGACTTTCTAA
GTAATTACCGTCAAATAAAGGCCAGTTACTACCTCTATCTTTCTTCACTACCAACAGAGCTTTACGAGCCGAAACCCTTC
TTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGG
CCGTGTCTCAGTCCCAATGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATTGCCTTGGTAAGCCGTTACCTTACCA
ACTAGCTAATGCACCGCAGGTCCATCCAAGAGTGATAGCAGAACCATCTTTCAAACTCTAGACATGCGTCTAGTGTTGT

SEQ ID NO: 44

S12KG277 GGDK 266-1 519R

ACTTTCTAAGTAATTACCGTCAAATAAAGGCCAGTTACTACCTCTATCTTTCTTCACTACCAACAGAGCTTTACGAGCCG
AAACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAG
GAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATTGCCTTGGTAAGCCGTT
ACCTTACCAACTAGCTAATGCACCGCAGGTCCATCCAAGAGTGATAGCAGAACCATCTTTCAAACTCTAGACATGCGTCT
AGTGTTGTTATCCGGTATTAGCATCTGTTTCCAGGTGTTATCCCAGTCTCTTGGGCAGGTTACCCACGTGTTACTCACCC
GTCCGCCGCTCGCTTGTATCTAGTTTCATTTAGTGCAAGCACTAAAATCATCTAGGCAAGCTCGCTCGACTTGCATGTAT
TAGGCACGCCGCCAGCGTTCGTCCTGAGCCATGATCAAACT

SEQ ID NO: 45

S12KG278 GGDK 266-1 RP2

CTACCTTAGACGGCTGACTCCTATAAAGGTTATCCCACCGGCTTTGGGTGTTACAGACTCTCATGGTGTGACGGGCGGTG
TGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTACTAGCGATTCCAGCTTCGTGTAGGCGAGTTG
CAGCCTACAGTCCGAACTGAGAACGGCTTTAAGAGATCCGCTTGCCTTCGCAGGTTCGCTTCTCGTTGTACCGTCCATTG

TAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGT
CTCATTAGAGTGCCCAACTTAATGATGGCAACTAATGACAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACG
ACACGAGCTGACGACAGCCATGCACCACCTGTCTCAGCGTCCCCGAAGGGAACACCTAATCTCTTAGGTTTGCACTGGAT
GTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCC
TTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAGAGGCGGAAACCTC
CCAACACTTAGCACTCATCGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCA
GCGTCAGTTGCAGACCAGAGAGCCGCCT

NCIMB 41850 GGDK 266 – contains both Lactobacillus johnsonii and Lactobacillus reuteri

Lactobacillus reuteri

SEQ ID NO: 46

S12KG279 GGDK-266-2 27F

GTGTGCCTAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACCTGATTGACGATGGATCACCAG
TGAGTGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGATGCTAATACCG
CATAACAACAAAAGCCACATGGCTTTTGTTTGAAAGATGGCTTCGGCTATCACTCTGGGATGGACCTGCGGTGCATTAGC
TAGTTGGTAAGGTAACGGCTTACCAAGGCGATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACAATGGAACTGAGAC
ACGGTCCATACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGCAAGCCTGATGGAGCAACACCGCGTGAG
TGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTGGAGAAGAACGTGCGTGAGAGTAACTGTTCACGCAGTGACGGTAT
CCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGG
CGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCGGAAACCGGGC
GACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAATGCGTA

SEQ ID NO: 47

S12KG280 GGDK-266-2 519F – repeat

CGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCA
TCGGAAACCGGGCGACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGA
ACACCAGTGGCGAAGGCGGCTGTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGAGCTAACGCATTAAG
CACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG
GTTTAATTCG

SEQ ID NO: 48

S12KG281 GGDK-266-2 926F – repeat

GAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCGT
TCCCTTCGGGGACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGG
GGACGACGTCAGATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGAGTCGCAAGCT
CGCGAGAGTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGTCGGAATCGC
TAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACC

SEQ ID NO: 49

S12KG282 GGDK-266-2 926R – repeat

ACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTCCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCACTCATCGTTTA
CGGCATGGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACCAGACAGCC
GCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCA
AGTCGCCCGGTTTCCGATGCACTTCTTCGGTTAAGCCGAAGGCTTTCACATCAGACCTAAGCAACCGCCTGCGCTCGCTT
TACGCCCAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGACTTTCTGG
TTGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTTCTTCTCCAACAACAGAGCTTTACGAGCCGAAACCCTTC
TTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGA
CCGTGTCTCAGTTCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCGTTACCTTACCA
ACTAGCTAATGCACCGCAGGT

SEQ ID NO: 50

S12KG283 GGDK-266-2 519R

TTTCTGGTTGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTTCTTCTCCAACAACAGAGCTTTACGAGCCGAA
ACCCTTCTTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGA
GTATGGACCGTGTCTCAGTTCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCGTTAC
CTTACCAACTAGCTAATGCACCGCAGGTCCATCCCAGAGTGATAGCCAAAGCCATCTTTCAAACAAAAGCCATGTGGCTT
TTGTTGTTATGCGGTATTAGCATCTGTTTCCAAATGTTATCCCCCGCTCCGGGGCAGGTTACCTACGTGTTACTCACCCG
TCCGCCACTCACTGGTGATCCATCGTCAATCAGGTGCAAGCACCATCAATCAGTTGGGCCAGTGCGTACGACTTGCATGT
ATTAGGCACACCGCCGGCGTTCATCCTGAGCCATGATCAAACTCT

SEQ ID NO: 51

S12KG284 GGDK-266-2 RP2

TCCCGCCTTAGGCGGCTCCCTCCATAATGGTTAGGCCACCGACTTTGGGCGTTACAAACTCCCATGGTGTGACGGGCGGT
GTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTT
GCAGCCTACAGTCCGAACTGAGAACGGCTTTAAGAGATTAGCTTACTCTCGCGAGCTTGCGACTCGTTGTACCGTCCATT
GTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATCTGACGTCGTCCCCACCTTCCTCCGGTTTGTCACCGGCAG
TCTCACTAGAGTGCCCAACTTAATGCTGGCAACTAGTAACAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCAC
GACACGAGCTGACGACGACCATGCACCACCTGTCATTGCGTCCCCGAAGGGAACGCCTTATCTCTAAGGTTAGCGCAAGA
TGTCAAGACCTGGTAAGGTTCTTCGCGTAGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC
CTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTCCGGCACTGAAGGGCGGAAACCC
TCCAACACCTAGCACTCATCGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTC
AGCGTCAGTTGCAGACCAGACAGCCGCCTTCGCCACTGGTG

NCIMB 41850 GGDK 266 – contains both Lactobacillus johnsonii and Lactobacillus reuteri

Lactobacillus reuteri

SEQ ID NO: 52

S12KG381 27F

GTGTGCCTAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACCTGATTGACGATGGATCACCAGTGAGTGGCGGACG
GGTGAGTAACACGTAGGTAACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAACAACAAAAGCCACATGGCTTT
TGTTTGAAAGATGGCTTTGGCTATCACTCTGGGATGGACCTGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGATGATGCAT
AGCCGAGTTGAGAGACTGATCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGC

GCAAGCCTGATGGAGCAACACCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTGGAGAAGAACGTGCGTGAGAGTAACTGT
TCACGCAGTGACGGTATCCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTAT
TGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCGGAAACCGGGCGACTTGAG
TGC

SEQ ID NO: 53

S12KG382 519F

TTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCGGAAAC
CGGGCAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGG
CTGTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCT
AGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGA
ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTT
AGAGATAAGGCGTCCCTTCGGGGACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACGTCA

SEQ ID NO: 54

S12KG383 926F

GGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCGTTCCCTTCGGG
GACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTACTAGTTG
CCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACGTCAGATCATCATGCCCCTTATGACCTG
GGCTACACACGTGCTACAATGGACGGTACAACGAGTCGCAAGCTCGCGAGAGTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGG
CTGCAACTCGCCTACACGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
ACACCATGGGAGTTTGTAACGCCCAAAGTCGGTGGCCTAACCATTATGGAGGGAGCCGCCTAAGGCGGGACAGATGACTGGGGTGAAGTCGT
AACAAGGTAGCCGTA

SEQ ID NO: 55

S12KG384 926R

TACTCCCCAGGCGGAGTGCTTAATGCGTGAGCTCCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCACTCATCGTTTACGGCATGGACTA
CCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATAT
ATCTACGCATTCCACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTCGCCCGGTTTCCGATGCACTTCTTCGGTTAAGCCGAAGG
CTTTCACATCAGACCTAAGCAACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCA
CGTAGTTAGCCGTGACTTTCTGGTTGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTTCTTCTCCAACAACAGAGCTTTACGAGCCGA
AACCCTTCTTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGACCGTGTC
TCAGTTCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATGCACCGCAGGTC
CATCCCAGAGTGATAGCCAAAGCCATCTTTCAAACAAAAGCC

SEQ ID NO: 56

S12KG385 519R

GTGACTTTCTGGTTGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTGCTTCTCCAACAACAGAGCTTTACGAGCCGAAACCCTTCTTC
ACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGACCGTGTCTCAGTTCCATT
GTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATGCACCGCAGGTCCATCCCAGAGT
GATAGCCAAAGCCATCTTTCAAACAAAAGCCATGTGGCTTTTGTTGTTATGCGGTATTAGCATCTGTTTCCAAATGTTATCCCCCGCTCCGGGGC

AGGTTACCTACGTGTTACTCACCCGTCCGCCACTCACTGGTGATCCATCGTCAATCAGGTGCAAGCACCATCAATCAGTTGGGCCAGTGCGTAC
GACTTGCATGTATTAGGCACACCGCCGGCGTTCATCCTGAGCCATGATCAAAC

SEQ ID NO: 57

S12KG386 RP2

TCCCGCACTTAGGCGGCTCCCTCCATAATGGTTAGGCCACCGACTTTGGGCGTTACAAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCC
CGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAAC
GGCTTTAAGAGATTAGCTTACTCTCGCGAGCTTGCGACTCGTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGA
TCTGACGTCGTCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCACTAGAGTGCCCAACTTAATGCTGGCAACTAGTAACAAGGGTTGCGCTCG
TTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACGACCATGCACCACCTGTCATTGCGTCCCCGAAGGGAACGCCTTATCTCTAAG
GTTAGCGCAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTAGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCT
TTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTCCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCACT
CATCGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCC

NCIMB 42008 GGDK266a – L. johnsonii (sample 4a)

SEQ ID NO: 58

S12KG399 27F

GCGTGCCTAATACATGCAAGTCGAGCGAGCTTGCCTAGATGATTTTAGTGCTTGCACTAAATGAAACTAGATACAAGCGAGCGGCGGACGGGT
GAGTAACACGTGGGTAACCTGCCCAAGAGACTGGGATAACACCTGGAAACAGATGCTAATACCGGATAACAACACTAGACGCATGTCTAGAG
TTTGAAAGATGGTTCTGCTATCACTCTTGGATGGACCTGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAATGATGCATAGCC
GAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAA
GTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGGTAGTGAAGAAAGATAGAGGTAGTAACTGGCCTT
TATTTGACGGTAATTACTTAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGG
CGTAAAGCGAGTGCAGGCGGTTCAATAAGTCTGATGTGAAAGCCTTCGGCTCAACCGGAGAATTGCATCAGAAACTGTTGAACTTGAGTGCAG
AAGAGGAGAGTGGAACTCCATGTGTAGCGGTGGAATGCGTA

SEQ ID NO: 59

S12KG400 519F

TGTCCGGATTTATTGGGCGTAAAGCGAGTGCAGGCGGTTCAATAAGTCTGATGTGAAAGCCTTCGGCTCAACCGGAGAATTGCATCAGAAACT
GTTGAACTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGC
TCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTA
AGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAAT
TGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCAGTGCAAACCTAA
GAGATTAGGTGTTCCCTTCGGGGACGCTGAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTTGTCATTAGTTGCCATCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT

SEQ ID NO: 60

S12KG401 926F

GGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCAGTGCAAACCTAAGAGATTAGGTGTTCCCTTCGG
GGACGCTGAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCATTAGTT

GCCATCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTG
GGCTACACACGTGCTACAATGGACGGTACAACGAGAAGCGAACCTGCGAAGGCAAGCGGATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAG
GCTGCAACTCGCCTACACGAAGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG
TCACACCATGAGAGTCTGTAACACCCAAAGCCGGTGGGATAACCTTTATAGGAGTCAGCCGTCTAAGGTAGGACAGATGATTAGGGTGAAGTC
GTAACAAGGTAG

SEQ ID NO: 61

S12KG402 926R

TACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAGAGGCGGAAACCTCCCAACACTTAGCACTCATCGTTTACGGCATGGACTAC
CAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCTTCCATATA
TCTACGCATTCCACCGCTACACATGGAGTTCCACTCTCCTCTTCTGCACTCAAGTTCAACAGTTTCTGATGCAATTCTCCGGTTGAGCCGAAGGCT
TTCACATCAGACTTATTGAACCGCCTGCACTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACG
TAGTTAGCCGTGACTTTCTAAGTAATTACCGTCAAATAAAGGCCAGTTACTACCTCTATCTTTCTTCACTACCAACAGAGCTTTACGAGCCGAAA
CCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTC
AGTCCCAATGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATTGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATGCACCGCAGGTCCA
TCCAAGAGTGATAGCAGANCCATCTTTCAAACTCTAGACATGCGTCTAGTG

SEQ ID NO: 62

S12KG403 519R

GTGACTTTCTAAGTAATTACCGTCAAATAAAGGCCAGTTACTACCTCTATCTTTCTTCACTACCAACAGAGCTTTACGAGCCGAAACCCTTCTTCA
CTCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATG
TGGCCGATCAGTCTCTCAACTCGGCTATGCATCATTGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATGCACCGCAGGTCCATCCAAGAGTG
ATAGCAGAACCATCTTTCAAACTCTAGACATGCGTCTAGTGTTGTTATCCGGTATTAGCATCTGTTTCCAGGTGTTATCCCAGTCTCTTGGGCAG
GTTACCCACGTGTTACTCACCCGTCCGCCGCTCGCTTGTATCTAGTTTCATTTAGTGCAAGCACTAAAATCATCTAGGCAAGCTCGCTCGACTTG
CATGTATTAGGCACGCCGCCAGCGTTCGTCCTGAGCCA

SEQ ID NO: 63

S12KG404 RP2

TCCTACACTTAGACGGCTGACTCCTATAAAGGTTATCCCACCGGCTTTGGGTGTTACAGACTCTCATGGTGTGACGGGCGGTGTGTACAAGGCC
CGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTACTAGCGATTCCAGCTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAAC
GGCTTTAAGAGATCCGCTTGCCTTCGCAGGTTCGCTTCTCGTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGA
CTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCATTAGAGTGCCCAACTTAATGATGGCAACTAATGACAAGGGTTGCGCTCG
TTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACCTGTCTCAGCGTCCCCGAAGGGAACACCTAATCTCTTAG
GTTTGCACTGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTT
TGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAGAGGCGGAAACCTCCCAACACTTAGCACTC
ATCGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGC

SEQ ID NO: 64

NCIMB 42009 GGDK266b – L.reuteri (sample 6a)

S12KG411 27F

GTGTGCCTAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACCTGATTGACGATGGATCACCAGTGAGTGGCGGACG
GGTGAGTAACACGTAGGTAACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAACAACAAAAGCCACATGGCTTT
TGTTTGAAAGATGGCTTTGGCTATCACTCTGGGATGGACCTGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGATGATGCAT
AGCCGAGTTGAGAGACTGATCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGC
GCAAGCCTGATGGAGCAACACCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTGGAGAAGAACGTGCGTGAGAGTAACTGT
TCACGCAGTGACGGTATCCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGT

SEQ ID NO: 65

S12KG412 519F

TATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCGGAAACC
GGGCGACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGC
TGTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTA
GGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAAT
TGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAG
AGATAAGGCGTTCCCTTCGGGGACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGA

SEQ ID NO: 66

S12KG413 926F

GTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCGTTCCCTTCGGGG
ACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTACTAGTTGC
CAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACGTCAGATCATCATGCCCCTTATGACCTGG
GCTACACACGTGCTACAATGGACGGTACAACGAGTCGCAAGCTCGCGAGAGTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGC
TGCAACTCGCCTACACGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
ACACCATGGGAGTTTGTAACGCCCAAAGTCGGTGGCCTAACCATTATGGAGGGAGCCGCCTAAGGCGGGACAGATGACTGGGGTGAAGTCGT

SEQ ID NO: 67

S12KG414 926R

TACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTCCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCACTCATCGTTTACGGCATGGACTAC
CAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATA
TCTACGCATTCCACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTCGCCCGGTTTCCGATGCACTTCTTCGGTTAAGCCGAAGGC
TTTCACATCAGACCTAAGCAACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCAC
GTAGTTAGCCGTGACTTTCTGGTTGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTTCTTCTCCAACAACAGAGCTTTACGAGCCGAA
ACCCTTCTTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGACCGTGTCT
CAGTTCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCC

SEQ ID NO: 68

S12KG415 519R

GTGACTTTCTGGTTGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTTCTTCTCCAACAACAGAGCTTTACGAGCCGAAACCCTTCTTC
ACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGACCGTGTCTCAGTTCCATT

GTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATGCACCGCAGGTCCATCCCAGAGT
GATAGCCAAAGCCATCTTTCAAACAAAAGCCATGTGGCTTTTGTTGTTATGCGGTATTAGCATCTGTTTCCAAATGTTATCCCCCGCTCCGGGGC
AGGTTACCTACGTGTTACTCACCCGTCCGCCACTCACTGGTGATCCATCGTCAATCAGGTGCAAGCACCATCAATCAGTTGGGCCAGTGCGTAC
GACTTGCATGTATTAGGCACACCGCCGGCGTTCAT

SEQ ID NO: 69

S12KG416 RP2

TCCCGCCTTAGGCGGCTCCCTCCATAATGGTTAGGCCACCGACTTTGGGCGTTACAAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCC
GGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAACG
GCTTTAAGAGATTAGCTTACTCTCGCGAGCTTGCGACTCGTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGAT
CTGACGTCGTCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCACTAGAGTGCCCAACTTAATGCTGGCAACTAGTAACAAGGGTTGCGCTCGT
TGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACGACCATGCACCACCTGTCATTGCGTCCCCGAAGGGAACGCCTTATCTCTAAGG
TTAGCGCAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTAGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTT
GAGTTTCAACCTTGCGGTCGTACTCC

SEQ ID NO: 70

NCIMB 42010 GGDK161a – L.plantarum (sample 7a)

S12KG417 27F

GTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACAC
GTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGTTTGAAAGA
TGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGAGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGA
GAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGAT
GGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGAC
GGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGC
GAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGA
CAGTGGAACTCATGTGT

SEQ ID NO: 71

S12KG418 519F

TCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGG
GAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTG
TCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAG
TGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTG
ACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAG
ATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCAT
CATGCCCCTTATGACCTGGGCTACACAC

SEQ ID NO: 72

S12KG419 926F

GGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGG
GACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATCAGTTG

CCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTG
GGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTAAGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGG
CTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
ACACCATGAGAGTTTGTAACACCCAAAGTCGGTGGGGTAACCTTTTAGGAACCAGCCGCCTAAGGTGGGACAGATGATTACGGTGAAGTCGTA
ACAAGGTAGCCCGTA

SEQ ID NO: 73

S12KG420 926R

GTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCATTCATCGTTTACGGTATGGACTA
CCAGGGTATCTAATCCTGTTTGCTACCCATACTTTCGAGCCTCAGCGTCAGTTACAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATAT
ATCTACGCATTTCACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCACTTCTTCGGTTGAGCCGAAGGC
TTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCAC
GTAGTTAGCCGTGGCTTTCTGGTTAAATACCGTCAATACCTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACGAGCCGAA
ACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCT
CAGTCCCAATGTGGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCCATGGTGAGCCGTTACCTCACCATCTAGCTAATACGCCGCGGGAC
CATCCAAAAGTGATA

SEQ ID NO: 74

S12KG421 519R

TGGCTTTCTGGTTAAATACCGTCAATACCTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACGAGCCGAAACCCTTCTTCAC
TCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGT
GGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCCATGGTGAGCCGTTACCTCACCATCTAGCTAATACGCCGCGGGACCATCTAAAAGTG
ATAGCCGAAGCCATCTTTCAAACTCGGACCATGCGGTCCAAGTTGTTATGCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGGCA
GGTTTCCCACGTGTTACTCACCAGTTCGCCACTCACTCAAATGTAAATCATGATGCAAGCACCAATCAATACCAGAGTTCGTTCGACTTGCATGT
ATTAGGCACGCCGCCAGCGTTCGTCCTGAGCCATGATCAAACTCTA

SEQ ID NO: 75

S12KG422 RP2

ACTTAGGCGGCTGGTTCCTAAAAGGTTACCCCACCGACTTTGGGTGTTACAAACTCTCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAA
CGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGAATGGCTTTA
AGAGATTAGCTTACTCTCGCGAGTTCGCAACTCGTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACG
TCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCACCAGAGTGCCCAACTTAATGCTGGCAACTGATAATAAGGGTTGCGCTCGTTGCGG
GACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTATCCATGTCCCCGAAGGGAACGTCTAATCTCTTAGATTTGC
ATAGTATGTCAAGACCTGGTAAGGTTCTTCGCGTAGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTT
TCAGCCTTGCGGCCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCA

NCIMB 42011 GGDK161b – L.reuteri (sample 11a)

SEQ ID NO: 76

S12KG441 27F

TAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACCTGATTGACGATGGATCACCAGTGAGTGGCGGACGGGTGAGT
AACACGTAGGTAACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAACAACAAAAGCCACATGGCTTTTGTTTGA

AAGATGGCTTTGGCTATCACTCTGGGATGGACCTGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGATGATGCATAGCCGAG
TTGAGAGACTGATCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGCAAGCC
TGATGGAGCAACACCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTGGAGAAGAACGTGCGTGAGAGTAACTGTTCACGCA
GTGACGGTATCCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTGATCCGGATTTATTGGGCG
TAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCGGAGACGGGCGACTTGAGTGCA

SEQ ID NO: 77

S12KG442 519F

TTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCGGAAAC
CGGGCGACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGG
CTGTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCT
AGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACGCAAAGGA
ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTT
ANAAGGCGTCCCCTTCGGGGACTCAATGACAGGTGGTGCATGGTT

SEQ ID NO: 78

S12KG443 926F

GGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCGTTCCCTTCGGG
GACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTACTAGTTG
CCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACGTCAGATCATCATGCCCCTTATGACCTG
GGCTACACACGTGCTACAATGGACGGTACAACGAGTCGCAAGCTCGCGAGAGTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGG
CTGCAACTCGCCTACACGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
ACACCATGGGAGTTTGTAACGCCCAAAGTCGGTGGCCTAACCTTTATGGAGGGAGCCGCCTAAGGCGGGACAGATGACTGGGGTGAAGTCGT
AACAAGGTAG

SEQ ID NO: 79

S12KG444 926R

No results

SEQ ID NO: 80

S12KG445 519R

GTGACTTTCTGGTTGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTTCTTCTCCAACAACAGAGCTTTACGAGCCGAAACCCTTCTTC
ACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGACCGTGTCTCAGTTCCATT
GTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATGCACCGCAGGTCCATCCCAGAGT
GATAGCCAAAGCCATCTTTCAAACAAAAGCCATGTGGCTTTTGTTGTTATGCGGTATTAGCATCTGTTTCCAAATGTTATCCCCCGCTCCGGGGC
AGGTTACCTACGTGTTACTCACCCGTCCGCCACTCACTGGTAATCCATCGTCAATCAGGTGCAAGCACCATCAATCAGTTGGGCCAGTGCGTAC
GACTTGCATGTATTAGGCACACCGCCGGCGTTCATCCTGAGCCA

SEQ ID NO: 81

S12KG446 RP2

CTCCCTCCATAAAGGTTAGGCCACCGACTTTGGGCGTTACAAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACC
GCGGCATGCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAACGGCTTTAAGAGATTAG

CTTACTCTCGCGAGCTTGCGACTCGTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATCTGACGTCGTCCCCA
CCTTCCTCCGGTTTGTCACCGGCAGTCTCACTAGAGTGCCCAACTTAATGCTGGCAACTAGTAACAAGGGTTGCGCTCGTTGCGGGACTTAACC
CAACATCTCACGACACGAGCTGACGACGACCATGCACCACCTGTCATTGCGTCCCCGAAGGGAACGCCTTATCTCTAAGGTTAGCGCAAGATGT
CAAGACCTGGTAAGGTTCTTCGCGTAGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTG
GCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTCCGGCACTGAAGGGCGGAA

NCIMB 42012 GGDK266c – L.reuteri (sample 1a)

SEQ ID NO: 82

S12KG381 27F

GTGTGCCTAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGCTTGCACCTGATTGACGATGGATCACCAGTGAGTGGCGGACG
GGTGAGTAACACGTAGGTAACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAACAACAAAAGCCACATGGCTTT
TGTTTGAAAGATGGCTTTGGCTATCACTCTGGGATGGACCTGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGATGATGCAT
AGCCGAGTTGAGAGACTGATCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGC
GCAAGCCTGATGGAGCAACACCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTGGAGAAGAACGTGCGTGAGAGTAACTGT
TCACGCAGTGACGGTATCCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTAT
TGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCGGAAACCGGGCGACTTGAG
TGC

SEQ ID NO: 83

S12KG382 519F

TTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCGGAAAC
CGGGCAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGG
CTGTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCT
AGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGA
ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTT
AGAGATAAGGCGTCCCTTCGGGGACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACGTCA

SEQ ID NO: 84

S12KG383 926F

GGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCGTTCCCTTCGGG
GACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTACTAGTTG
CCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACGTCAGATCATCATGCCCCTTATGACCTG
GGCTACACACGTGCTACAATGGACGGTACAACGAGTCGCAAGCTCGCGAGAGTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGG
CTGCAACTCGCCTACACGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
ACACCATGGGAGTTTGTAACGCCCAAAGTCGGTGGCCTAACCATTATGGAGGGAGCCGCCTAAGGCGGGACAGATGACTGGGGTGAAGTCGT
AACAAGGTAGCCGTA

SEQ ID NO: 85

S12KG384 926R

TACTCCCCAGGCGGAGTGCTTAATGCGTGAGCTCCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCACTCATCGTTTACGGCATGGACTA
CCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATAT
ATCTACGCATTCCACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTCGCCCGGTTTCCGATGCACTTCTTCGGTTAAGCCGAAGG
CTTTCACATCAGACCTAAGCAACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCA
CGTAGTTAGCCGTGACTTTCTGGTTGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTTCTTCTCCAACAACAGAGCTTTACGAGCCGA
AACCCTTCTTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGACCGTGTC
TCAGTTCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATGCACCGCAGGTC
CATCCCAGAGTGATAGCCAAAGCCATCTTTCAAACAAAAGCC

SEQ ID NO: 86

S12KG385 519R

GTGACTTTCTGGTTGGATACCGTCACTGCGTGAACAGTTACTCTCACGCACGTGCTTCTCCAACAACAGAGCTTTACGAGCCGAAACCCTTCTTC
ACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGACCGTGTCTCAGTTCCATT
GTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATGCACCGCAGGTCCATCCCAGAGT
GATAGCCAAAGCCATCTTTCAAACAAAAGCCATGTGGCTTTTGTTGTTATGCGGTATTAGCATCTGTTTCCAAATGTTATCCCCCGCTCCGGGGC
AGGTTACCTACGTGTTACTCACCCGTCCGCCACTCACTGGTGATCCATCGTCAATCAGGTGCAAGCACCATCAATCAGTTGGGCCAGTGCGTAC
GACTTGCATGTATTAGGCACACCGCCGGCGTTCATCCTGAGCCATGATCAAAC

SEQ ID NO: 87

S12KG386 RP2

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GGCTTTAAGAGATTAGCTTACTCTCGCGAGCTTGCGACTCGTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGA
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GTTAGCGCAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTAGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCT
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CATCGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCC

REFERENCES

Blandino, G., Fazio, D., Di Marco, R. Probiotics: Overview of microbiological and immunological characteristics (2008). *Expert Review of Anti-Infective Therapy*, 6 (4), pp. 497-508.

Cintas LM, Casaus MP, Herranz C, Nes IF, Hernandez PE. Review: bacteriocins of lactic acid bacteria (2001). Food Sci Technol Int. 7(4):281-305.

Clarridge III, J.E. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases (2004). Clinical Microbiology Reviews, 17 (4), pp. 840-862.

Cotter, P.D., Hill, C., Ross, R.P. Food microbiology: Bacteriocins: Developing innate immunity for food (2005). Nature Reviews Microbiology, 3 (10), pp. 777-788.

De Angelis, M., Siragusa, S., Berloco, M., Caputo, L., Settanni, L., Alfonsi, G., Amerio, M., Grandi, A., Ragni, A., Gobbetti, M. Selection of potential probiotic lactobacilli from pig feces to be used as additives in pelleted feeding (2006). *Research in Microbiology*, 157 (8), pp. 792-801

Elmadfa, I., Klein, P., Meyer, A.L. Immune-stimulating effects of lactic acid bacteria *in vivo* and *in vitro* (2010). *Proceedings of the Nutrition Society*, 69 (3), pp. 416-420.

Gopal, P.K., Sullivan, P.A., Smart, J.B.Utilisation of galacto-oligosaccharides as selective substrates for growth by lactic acid bacteria including Bifidobacterium lactis DR10 and Lactobacillus rhamnosus DR20 (2001). International Dairy Journal, 11 (1-2), pp. 19-25.

Gousia, P., Economou, V., Sakkas, H., Leveidiotou, S., Papadopoulou, C. Antimicrobial resistance of major foodborne pathogens from major meat products (2011). *Foodborne Pathogens and Disease*, 8 (1), pp. 27-38.

Jackson MS, Bird AR, McOrist AL. Comparison of two selective media for the detection and enumeration of Lactobacilli in human faeces (2002). J Microbiol Methods. 51(3):313-21. 65

Korhonen, J.M., Sclivagnotis, Y., Wright, A.V. Characterization of dominant cultivable lactobacilli and their antibiotic resistance profiles from faecal samples of weaning piglets (2007). *Journal of Applied Microbiology*, 103 (6), pp. 2496-2503.

Lähteinen, T., Malinen, E., Koort, J.M.K., Mertaniemi-Hannus, U., Hankimo, T., Karikoski, N., Pakkanen, S., Laine, H., Sillanpää, H., Söderholm, H., Palva, A. Probiotic properties of Lactobacillus isolates originating from porcine intestine and feces (2010). Anaerobe, 16 (3), pp. 293-300

Liu, Y., Fatheree, N.Y., Mangalat, N., Rhoads, J.M. Human-derived probiotic Lactobacillus reuteri strains differentially reduce intestinal inflammation (2010). *American Journal of Physiology - Gastrointestinal and Liver Physiology*, 299 (5), pp. G1087-G1096.

Ljungh, A., Wadström, T. Lactic acid bacteria as probiotics (2006). *Current Issues in Intestinal Microbiology*, 7 (2), pp. 73-90.

Martín, R, Delgado, S, Maldonado, A, Jiménez, E, Olivares, M, Fernández, L, Sobrino, OJ, Rodríguez, JM. Isolation of lactobacilli from sow milk and evaluation of their probiotic potential (2009). Journal of Dairy Research, 76 (4), pp. 418-425.

Mulder IE, Schmidt B, Stokes CR, Lewis M, Bailey M, Aminov RI, Prosser JI, Gill BP, Pluske JR, Mayer CD, Musk CC, Kelly D. Environmentally-acquired bacteria influence microbial diversity and natural innate immune responses at gut surfaces (2009). BMC Biol. 7:79.

Naughton PJ; Grant G. (2005) Modelling of salmonellosis In: Microbial Ecology of the Growing Animal Holzapfel WH, Naughton PJ. (Eds). London, Elsevier. pp. 235-257

Neeser, J.-R., Granato, D., Rouvet, M., Servin, A., Teneberg, S., Karlsson, K.-A. Lactobacillus johnsonii La1 shares carbohydrate-binding specificities with several enteropathogenic bacteria (2000). Glycobiology, 10 (11), pp. 1193-1199.

Nicolau, D.P. Current challenges in the management of the infected patient (2011). *Current Opinion in Infectious Diseases*, 24 (Suppl 1), pp. S1-S10.

Ohashi, Y., Ushida, K. Health-beneficial effects of probiotics: Its mode of action (2009). Animal Science Journal, 80 (4), pp. 361-371.

Reddy, K.B.P.K., Awasthi, S.P., Madhu, A.N., Prapulla, S.G. Role of cryoprotectants on the viability and functional properties of probiotic lactic acid bacteria during freeze drying (2009). *Food Biotechnology*, 23 (3), pp. 243-265.

Robertson, J.M.C., McKenzie, N.H., Duncan, M., Allen-Vercoe, E., Woodward, M.J., Flint, H.J., Grant, G. Lack of flagella disadvantages Salmonella enterica serovar Enteritidis during the early stages of infection in the rat (2003). Journal of Medical Microbiology, 52 (1), pp. 91-99.

Schreiber, O., Petersson, J., Phillipson, M., Perry, M., Roos, S., Holm, L. Lactobacillus reuteri prevents colitis by reducing P-selectin-associated leukocyte- and platelet-endothelial cell interactions (2009). American Journal of Physiology - Gastrointestinal and Liver Physiology, 296 (3), pp. G534-G542.

Smith, C.L., Geier, M.S., Yazbeck, R., Torres, D.M., Butler, R.N., Howarth, G.S. Lactobacillus fermentum BR11 and fructo-oligosaccharide partially reduce jejunal

inflammation in a model of intestinal mucositis in rats (2008). Nutrition and Cancer, 60 (6), pp. 757-767.

Strasser, S., Neureiter, M., Geppl, M., Braun, R., Danner, H. Influence of lyophilization, fluidized bed drying, addition of protectants, and storage on the viability of lactic acid bacteria (2009). *Journal of Applied Microbiology*, 107 (1), pp. 167-177.

Tomás, M.S.J., Bru, E., Martos, G., Nader-Macías, M.E. Stability of freeze-dried vaginal Lactobacillus strains in the presence of different lyoprotectors (2009). *Canadian Journal of Microbiology*, 55 (5), pp. 544-552.

Tzortzis, G., Baillon, M.-L.A., Gibson, G.R., Rastall, R.A. Modulation of anti-pathogenic activity in canine-derived Lactobacillus species by carbohydrate growth substrate (2004). Journal of Applied Microbiology, 96 (3), pp. 552-559.

Williams, N.T. Probiotics (2010). *American Journal of Health-System Pharmacy*, 67 (6), pp. 449-458.

Yao, W., Zhu Wei-yun, W.-Y., Smidt, H., Verstegen, M.W.A. Cultivation-Independent Analysis of the Development of the Lactobacillus spp. Community in the Intestinal Tract of Newborn Piglets (2011) *Agricultural Sciences in China*, 10 (3), pp. 438-447.

Yun, J.H., Lee, K.B., Sung, Y.K., Kim, E.B., Lee, H.-G., Choi, Y.J.
Isolation and characterization of potential probiotic lactobacilli from pig feces (2009). Journal of Basic Microbiology, 49 (2), pp. 220-226.

CLAIMS

1. A porcine lactic acid bacterial strain, wherein said bacterial strain is characterised by one or more of the following characteristics:

(i) the ability to exhibit antimicrobial activity against *E. coli*;

(ii) the ability to exhibit antimicrobial activity against *S. enteritidis*;

(iii) the ability to suppress inflammation in IPEC cells induced by 12-O-tetradecaboylphorbol-13-acetate (PMA);

(iv) the ability to block the attachment or invasion of IPEC cells by *S. enteritidis*;

(v) the ability to block the attachment or invasion of IPEC cells by *E. coli*;

(vi) the absence of antibiotic resistance to one or more antibiotics selected from the following: ampicillin; cefotaxime; chloramphenicol; erythromycin; gentamicin; tetracycline; vancomycin; metronizadole; nalidixic acid; and kanamycin; and

(vii) the ability to exhibit heat stability when subjected to three cycles of heating, each cycle comprising heating at a temperature of 70 °C for a period of 15 minutes.

2. A lactic acid bacterial strain according to claim 1 having any two characteristics selected from the group consisting of (i), (ii), (iii), (iv), (v), (vi) and (vii).

3. A lactic acid bacterial strain according to claim 1 having any three characteristics selected from the group consisting of (i), (ii), (iii), (iv), (v), (vi) and (vii).

4. A lactic acid bacterial strain according to claim 1 having any four characteristics selected from the group consisting of (i), (ii), (iii), (iv), (v), (vi) and (vii).

5. A lactic acid bacterial strain according to claim 1 having any five characteristics selected from the group consisting of (i), (ii), (iii), (iv), (v), (vi) and (vii).

6. A lactic acid bacterial strain according to claim 1 having any six characteristics selected from the group consisting of (i), (ii), (iii), (iv), (v), (vi) and (vii).

7. A lactic acid bacterial strain according to claim 1 having all seven characteristics (i), (ii), (iii), (iv), (v), (vi) and (vii).

8. A lactic acid bacterial strain according to any preceding claim which is selected from *L. johnsonii*, *L. reuteri*, *L. plantarum*, *L. gasseri*, *L. pentosus*, *L. acidophilus*, *L. vaginalis* and *L. mucosae*.

9. A lactic acid bacterial strain according to any preceding claim which is selected from *L. johnsonii*, *L. reuteri* and *L. plantarum*.

10. A lactic acid bacterial strain according to any preceding claim which is selected from:
NCIMB 41846: *Lactobacillus reuteri* GGDK31;
NCIMB 41847: *Lactobacillus paraplantarum* and *Lactobacillus reuteri* GGDK161;
NCIMB 41848: *Lactobacillus reuteri* GGDK255;
NCIMB 41849: *Lactobacillus plantarum/pentosus/helveticus/paraplantarum* GGDK258;
NCIMB 41850: *Lactobacillus johnsonii* and *Lactobacillus reuteri* GGDK266;
NCIMB 42008 *Lactobacillus johnsonii*;
NCIMB 42009 *Lactobacillus reuteri*;
NCIMB 42010 *Lactobacillus plantarum*;
NCIMB 42011 *Lactobacillus reute*ri; and
NCIMB 42012 *Lactobacillus reuteri.*

11. A composition comprising one or more lactic acid bacterial strains according to any one of claims 1 to 10 and a pharmaceutically acceptable excipient, carrier or diluent.

12. A probiotic composition comprising one or more lactic acid bacterial strains according to any one of claims 1 to 10.

13. One or more lactic acid bacterial strains according to any one of claims 1 to 10 for use in medicine.

14. One or more lactic acid bacterial strains according to any one of claims 1 to 10 for use in treating an intestinal disorder in a subject.

15. Use of one or more lactic acid bacterial strains according to any one of claims 1 to 10 in the preparation of a medicament for treating an intestinal disorder in a subject.

16. One or more lactic acid bacterial strains for use according to claim 14, or a use according to claim 15, wherein the subject is a mammal, preferably a human.

17. One or more lactic acid bacterial strains for use according to claim 14, or a use according to claim 15, wherein the intestinal disorder is salmonellosis, irritable bowel syndrome (IBS), inflammatory bowel disorder (IBD), functional dyspepsia, functional constipation, functional diarrhoea (including antibiotic associated diarrhoea, traveller's diarrhoea and pediatric diarrhoea), functional abdominal pain, functional bloating, Epigastric Pain Syndrome, Postprandial Distress Syndrome, Crohn's disease, ulcerative colitis, gastrointestinal reflux disease (GERD), allergies, atopic diseases e.g. atopic dermatitis, necrotising enterocolitis, other infections, and combinations thereof.

18. A method of treating an intestinal disorder in a subject, said method comprising administering to the subject a pharmaceutically effective amount of one or more lactic acid bacterial strains according to any one of claims 1 to 10 or composition according to claim 11 or claim 12.

19. One or more lactic acid bacterial strains according to any one of claims 1 to 10 for improving intestinal microbiota.

20. A method of improving intestinal microbiota in a subject, said method comprising administering to the subject a composition comprising one or more lactic acid bacterial strains according to any one of claims 1 to 10 or a composition according to claim 11 or claim 12.

21. Use of one or more lactic acid bacterial strains according to any one of claims 1 to 10 in the preparation of a medicament for improving intestinal microbiota.

22. A feedstuff comprising one or more bacterial strains according to any one of claims 1 to 10.

23. A food product comprising one or more bacterial strains according to any one of claims 1 to 10.

24. A dietary supplement comprising one or more bacterial strains according to any one of claims 1 to 10.

25. A food additive comprising one or more bacterial strains according to any one of claims 1 to 10.

26. A process for producing a probiotic, said process comprising culturing a bacterial strain according to any one of claims 1 to 10.

27. A method of preparing one or more bacterial strains according to any one of claims 1 to 10, said method comprising the steps of:

(i) obtaining faeces from an organically reared pig;

(ii) freezing the faeces and dispersing in a suitable diluent;

(iii) applying the dispersed faeces obtained in step (ii) to a suitable agar, optionally in the presence of supplemental pig colostrum carbohydrates, and incubating under an anaerobic conditions;

(v) selecting off distinct colonies of bacteria formed during step (iv) and seeding into a suitable broth, optionally in the presence of supplemental pig colostrum carbohydrates;

(vi) incubating the seeded colonies obtained in step (v).

28. A process for obtaining a porcine lactic acid bacterial strain, said process comprising obtaining faeces from an organically reared pig and extracting one or more porcine lactic acid bacterial strains from said faeces.

29. A process according to claim 28 which comprises the steps of:

(i) obtaining faeces from an organically reared pig;

(ii) freezing the faeces and dispersing in a suitable diluent;

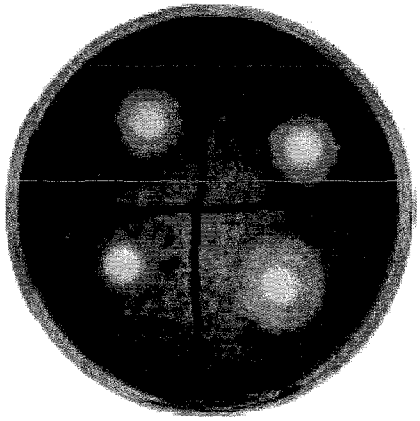
(iii) applying the dispersed faeces obtained in step (ii) to a suitable agar, optionally in the presence of supplemental pig colostrum carbohydrates, and incubating under an anaerobic conditions;

(v) selecting off distinct colonies of bacteria formed during step (iv) and seeding into a suitable broth, optionally in the presence of supplemental pig colostrum carbohydrates;

(vi) incubating the seeded colonies obtained in step (v).

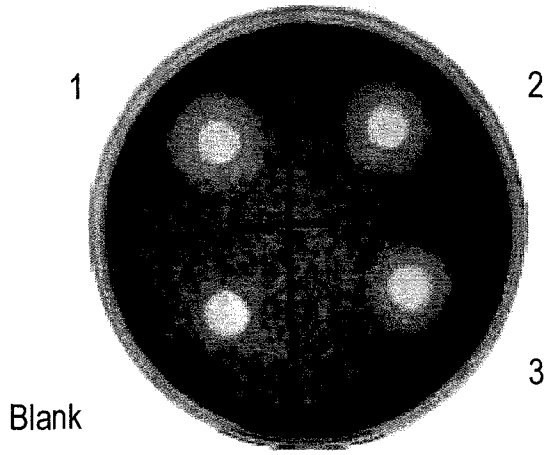
30. One or more porcine lactic acid bacterial strains obtained by, or obtainable by, the process of claim 28 or claim 29.

31. A lactic acid bacterial strain comprising a 16S rRNA gene sequence selected from SEQ ID NOs 1-87, or a variant or homologue thereof of said sequence.

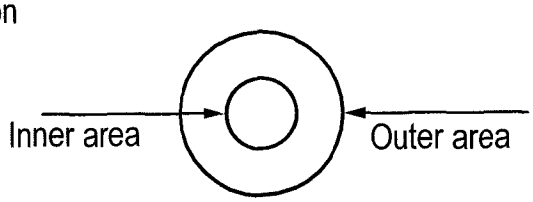


XLD agar containing S. enteritidis S1400 [10^6 cfu/ml].
Approximately 5 mm wells cut in agar
An aliquot (60µl) of conditionel media or MRS broth added to the wells.
Plates incubated aerobically for 16 hours at 37°C.
Image captured and area of inhibition measured.

Group 1	<20000 units of inhibition
Group 2	20000-40000 units of inhibition
Group 3	40000-60000 units of inhibition
Group 4	60000-80000 units of inhibition
Group 5	80000-100000 units of inhibition
Group 6	>>100000 units of inhibition



Bacterial Sample	Inner circle Diameter	Outer circle Diameter	Inhibition Area
1	174	366	81430
2	174	354	74644
3	174	336	64889



Inhibition area = $[(\pi R^2) - (\pi r^2)]$

FIG. 1

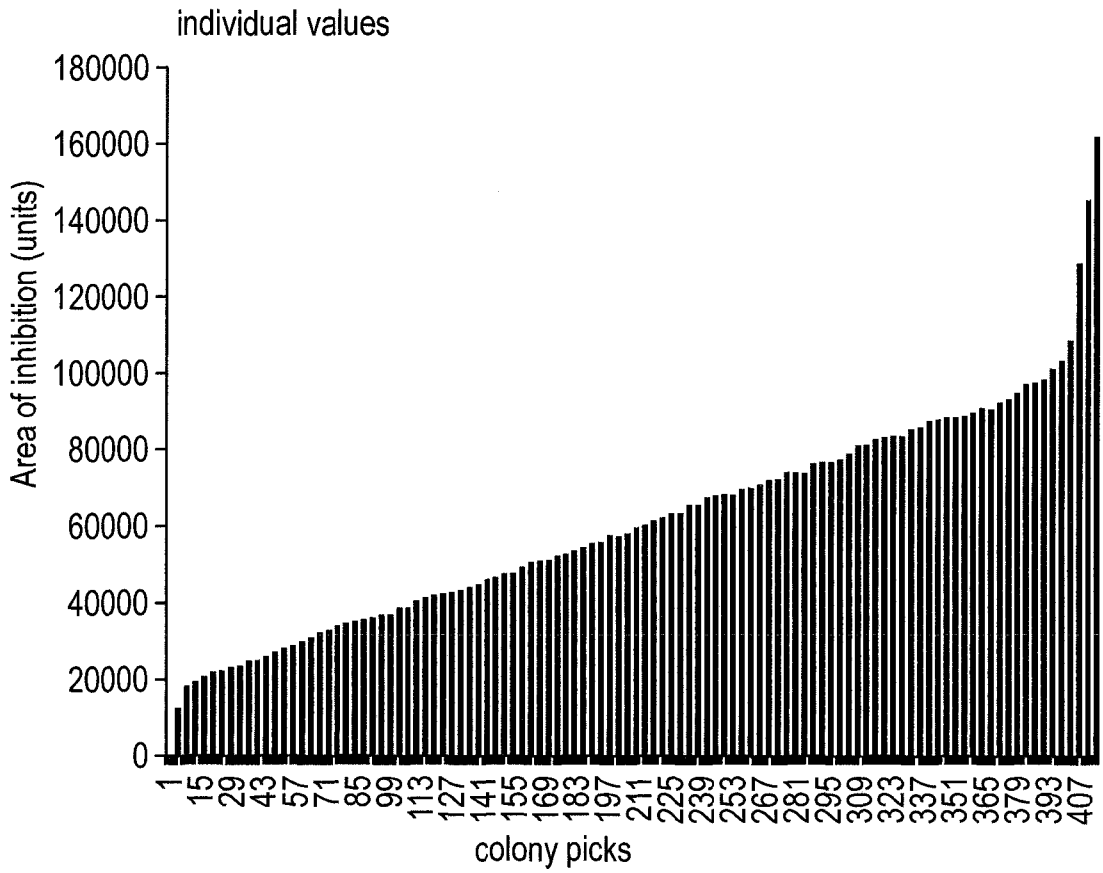

individual values
Area of inhibition (units)
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
1
15
29
43
57
71
85
99
113
127
141
155
169
183
197
211
225
239
253
267
281
295
309
323
337
351
365
379
393
407
colony picks

FIG. 2a

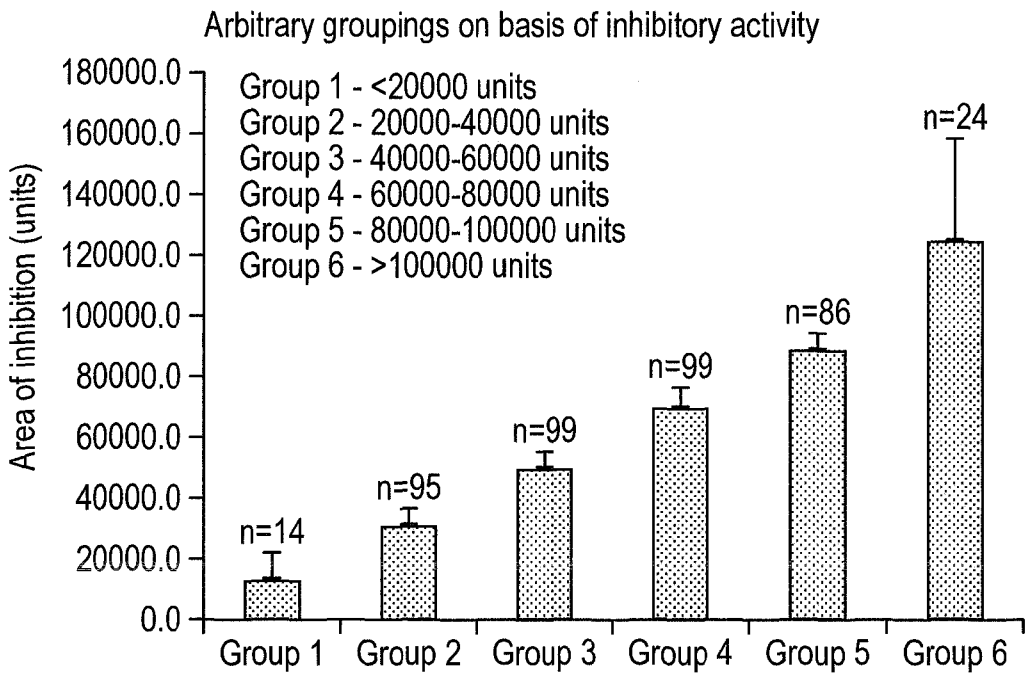

Arbitrary groupings on basis of inhibitory activity
Area of inhibition (units)
180000.0
160000.0
140000.0
120000.0
100000.0
80000.0
60000.0
40000.0
20000.0
0.0
Group 1 - <20000 units
Group 2 - 20000-40000 units
Group 3 - 40000-60000 units
Group 4 - 60000-80000 units
Group 5 - 80000-100000 units
Group 6 - >100000 units
n=14
n=95
n=99
n=99
n=86
n=24
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6

FIG. 2b

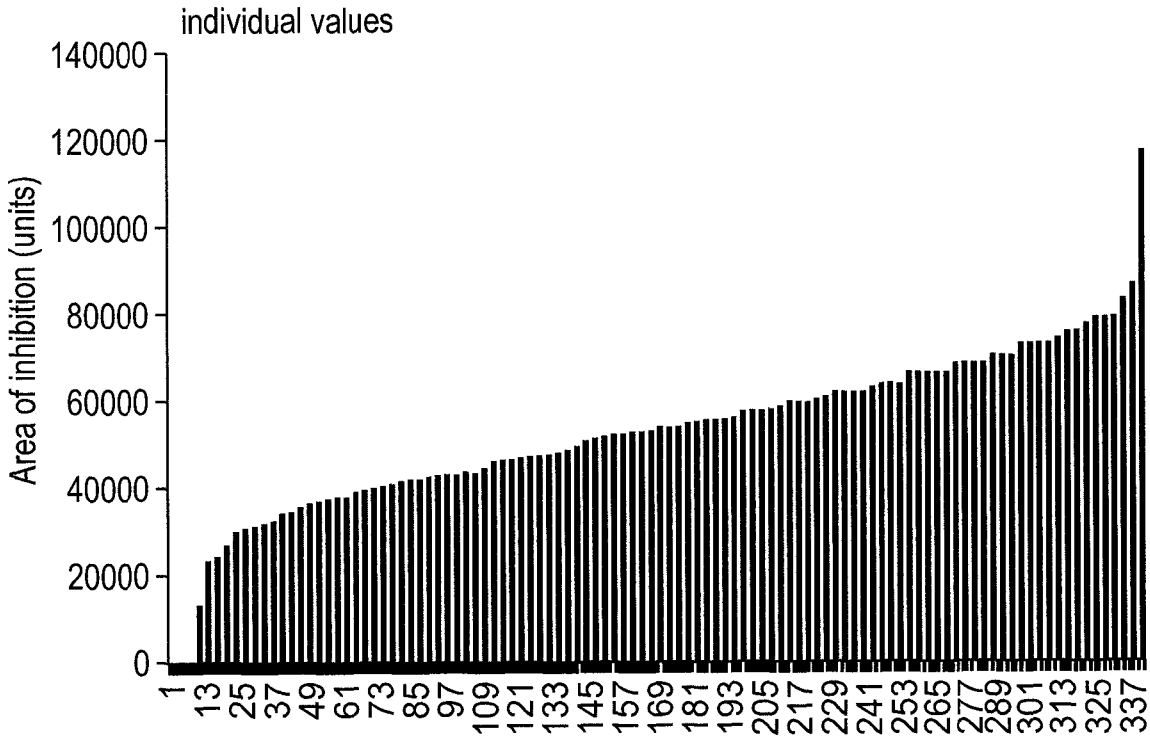

individual values
Area of inhibition (units)
140000
120000
100000
80000
60000
40000
20000
0
1
13
25
37
49
61
73
85
97
109
121
133
145
157
169
181
193
205
217
229
241
253
265
277
289
301
313
325
337

FIG. 3a

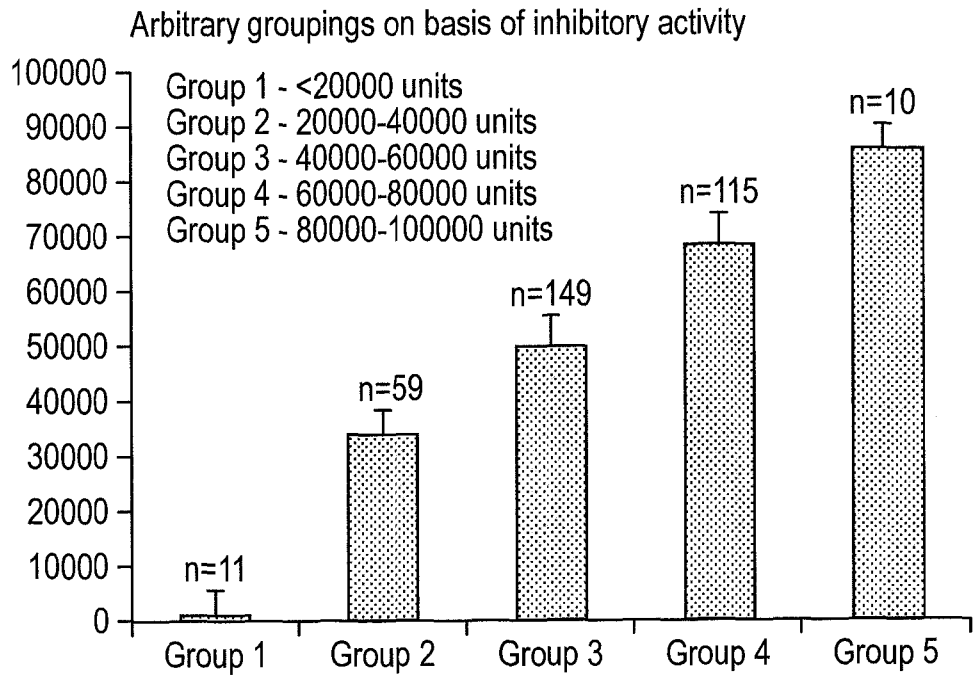

Arbitrary groupings on basis of inhibitory activity
Group 1 - <20000 units
Group 2 - 20000-40000 units
Group 3 - 40000-60000 units
Group 4 - 60000-80000 units
Group 5 - 80000-100000 units
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
n=11
n=59
n=149
n=115
n=10
Group 1
Group 2
Group 3
Group 4
Group 5

FIG. 3b

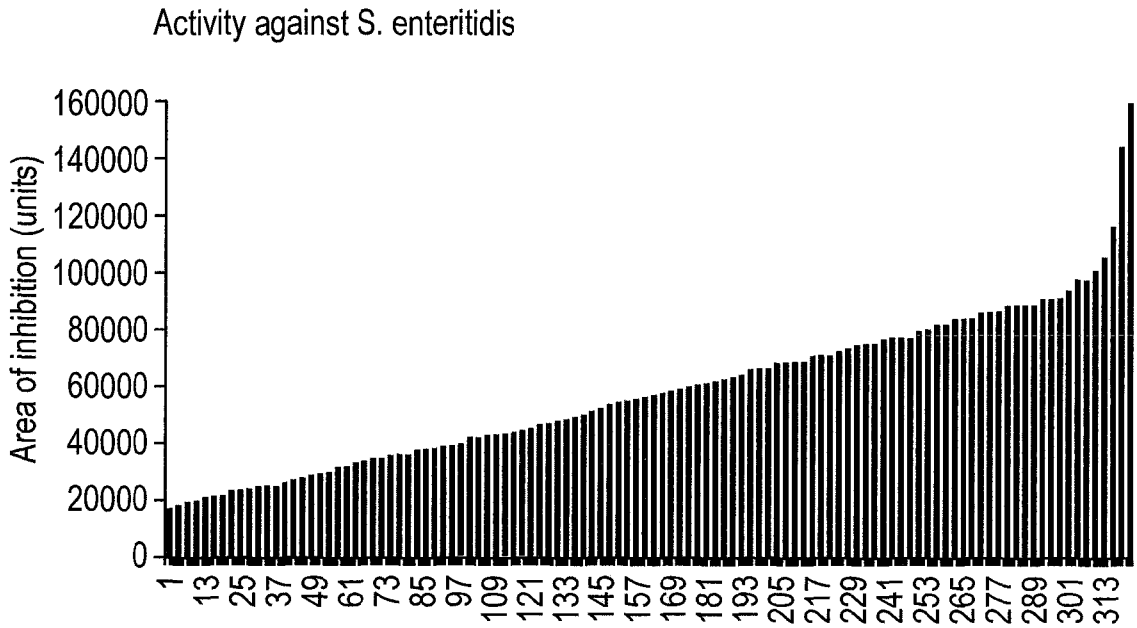

Activity against S. enteritidis
Area of inhibition (units)
160000
140000
120000
100000
80000
60000
40000
20000
0
1
13
25
37
49
61
73
85
97
109
121
133
145
157
169
181
193
205
217
229
241
253
265
277
289
301
313

FIG. 3c

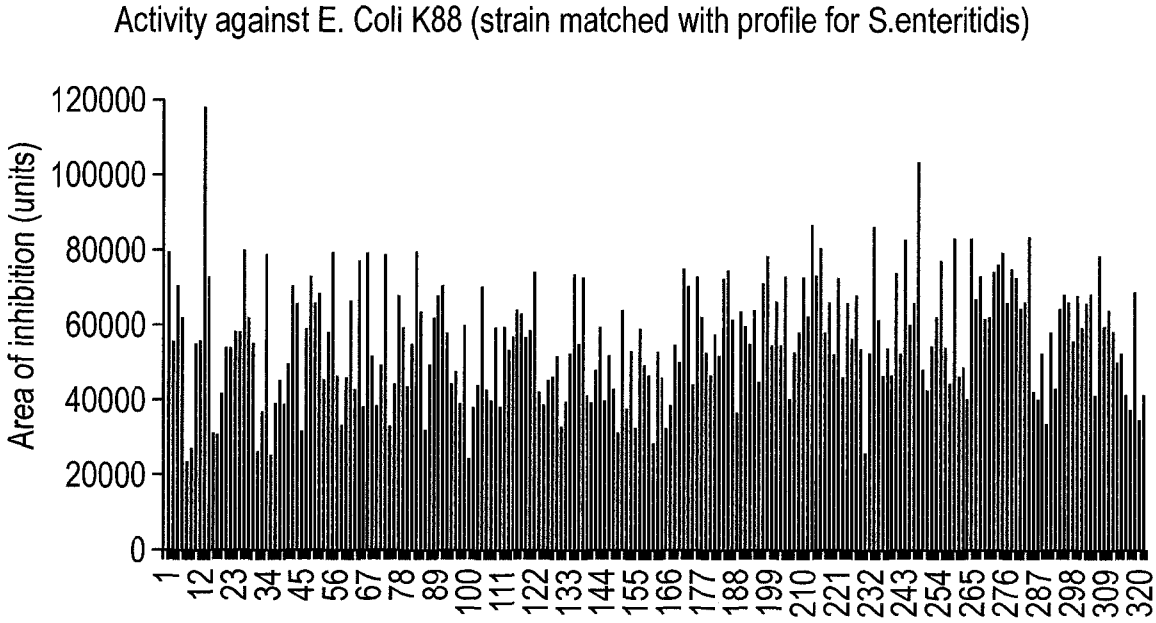

Activity against E. Coli K88 (strain matched with profile for S.enteritidis)
Area of inhibition (units)
120000
100000
80000
60000
40000
20000
0
1
12
23
34
45
56
67
78
89
100
111
122
133
144
155
166
177
188
199
210
221
232
243
254
265
276
287
298
309
320

FIG. 3d

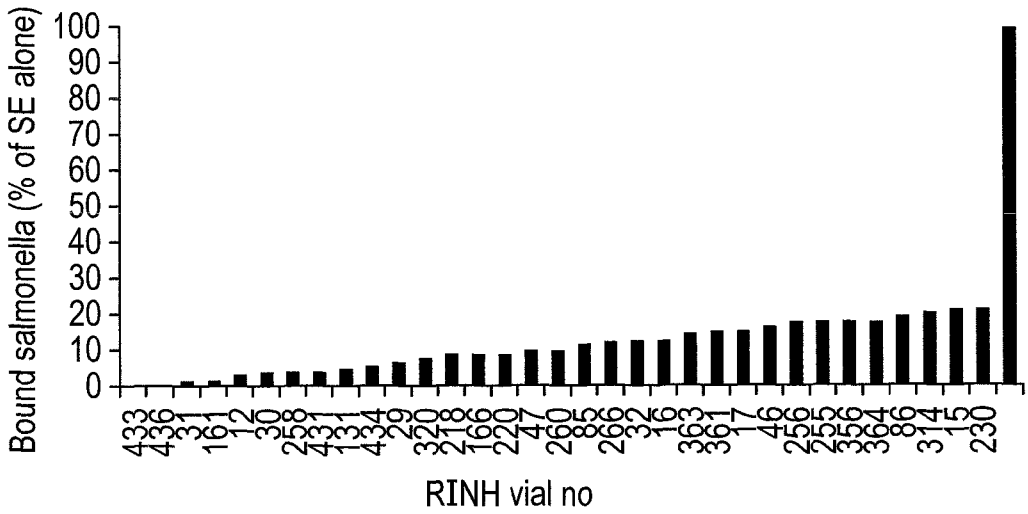


FIG. 4a

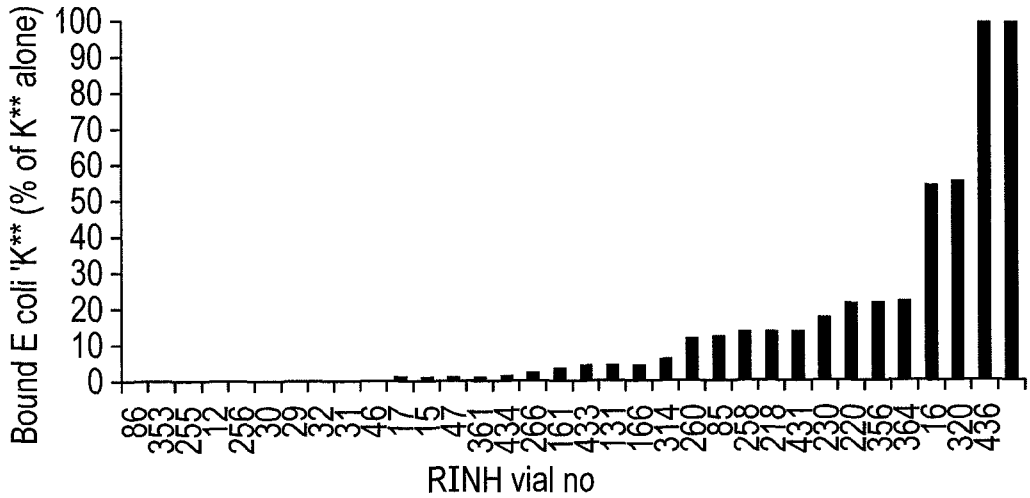


FIG. 4b

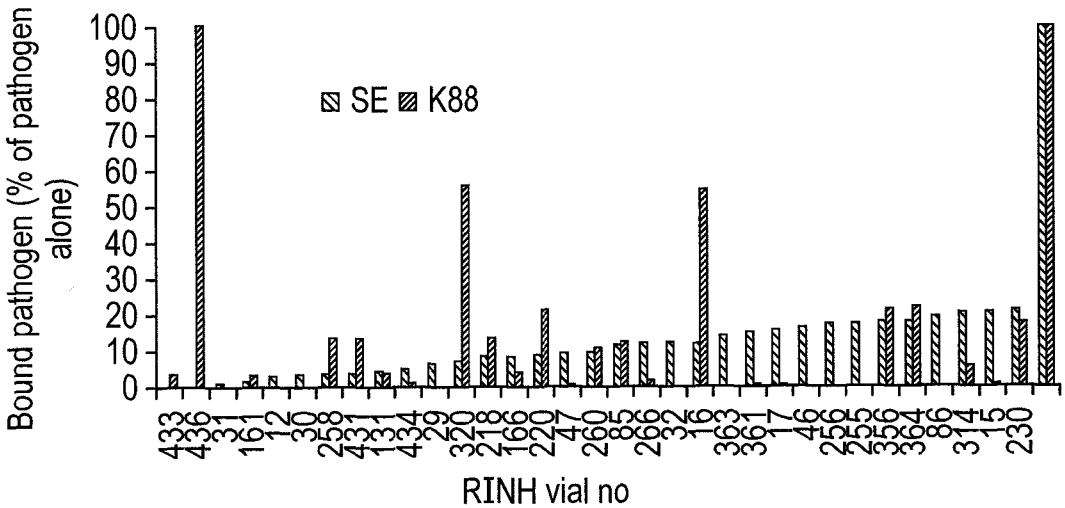
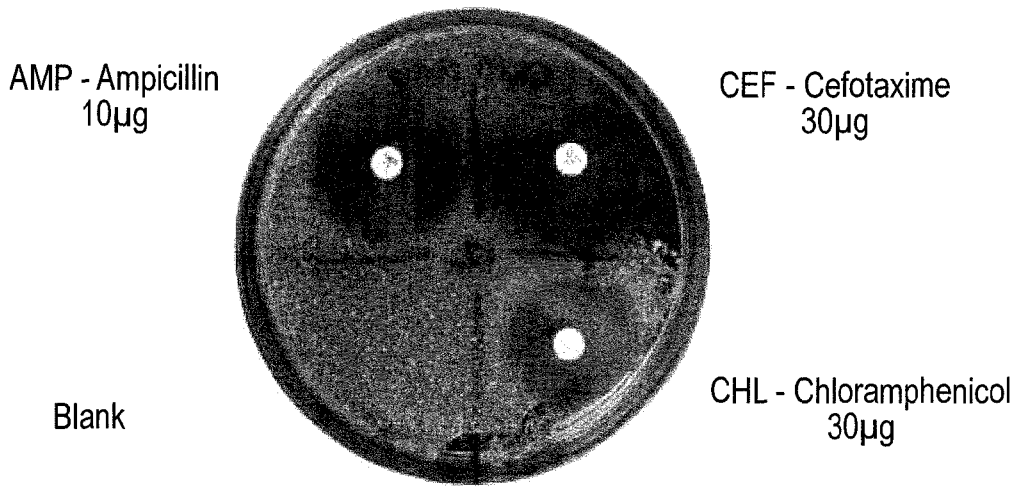
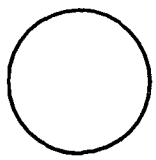


FIG. 4c

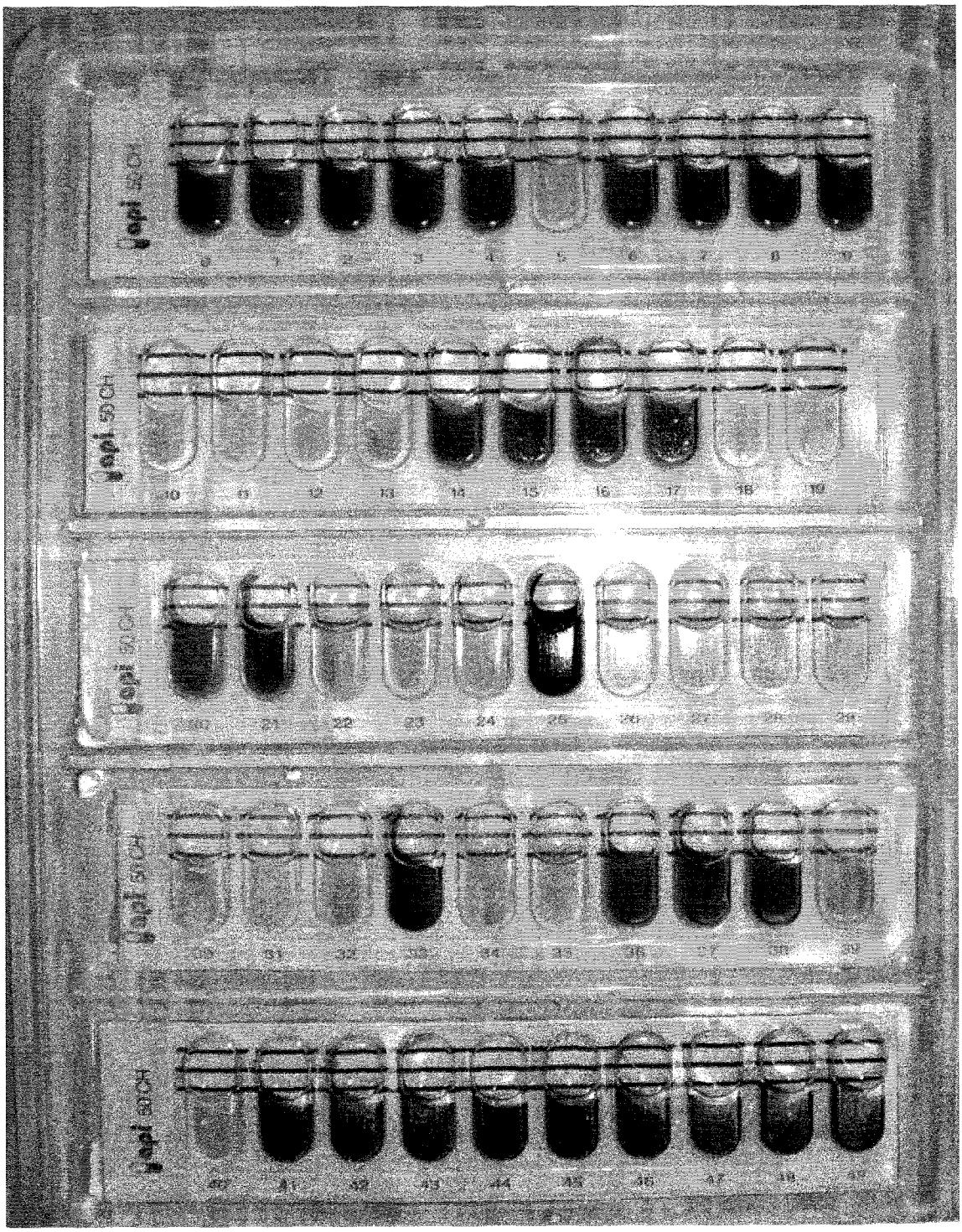


Inhibition area = πR^2



Ampicillin
Cefotaxime
Chloramphenicol
Erythromycin
Tetracycline
Vancomycin
Gentamicin
Kanamycin
Metronizadole
Nalidixic acid

FIG. 5



Bacterium evaluated: Lactobacillus plantarum

FIG. 6

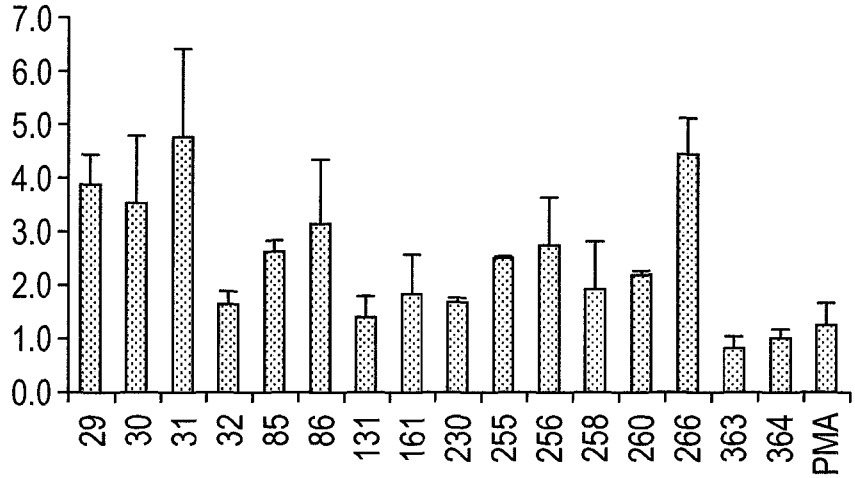


FIG. 7a

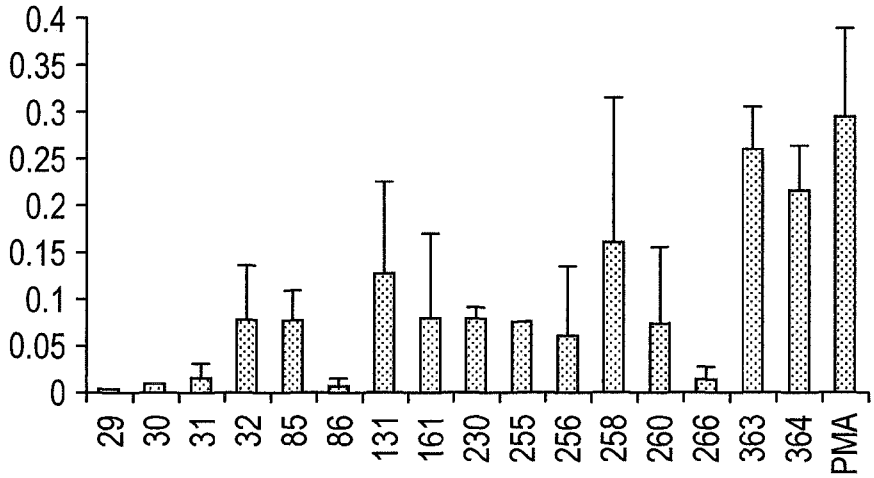


FIG. 7b

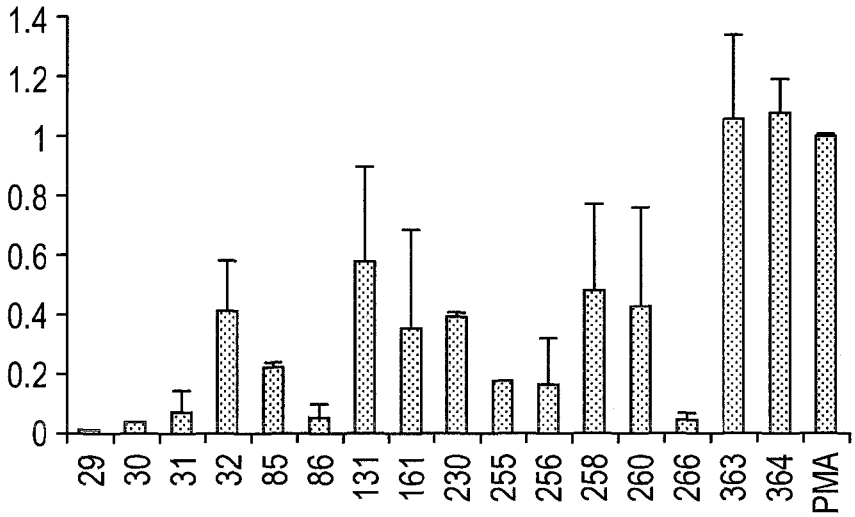


FIG. 7c

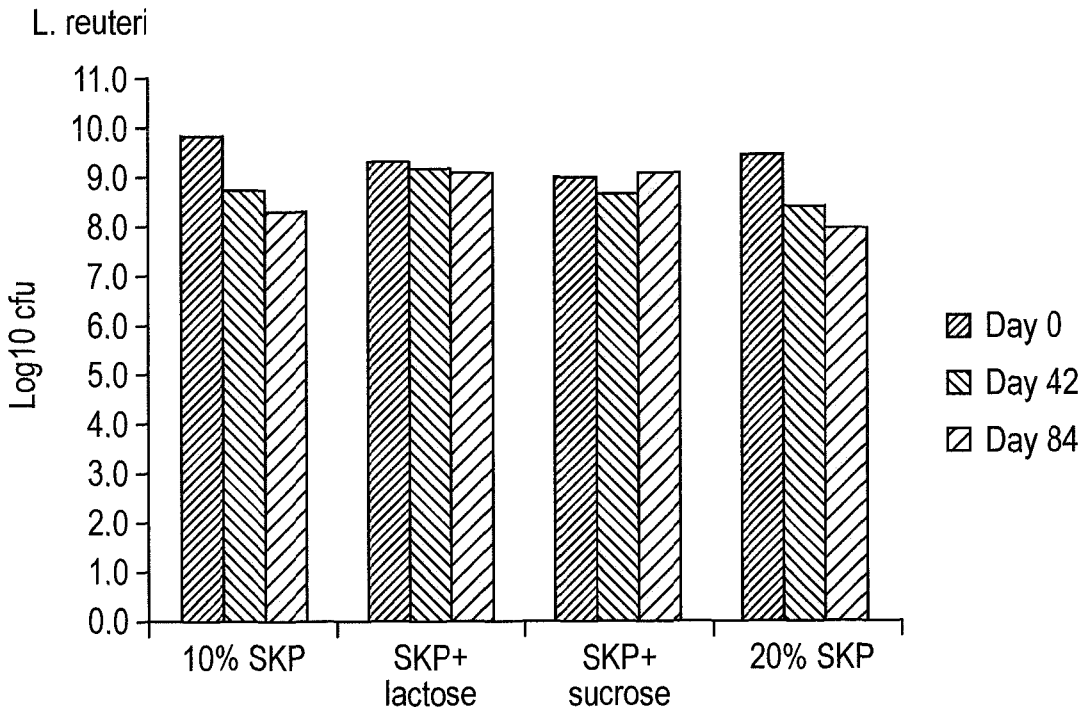

L. reuteri
Log10 cfu
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
10% SKP
SKP+ lactose
SKP+ sucrose
20% SKP
Day 0
Day 42
Day 84

FIG. 8a

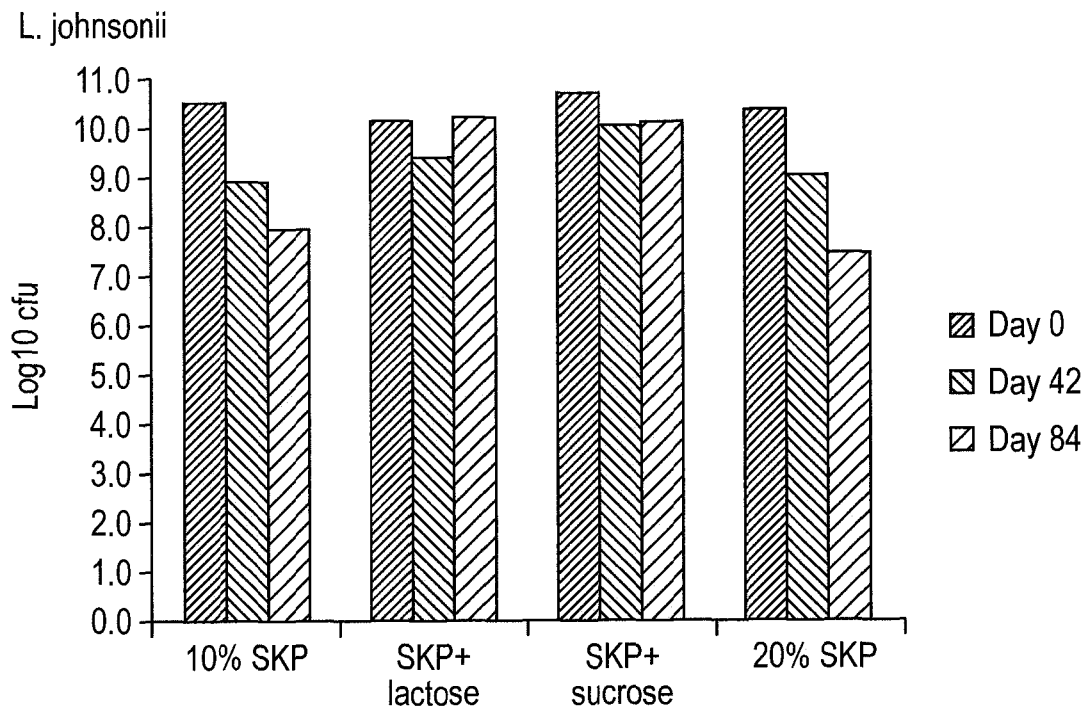

L. johnsonii
Log10 cfu
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
10% SKP
SKP+ lactose
SKP+ sucrose
20% SKP
Day 0
Day 42
Day 84

FIG. 8b

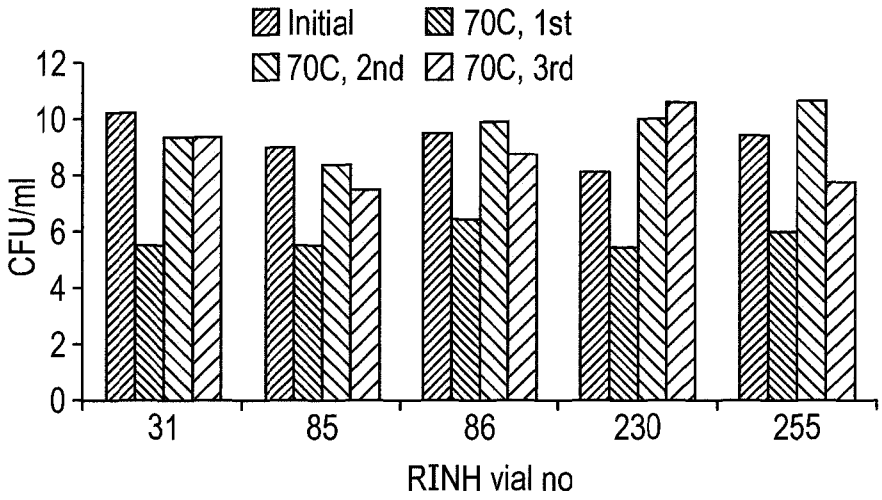


FIG. 9a

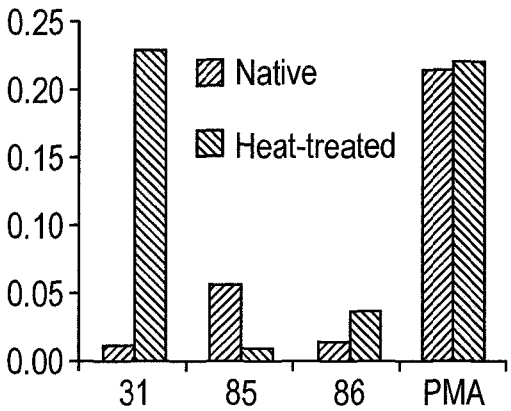


FIG. 9b

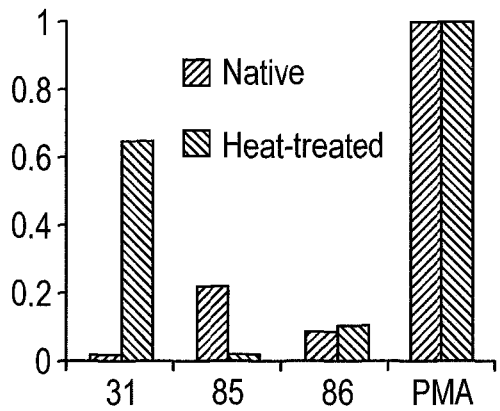


FIG. 9c

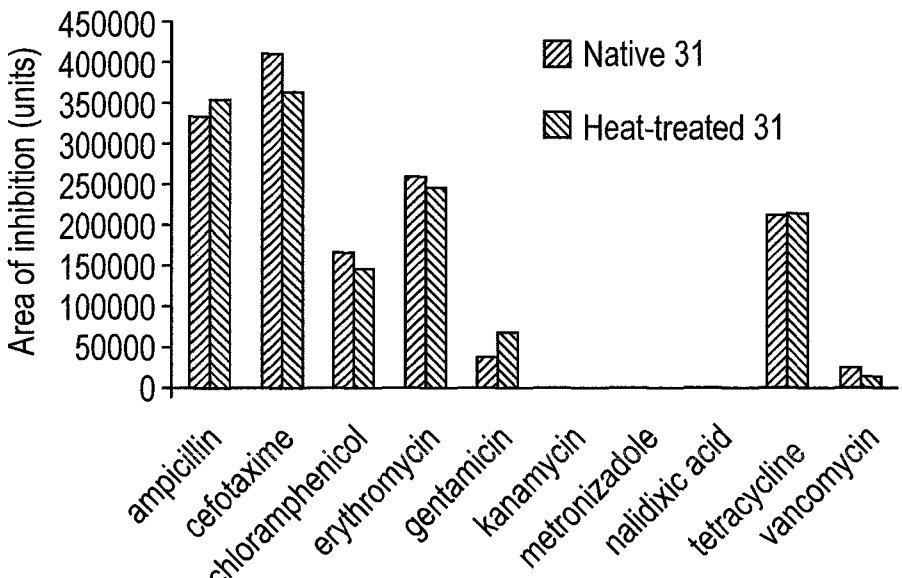


FIG. 9d

Day		Control	Salmonella	L. mucosae	L. mucosae + salmonella
-7	AM	MRS broth	MRS broth	L. mucosae	L. mucosae
-4	AM	MRS broth	MRS broth	L. mucosae	L. mucosae
-2	AM	MRS broth	MRS broth	L. mucosae	L. mucosae
0	AM	MRS broth	MRS broth	L. mucosae	L. mucosae
	PM	LB media	SE S1400	LB media	SE S140
1	AM	MRS broth	MRS broth	L. mucosae	L. mucosae
2	AM	MRS broth	MRS broth	L. mucosae	L. mucosae
3	AM	MRS broth	MRS broth	L. mucosae	L. mucosae
4	AM	MRS broth	MRS broth	L. mucosae	L. mucosae
5	AM	MRS broth	MRS broth	L. mucosae	L. mucosae
6	AM	MRS broth	MRS broth	L. mucosae	L. mucosae
7	AM	MRS broth	MRS broth	L. mucosae	L. mucosae
8	AM	MRS broth	MRS broth	L. mucosae	L. mucosae
9	AM	MRS broth	MRS broth	L. mucosae	L. mucosae
10		Euthanase	Euthanase	Euthanase	Euthanase

SE S1400, S enteritidis S1400. LB media, Luria Bertani broth

FIG. 10

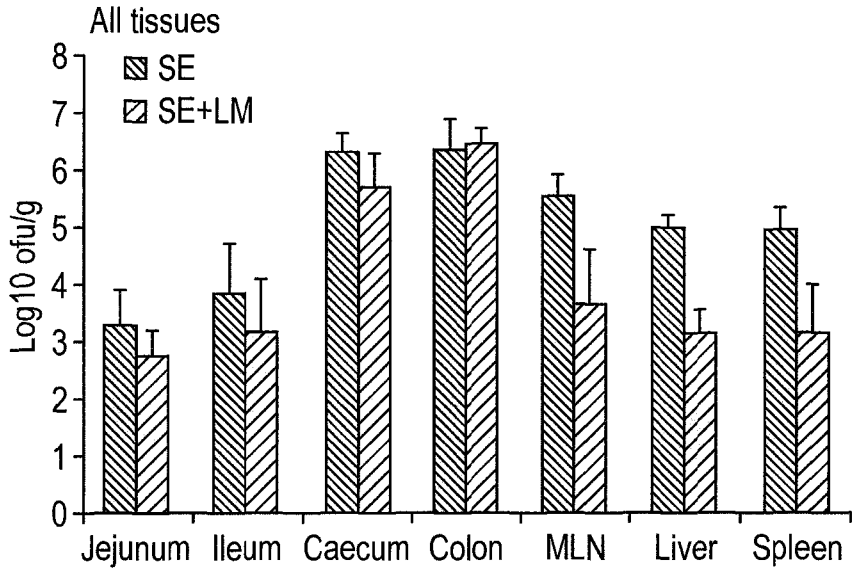
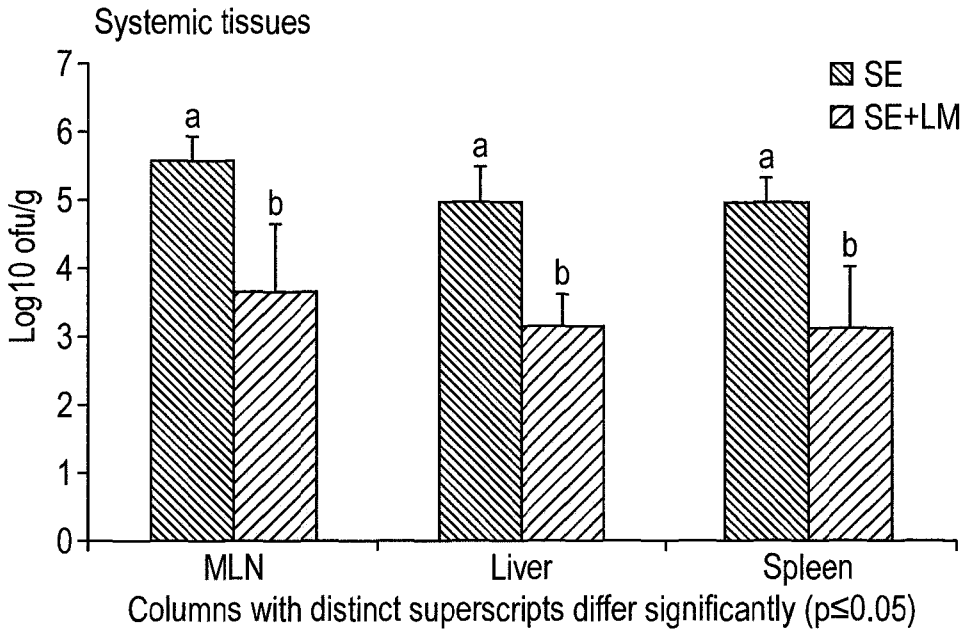


FIG. 11a



Columns with distinct superscripts differ significantly ($p \leq 0.05$)

FIG. 11b

Statistical analysis

S. enteritidis vs S. enteritidis + L. mucosae

Jejunum	$p>0.05$
Ileum	$p>0.05$
Caecum	$p>0.05$
Colon	$p>0.05$
MLN	$p<0.01$
Liver	$p<0.01$
Spleen	$p<0.01$

FIG. 11c

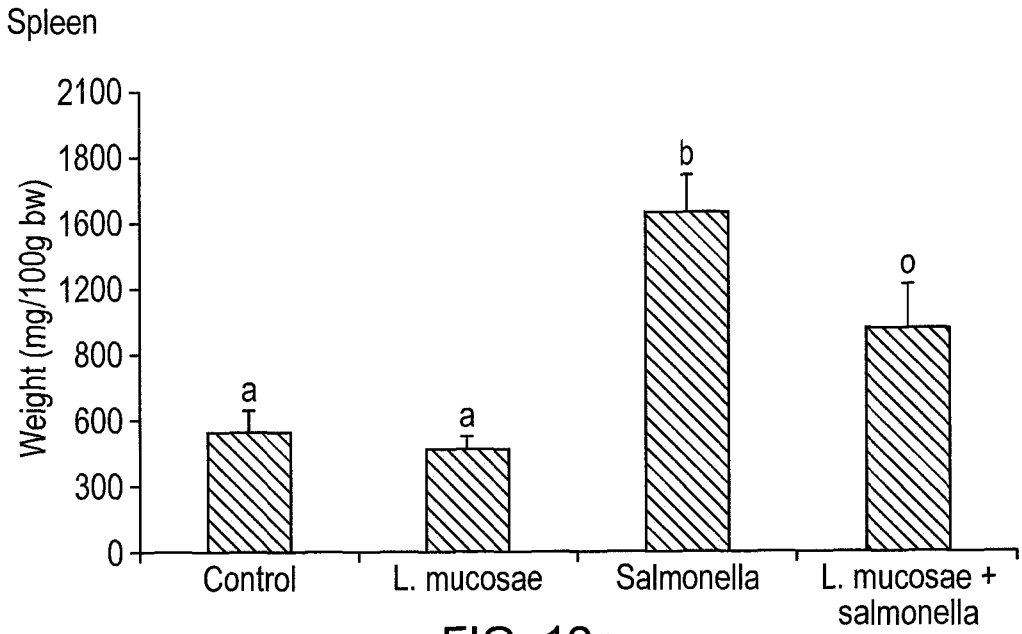
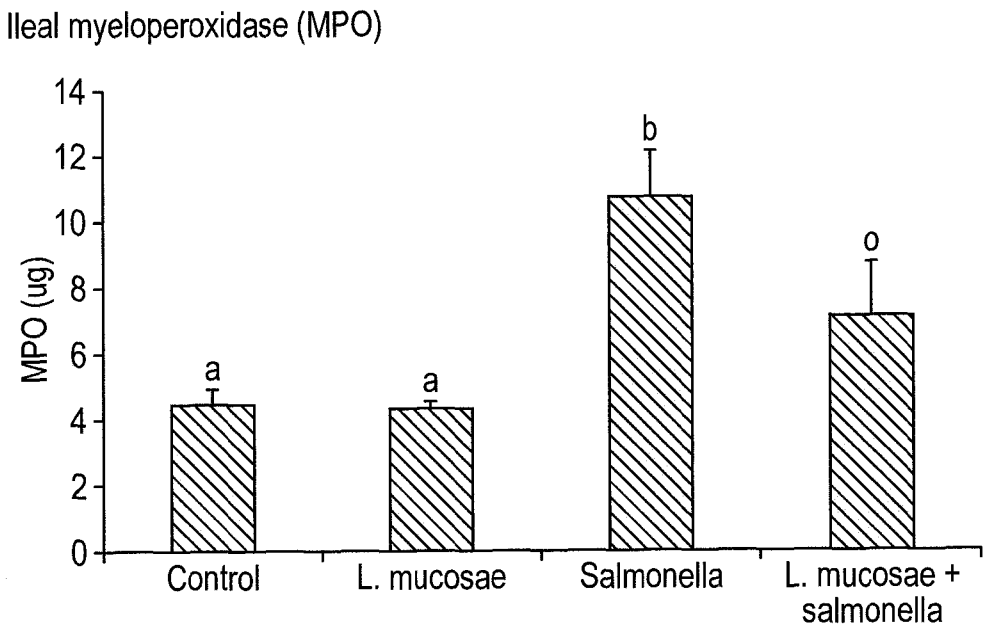


FIG. 12a



Columns with distinct superscripts differ significantly ($p \leq 0.05$)

FIG. 12b

Day		Salmonella	L. mucosae + salmonella
-7	AM	MRS broth	L. mucosae
-4	AM	MRS broth	L. mucosae
-2	AM	MRS broth	L. mucosae
		↓	↓
0	AM	MRS broth	L. mucosae
	PM	SE S1400	SE S1400
		↓	↓
1	AM	MRS broth	L. mucosae
2	AM	MRS broth	L. mucosae
3	AM	MRS broth	L. mucosae
4	AM	MRS broth	L. mucosae
5	AM	MRS broth	L. mucosae
6		Euthanase	Euthanase

SE S1400, S enteritidis S1400. LB media, Luria Bertani broth

FIG. 13

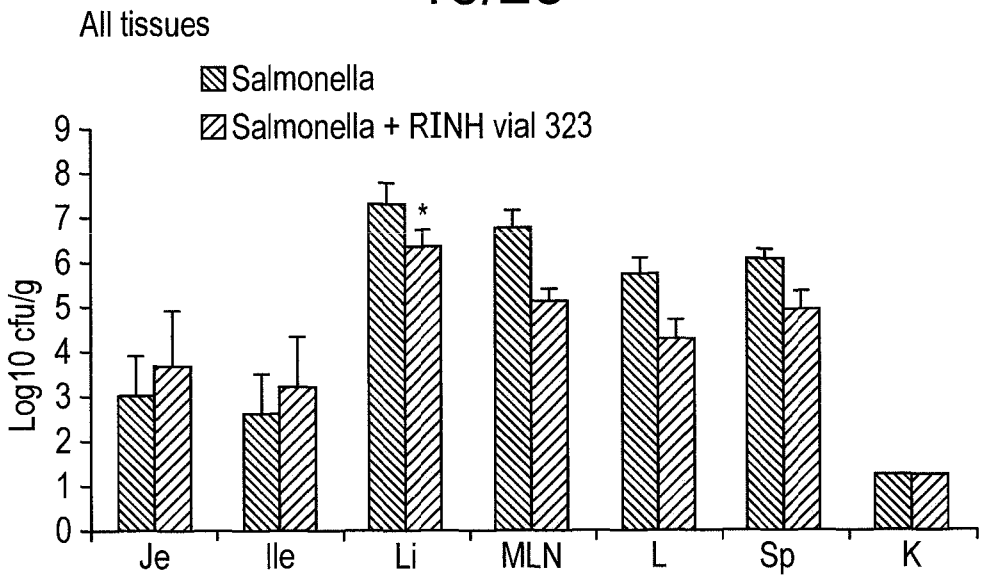


FIG. 14a

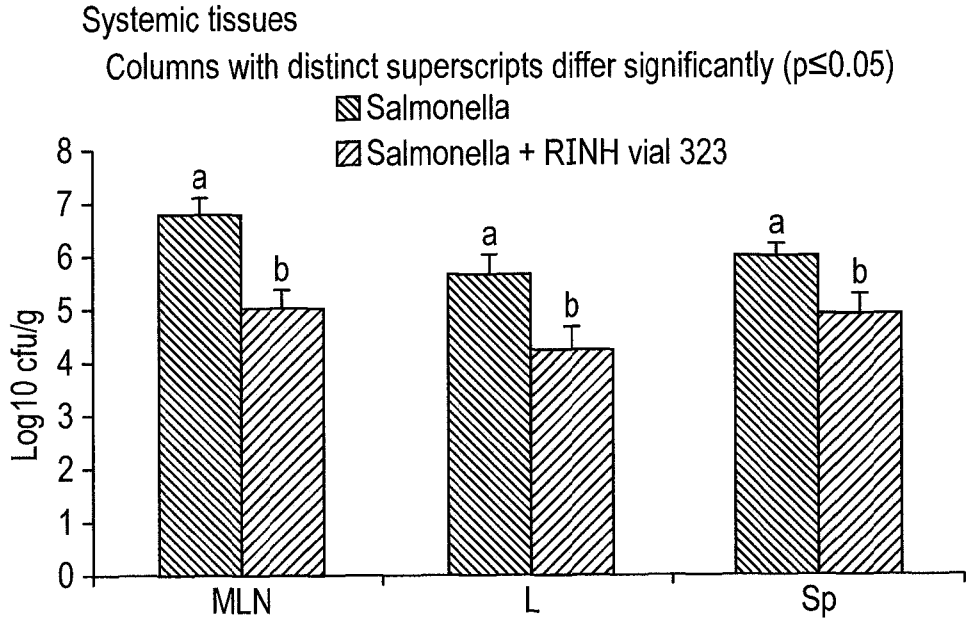
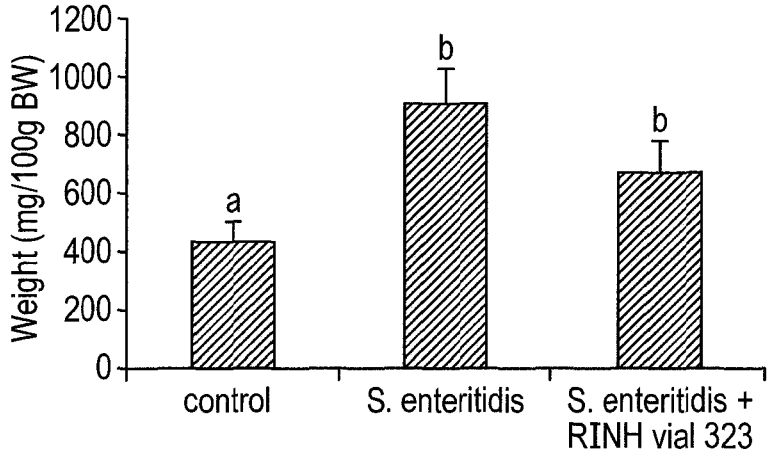


FIG. 14b

Statistical analysis

S. enteritidis vs S. enteritidis + 323	
Jejunum	p>0.05
Ileum	p>0.05
Large intestine	p<0.05
MLN	p<0.05
Liver	p<0.05
Spleen	p<0.05

FIG. 14c



Columns with distinct superscripts differ significantly ($p \leq 0.05$)

FIG. 15

LAB Challenge Day		Control	Salmonella	Group 6 L. reuteri 31 Salmonella	Group 6 L. reuteri 32 Salmonella	Group 3 L. mucosae Salmonella	Group 1 L. reuteri 46 Salmonella	Group 1; L. reuteri 47 Salmonella
-6	AM	MRS broth	MRS broth	LR 31	LR 32	L. mucosae	LR 46	LR 47
-4	AM	MRS broth	MRS broth	LR 31	LR 32	L. mucosae	LR 46	LR 47
-2	AM	MRS broth	MRS broth	LR 31	LR 32	L. mucosae	LR 46	LR 47
0	AM	MRS broth	MRS broth	LR 31	LR 32	L. mucosae	LR 46	LR 47
	PM	LB media	SE S1400	SE S1400	SE S1400	SE S1400	SE S1400	SE S1400
1	AM	MRS broth	MRS broth	LR 31	LR 32	L. mucosae	LR 46	LR 47
2	AM	MRS broth	MRS broth	LR 31	LR 32	L. mucosae	LR 46	LR 47
3	AM	MRS broth	MRS broth	LR 31	LR 32	L. mucosae	LR 46	LR 47
4	AM	MRS broth	MRS broth	LR 31	LR 32	L. mucosae	LR 46	LR 47
5	AM	MRS broth	MRS broth	LR 31	LR 32	L. mucosae	LR 46	LR 47
6	AM	MRS broth	MRS broth	LR 31	LR 32	L. mucosae	LR 46	LR 47
7	AM	MRS broth	MRS broth	LR 31	LR 32	L. mucosae	LR 46	LR 47
8	AM	MRS broth	MRS broth	LR 31	LR 32	L. mucosae	LR 46	LR 47
9	AM	MRS broth	MRS broth	LR 31	LR 32	L. mucosae	LR 46	LR 47
10		Euthanase	Euthanase	Euthanase	Euthanase	Euthanase	Euthanase	Euthanase

LR 31. Pig L. reuteri vial 3. LR 32, Pig L. reuteri vial 32. LR 46. Pig L reuteri vial 46. LR 47, Pig L. reuteri vial 47. SE S1400. S. enteritidis S1400. LB media, Luria Bertani broth

FIG. 16

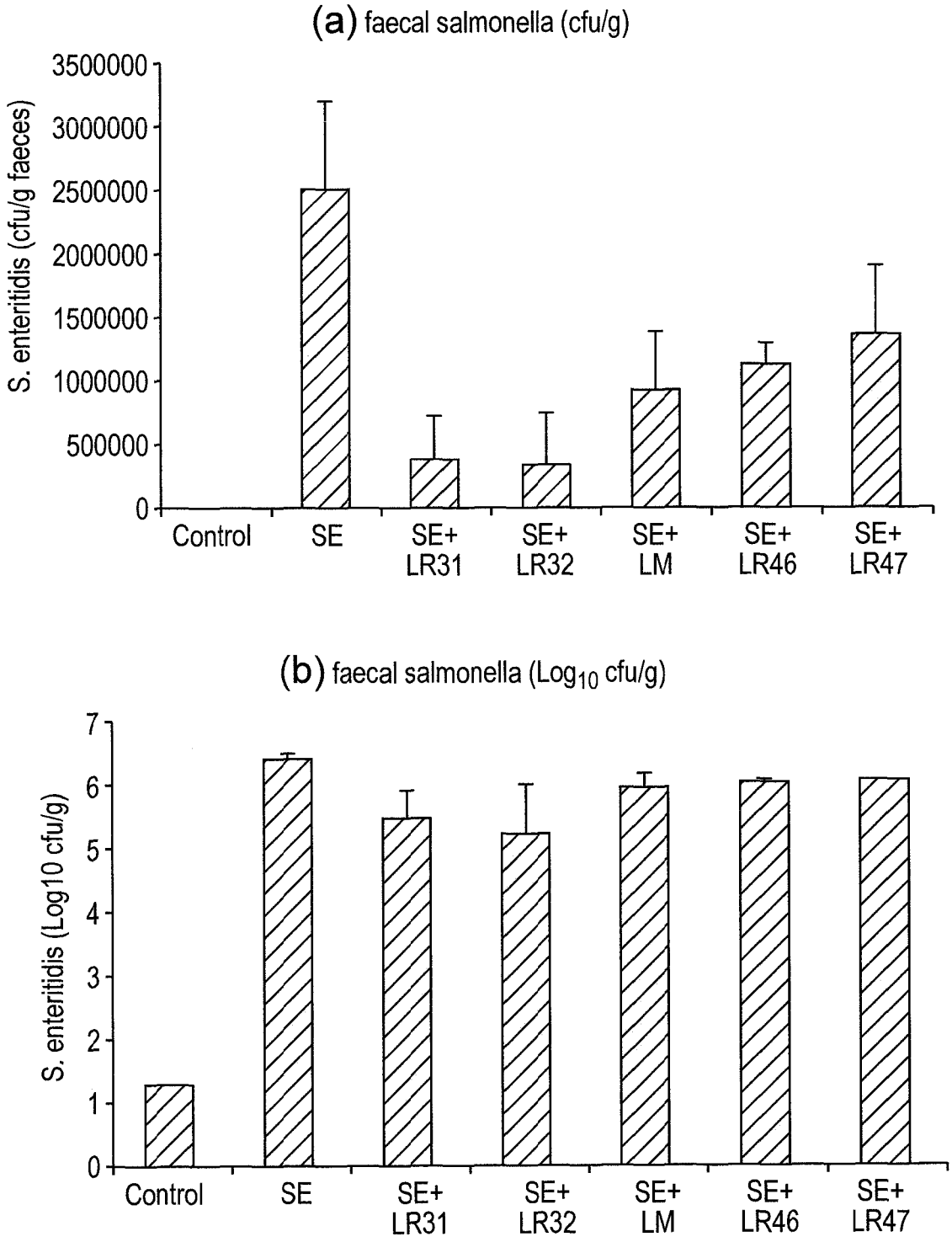

(a) faecal salmonella (cfu/g)
S. enteritidis (cfu/g faeces)
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
Control
SE
SE+ LR31
SE+ LR32
SE+ LM
SE+ LR46
SE+ LR47
(b) faecal salmonella (Log10 cfu/g)
S. enteritidis (Log10 cfu/g)
7
6
5
4
3
2
1
0
Control
SE
SE+ LR31
SE+ LR32
SE+ LM
SE+ LR46
SE+ LR47

FIG. 17

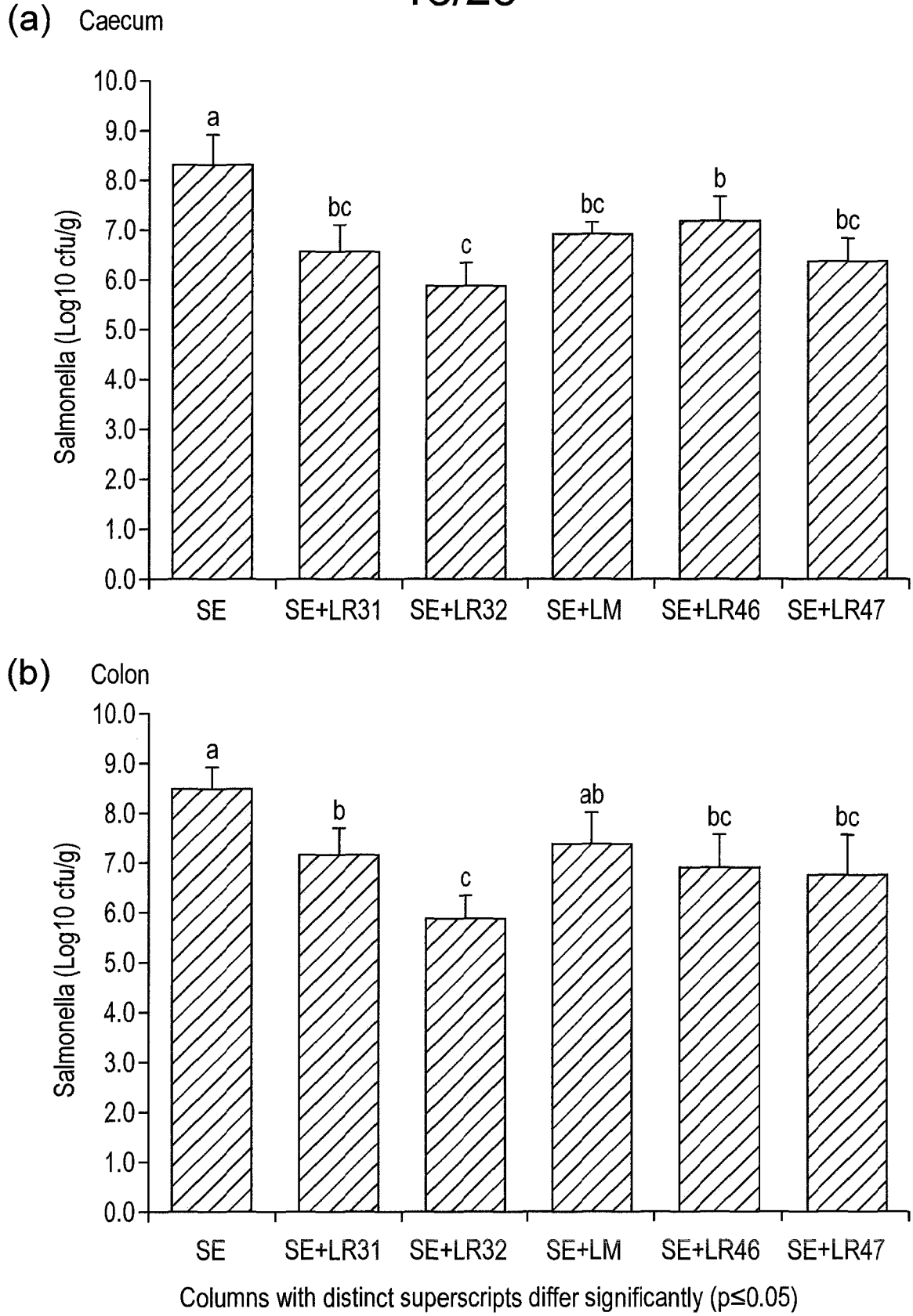
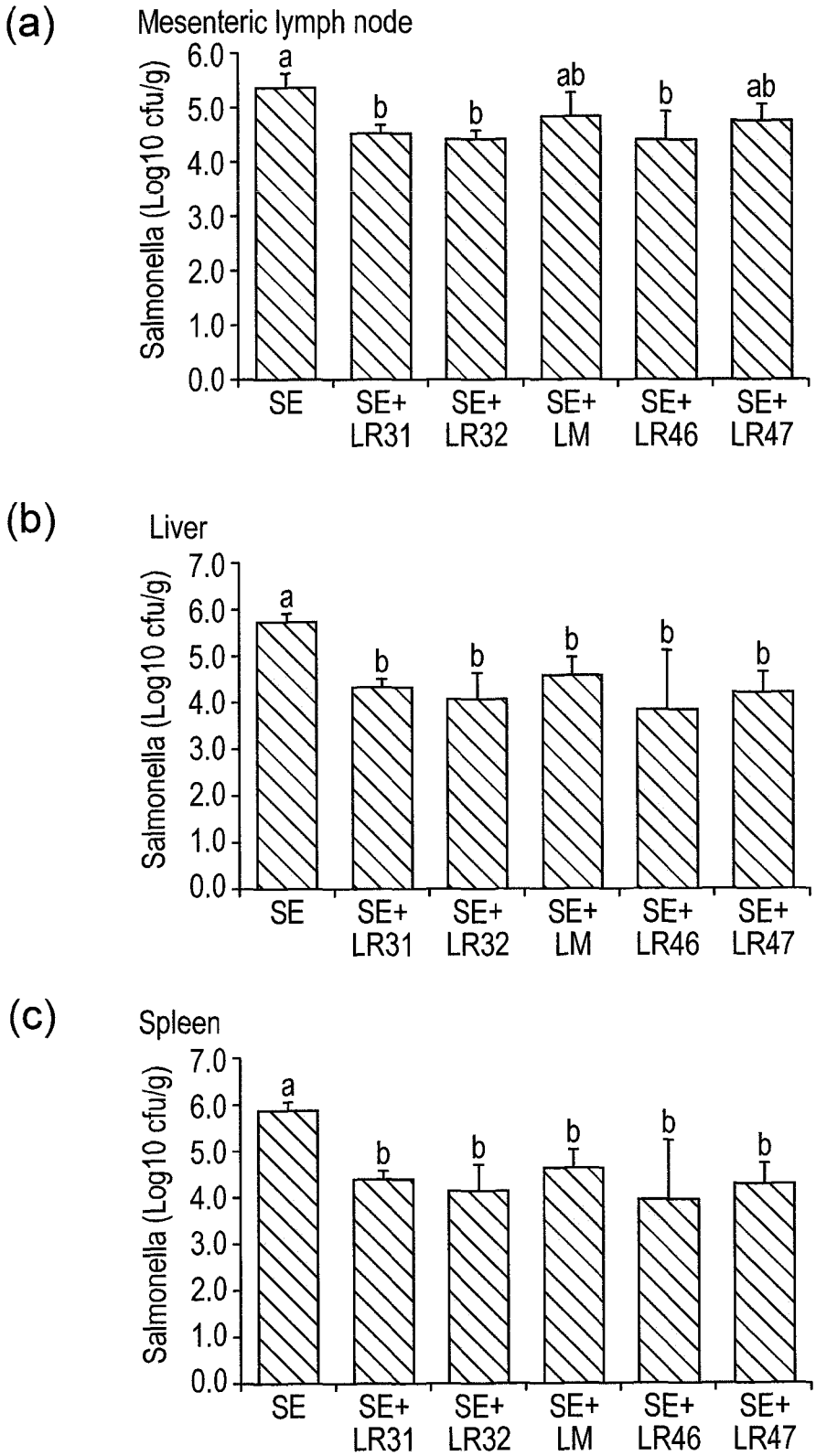

(a) Caecum
Salmonella (Log10 cfu/g)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
a
bc
c
bc
b
bc
SE
SE+LR31
SE+LR32
SE+LM
SE+LR46
SE+LR47
(b) Colon
Salmonella (Log10 cfu/g)
a
b
c
ab
bc
bc
SE
SE+LR31
SE+LR32
SE+LM
SE+LR46
SE+LR47
Columns with distinct superscripts differ significantly (p≤0.05)

FIG. 18



Columns with distinct superscripts differ significantly ($p \leq 0.05$)

FIG. 19

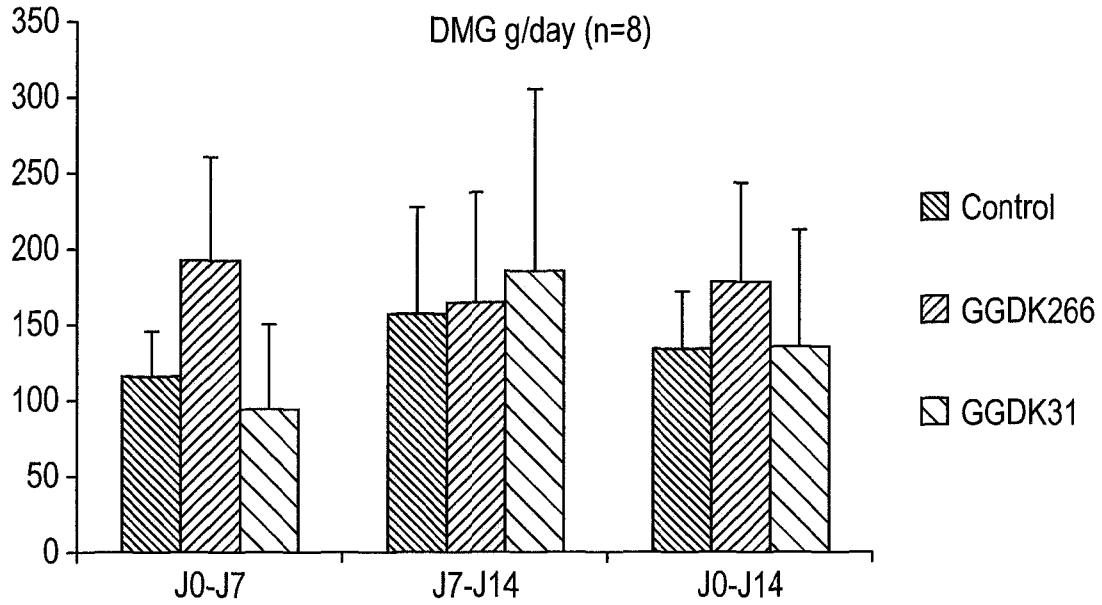

DMG g/day (n=8)
350
300
250
200
150
100
50
0
J0-J7
J7-J14
J0-J14
Control
GGDK266
GGDK31

FIG. 20

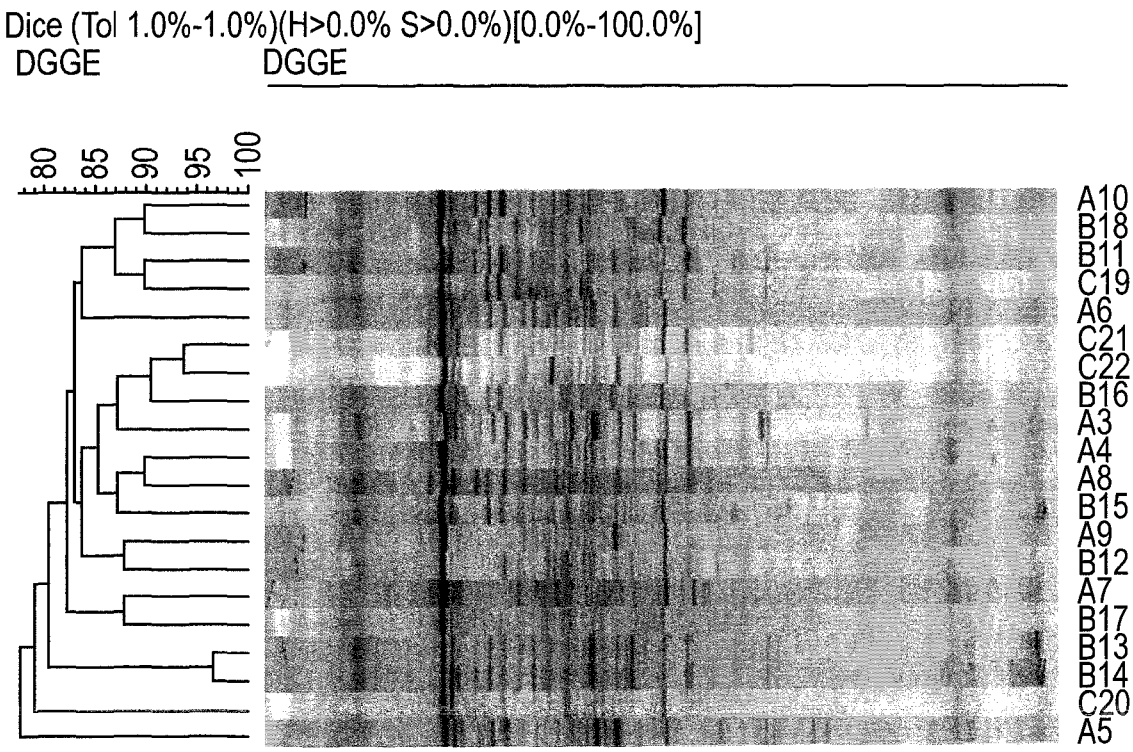

Dice (Tol 1.0%-1.0%)(H>0.0% S>0.0%)[0.0%-100.0%]
DGGE
DGGE
80
85
90
95
100
A10
B18
B11
C19
A6
C21
C22
B16
A3
A4
A8
B15
A9
B12
A7
B17
B13
B14
C20
A5

FIG. 21

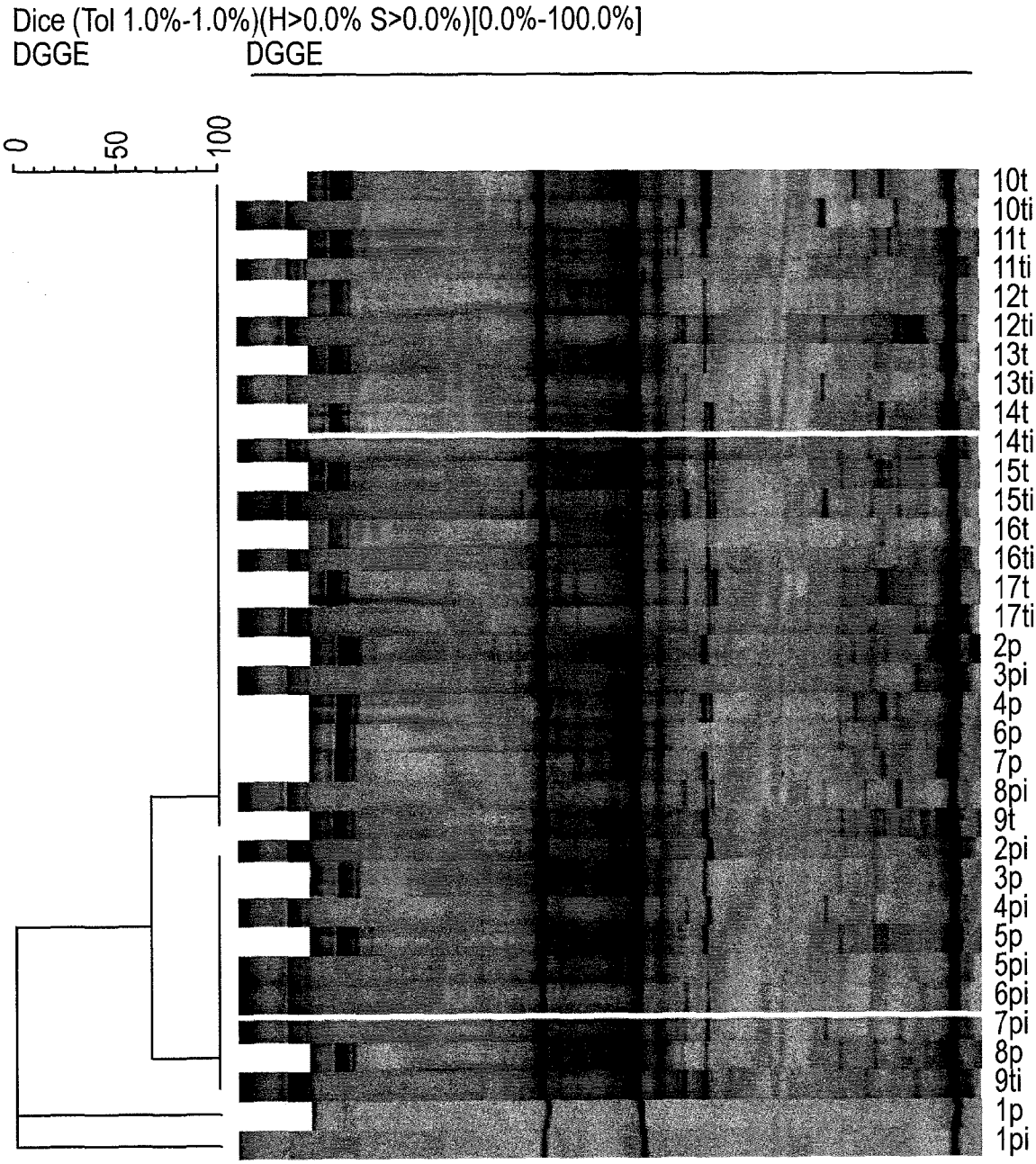

Dice (Tol 1.0%-1.0%)(H>0.0% S>0.0%)[0.0%-100.0%]
DGGE
DGGE
0
50
100
10t
10ti
11t
11ti
12t
12ti
13t
13ti
14t
14ti
15t
15ti
16t
16ti
17t
17ti
2p
3pi
4p
6p
7p
8pi
9t
2pi
3p
4pi
5p
5pi
6pi
7pi
8p
9ti
1p
1pi

FIG. 22

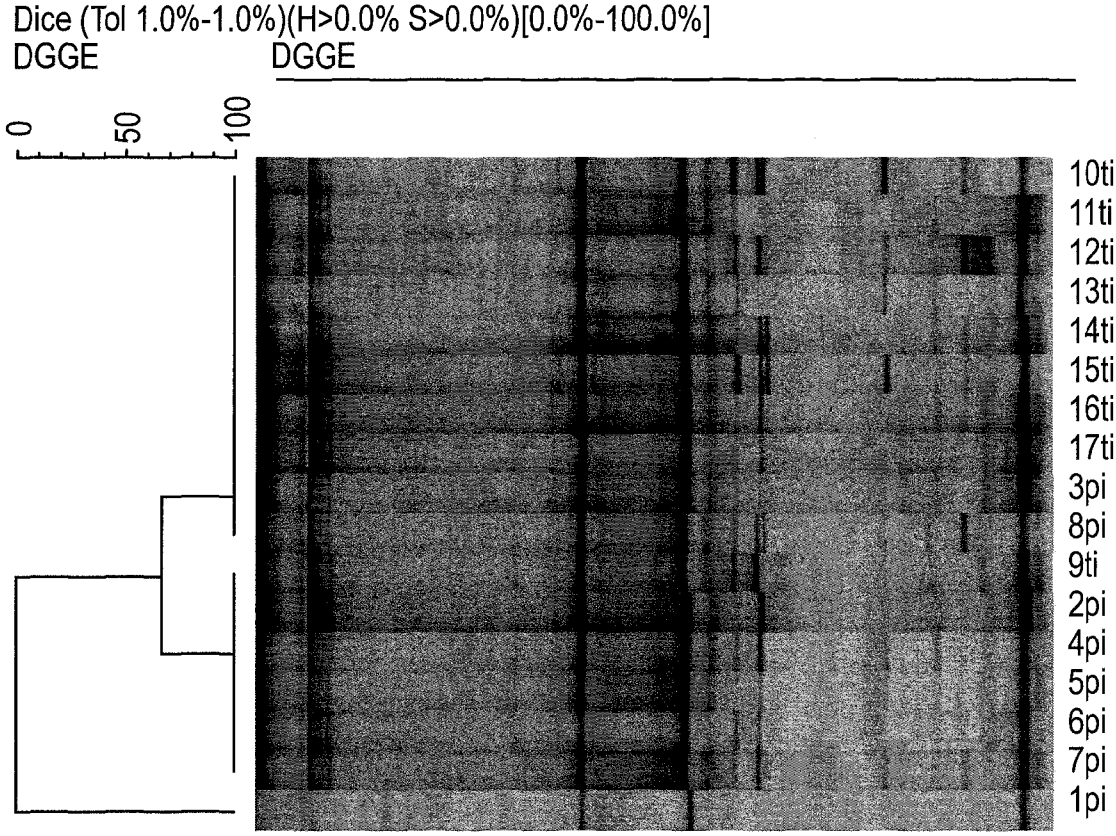

Dice (Tol 1.0%-1.0%)(H>0.0% S>0.0%)[0.0%-100.0%]
DGGE
DGGE
0
50
100
10ti
11ti
12ti
13ti
14ti
15ti
16ti
17ti
3pi
8pi
9ti
2pi
4pi
5pi
6pi
7pi
1pi

FIG. 23

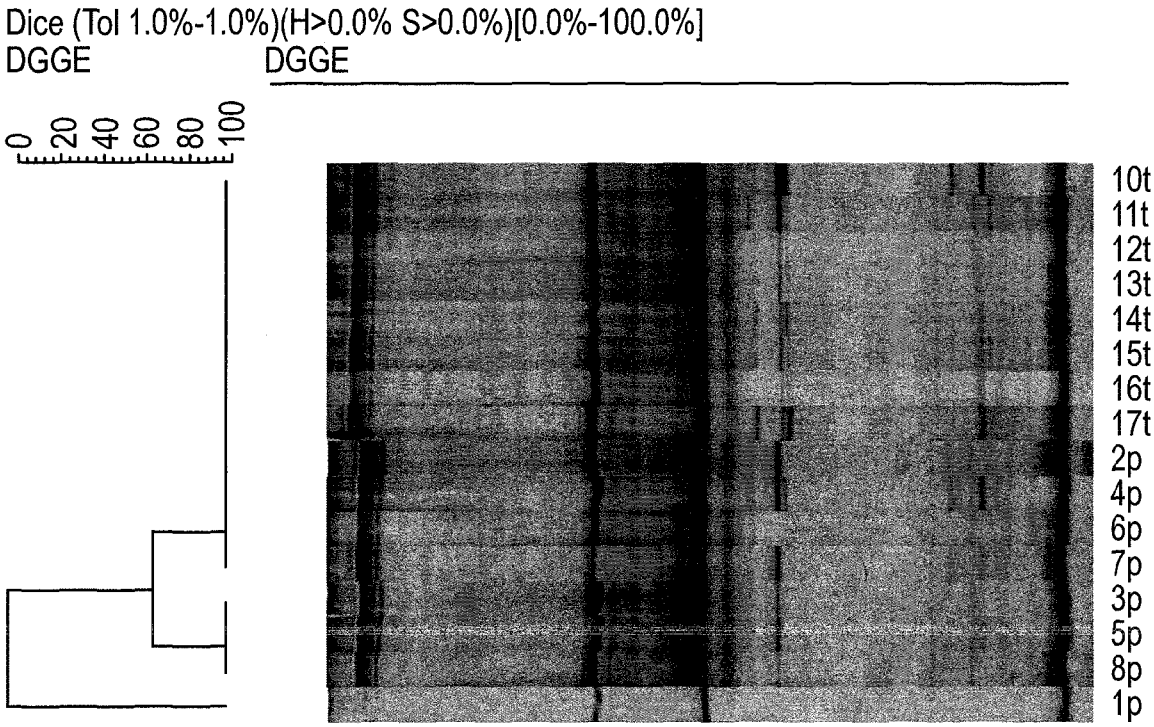

Dice (Tol 1.0%-1.0%)(H>0.0% S>0.0%)[0.0%-100.0%]
DGGE
DGGE
0
20
40
60
80
100
10t
11t
12t
13t
14t
15t
16t
17t
2p
4p
6p
7p
3p
5p
8p
1p

FIG. 24

23/25

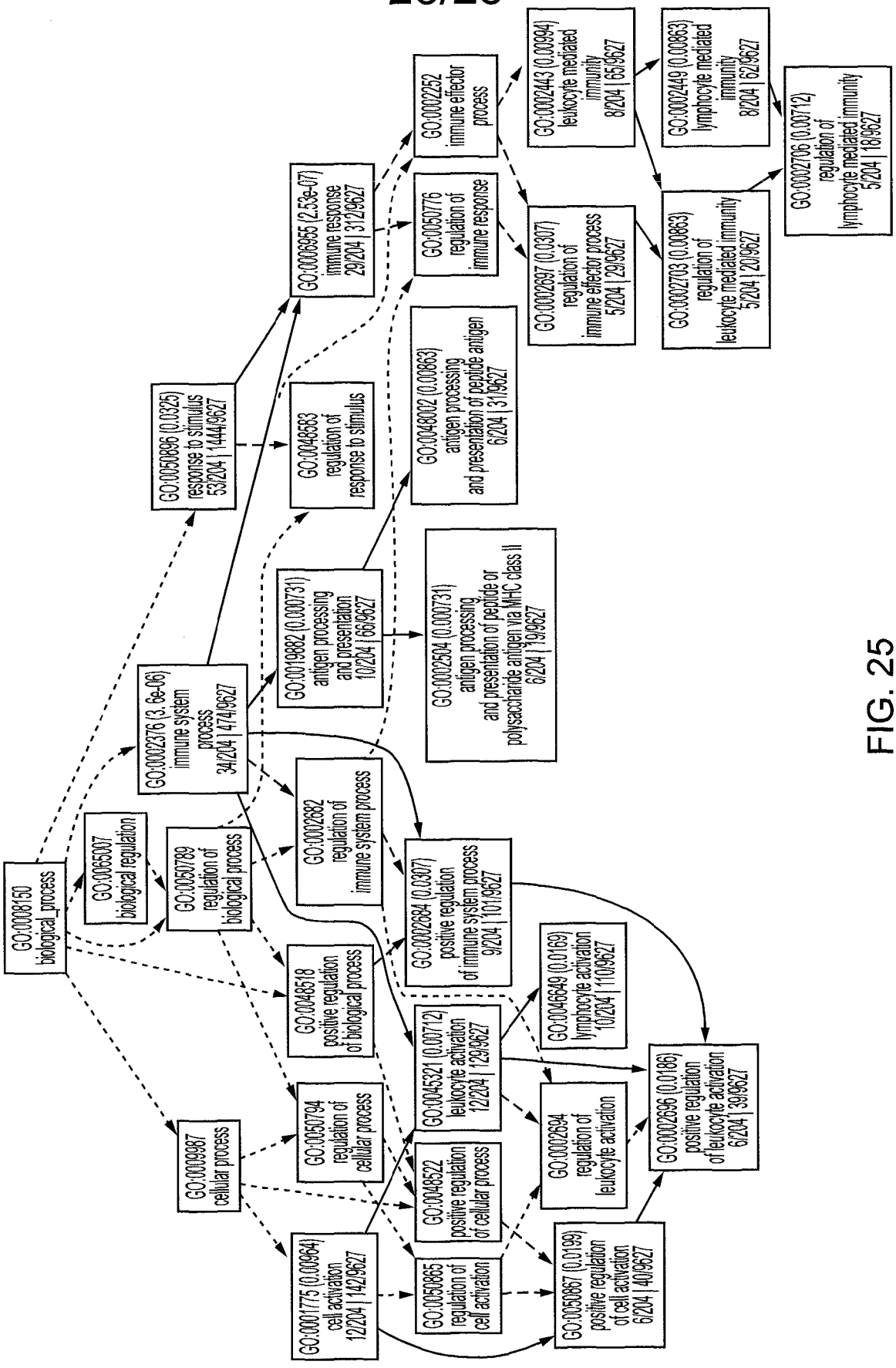

GO:0008150 biological_process
GO:0065007 biological regulation
GO:0050789 regulation of biological process
GO:0002376 (3. 6e-06) immune system process 34/204 | 474/9627
GO:0050896 (0.0325) response to stimulus 53/204 | 1444/9627
GO:0009987 cellular process
GO:0001775 (0.00964) cell activation 12/204 | 142/9627
GO:0009955 (2.53e-07) immune response 29/204 | 312/9627
GO:0048583 regulation of response to stimulus
GO:0019882 (0.000731) antigen processing and presentation 10/204 | 66/9627
GO:0002682 regulation of immune system process
GO:0048518 positive regulation of biological process
GO:0050794 regulation of cellular process
GO:0048522 positive regulation of cellular process
GO:0050865 regulation of cell activation
GO:0002252 immune effector process
GO:0050776 regulation of immune response
GO:0048002 (0.00863) antigen processing and presentation of peptide antigen 6/204 | 31/9627
GO:0002504 (0.000731) antigen processing and presentation of peptide or polysaccharide antigen via MHC class II 6/204 | 19/9627
GO:0002684 (0.0307) positive regulation of immune system process 9/204 | 101/9627
GO:0045321 (0.00712) leukocyte activation 12/204 | 129/9627
GO:0050867 (0.0199) positive regulation of cell activation 6/204 | 40/9627
GO:0002443 (0.00994) leukocyte mediated immunity 8/204 | 65/9627
GO:0002697 (0.0307) regulation of immune effector process 5/204 | 29/9627
GO:0046649 (0.0169) lymphocyte activation 10/204 | 110/9627
GO:0002694 regulation of leukocyte activation
GO:0002449 (0.00863) lymphocyte mediated immunity 8/204 | 62/9627
GO:0002703 (0.00863) regulation of leukocyte mediated immunity 5/204 | 20/9627
GO:0002706 (0.00712) regulation of lymphocyte mediated immunity 5/204 | 18/9627
GO:0002696 (0.0186) positive regulation of leukocyte activation 6/204 | 39/9627

FIG. 25

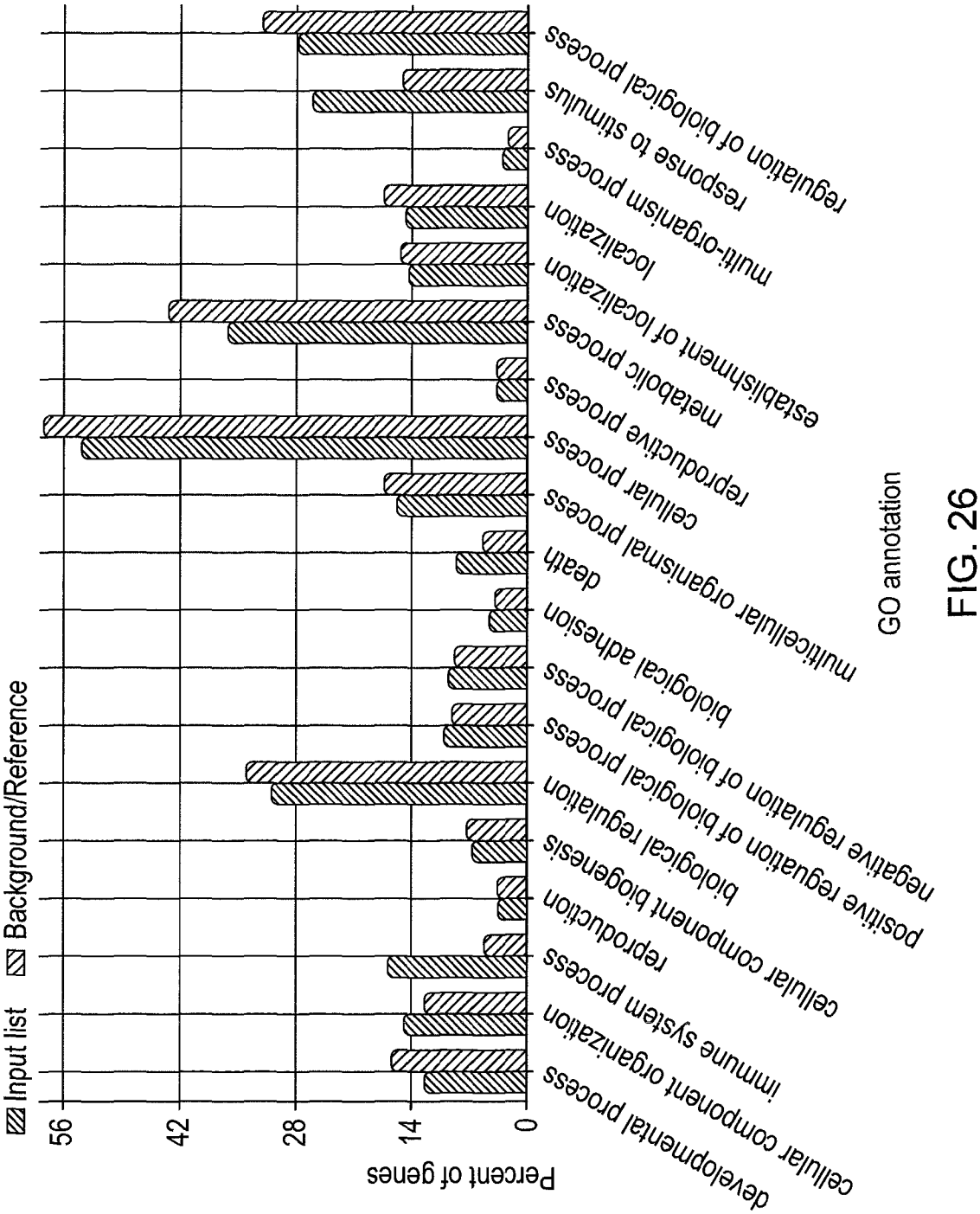

Input list
Background/Reference
Percent of genes
56
42
28
14
0
developmental process
cellular component organization
immune system process
reproduction
cellular component biogenesis
biological regulation
positive regulation of biological process
negative regulation of biological process
biological adhesion
death
multicellular organismal process
cellular process
reproductive process
metabolic process
establishment of localization
localization
multi-organism process
response to stimulus
regulation of biological process
GO annotation

FIG. 26

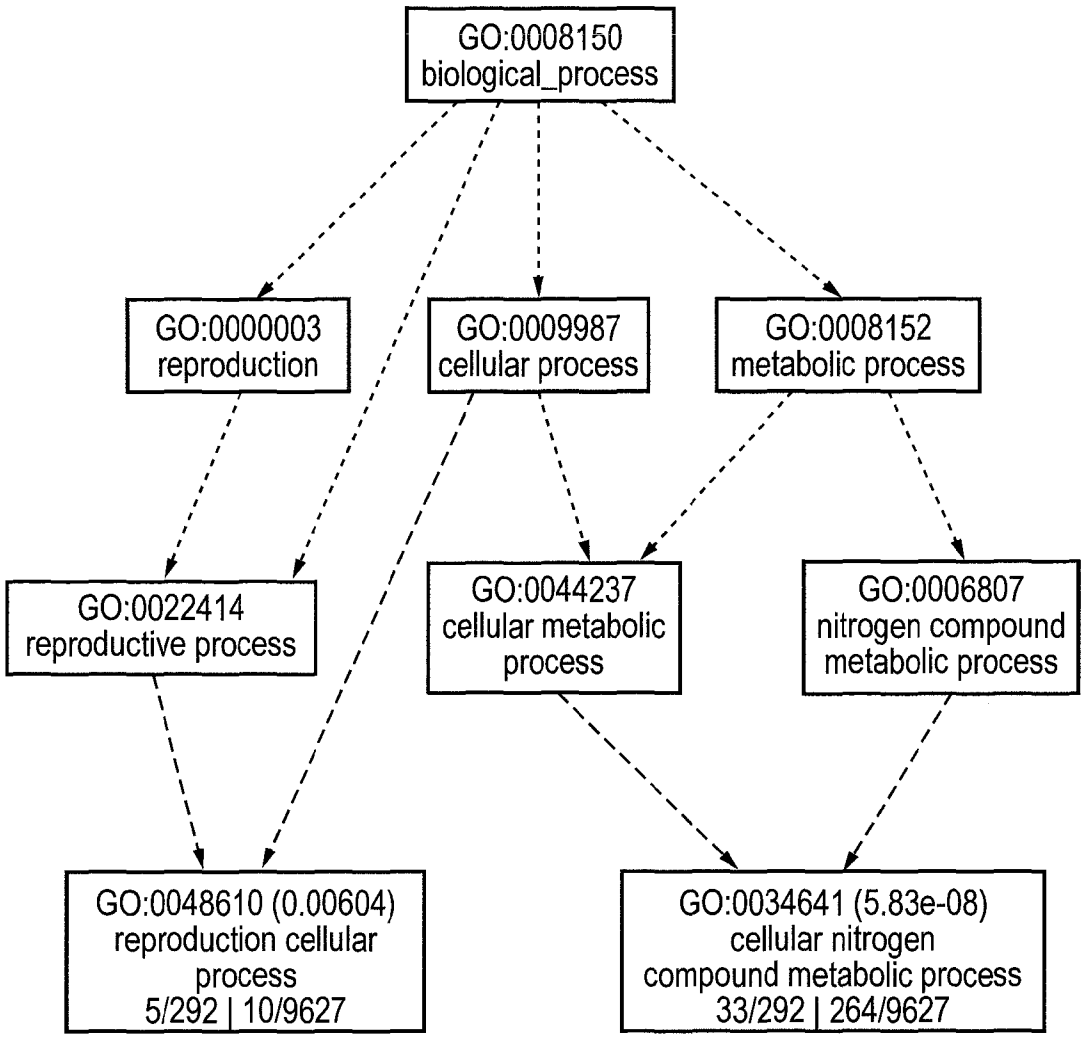

GO:0008150
biological_process
GO:0000003
reproduction
GO:0009987
cellular process
GO:0008152
metabolic process
GO:0022414
reproductive process
GO:0044237
cellular metabolic
process
GO:0006807
nitrogen compound
metabolic process
GO:0048610 (0.00604)
reproduction cellular
process
5/292 | 10/9627
GO:0034641 (5.83e-08)
cellular nitrogen
compound metabolic process
33/292 | 264/9627

FIG. 27